BMJ Open

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Journal:	*BMJ Open*
Manuscript ID	bmjopen-2015-009635
Article Type:	Research
Date Submitted by the Author:	04-Aug-2015
Complete List of Authors:	Taylor, Caroline; University of Bristol, Centre for Child and Adolescent Health Humphriss, Rachel; University Hospitals Bristol NHS Foundation Trust, Children's Hearing Centre; University of Bristol, School of Social and Community Medicine Hall, Amanda; University of Bristol, Centre for Hearing and Balance Studies Golding, Jean; University of Bristol, Centre for Child and Adolescent Health Emond, Alan; University of Bristol, Centre for Child and Adolescent Health; University of Bristol, School of Social and Community Medicine
Primary Subject Heading:	Epidemiology
Secondary Subject Heading:	Epidemiology, Paediatrics, Public health, Reproductive medicine
Keywords:	EPIDEMIOLOGY, Fetal medicine < OBSTETRICS, PAEDIATRICS, Community child health < PAEDIATRICS, Developmental neurology & neurodisability < PAEDIATRICS, TOXICOLOGY

Balance ability in 7- and 10-year-old children: associations with prenatal lead and cadmium exposure and with blood lead levels in children in a prospective birth cohort study

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Keywords: Lead, Cadmium, Vestibular function, Balance, Children, Pregnancy, ALSPAC

Word count: (excluding title page, abstract, references, figures and tables): 3429

Abstract

Objectives Most studies reporting evidence of adverse effects of lead and cadmium on balance have been conducted in high-exposure groups or have included adults. The effects of prenatal exposure have not been well studied, nor have the effects directly in children. The aim of the study was to identify the associations of lead (in utero and in childhood) and cadmium (in utero) exposure with balance ability in 7- and 10-year-old children.

Design Prospective birth cohort study

Participants Maternal lead and cadmium levels were measured in 4285 women enrolled in ALSPAC during pregnancy. Child lead levels were measured in a subsample of 582 children at age 30 months.

Main outcome measures A total of 5042 children completed a heel-to-toe walking test at 7 years. At 10 years 6915 children underwent clinical tests of static and dynamic balance. Statistical analysis included logistic regression modelling comparing categories of ≥5 vs <5 µg/dl for lead and ≥1 vs <1 µg/l for cadmium with SPSS v19.

Results Balance at age 7 years was not associated with elevated in utero lead or cadmium exposure (adjusted OR for balance dysfunction: Pb 1.02 (95% CI 0.96, 1.08); Cd 0.99 (0.80, 1.22)), or with elevated child blood lead level at age 30 months (adjusted OR 0.99 (0.93, 1.06)). Similarly, neither measures of static nor dynamic balance at age 10 years were associated with in utero lead or cadmium exposure, or child lead level.

Conclusions These findings do not provide any evidence of an association of prenatal exposure to lead or cadmium, or lead levels in childhood, on balance ability in children. Confirmation in other cohorts is needed.

Word count: 264

Strengths and limitations of the study

- Data were collected prospectively in a population-based study
- The number of participants was large compared with several comparable studies
- Measures of Pb and Cd do not necessarily reflect lifetime exposure
- Balance measures have a poor test–retest reliability

INTRODUCTION

Balance, or postural stability, is defined as the ability to keep the centre of gravity over the base of support.[1] The maintenance of balance underpins the ability to carry out nearly all daily activities. The control of balance is complex and is dependent on sensory inputs from the vestibular and visual systems, neural processing centres in the central nervous system, and motor inputs from the proprioceptive centre. Functional damage or deficits in any of these systems can lead to balance dysfunction, which can be associated with low self-esteem, anxiety and loss of confidence in children.[2]

Lead and cadmium are toxic metals and their effects on neurocognitive and behavioural functions are well documented.[3-6] Lead passes freely through the placenta so that ratio of fetal to maternal blood lead is about 0.8, although the placenta can act as a partial barrier to cadmium.[7] The fetus is particularly vulnerable to the effects of these metals because of high rates of cell division and development. The development of the inner ear and vestibular function spans the whole of the period of gestation (for example, the membranous labyrinth is complete by week 7 with development of the bony labyrinth from week 9 to 23; the vestibular apparatus is in an adult-like form by week 25, and is active by week 32; vestibular ganglions develop from week 12 and reach maturity at week 39, and so on[8]). Thus, prenatal exposure to lead and cadmium may have adverse effects on the development of the inner ear, and hence on vestibular function and balance ability in later childhood.

It was noted in the 1980s that children who survived acute lead encephalopathy had ataxia and experienced difficulties in maintaining postural balance.[9] This led to a series of studies in children with more moderate levels of lead exposure showing that the child's lead level was associated with balance dysfunction and sway oscillation.[10-14] To our knowledge, there are

no reports of the effect of cadmium on balance ability in children. However, a recent study of lead and cadmium levels in adults in the US National Health and Nutrition Examination Survey (NHANES) found preliminary evidence of an association of lead and cadmium with balance and vestibular function.[15] In addition, altered postural balance response has been reported in adult workers occupationally exposed to lead.[16-18] and cadmium.[19] These results require confirmation in other cohorts and particularly in children.

The aims of our study were to investigate the associations of in utero exposure to lead and cadmium, and lead levels in children, on balance in childhood using data obtained from the Avon Longitudinal Study of Parents and Children (ALSPAC).

Methods

We first modelled associations of in utero exposure to lead and cadmium, using maternal blood levels during pregnancy, with clinical measures of balance (dynamic and static) at 7 and 10 years. We also investigated associations with questionnaire items related to balance repeated at 30 months, 42 and 81 months, and further items at 10 years. We also modelled the associations of child levels of lead with the balance variables.

The ALSPAC study

The study sample was derived from the ALSPAC study, a population-based study investigating environmental and genetic influences on the health, behaviour and development of children. All pregnant women in the former Avon Health Authority with an expected delivery date between 1 April 1991 and 31 December 1992 were eligible for the study; 14,541 pregnant women were initially enrolled, resulting in a cohort of 14,062 live births.[20]

The social and demographic characteristics of this cohort were similar to those found in UK national census surveys [21]. Further details of ALSPAC are available at www.bris.ac.uk/alspac.

Collection, storage and analysis of blood samples

Maternal blood samples Whole blood samples were collected in acid-washed vacutainers (Becton and Dickinson, Oxford, UK) by midwives as early as possible in pregnancy. The median gestational age at the time of blood sampling was 11 weeks. The interquartile range was 9–13 weeks, and 93% of the samples were collected at <18 weeks gestation. Whole blood samples were stored in the original tube at 4ºC at the collection site before being transferred to the central Bristol laboratory within 1–4 days. Samples were at ambient temperature during transfer (up to 3 h). They were then stored at 4ºC until analysis. Samples were analysed for lead using inductively-coupled plasma mass spectrometry in standard mode (R. Jones; Centers for Disease Control (CDC), Bethesda, MD, USA; CDC Method 3009.1). The analyses were completed on 4284 women. One sample had a Pb level below the limit of detection (0.29 µg/dl); 1119 samples were below the lower limit of detection for Cd (0.20 µg/l). These samples were assigned a value of 0.7 times the lower limit of detection.

Child blood samples Details of the selection of the subsample of children and analysis of the blood samples have been reported previously in detail.[3 22] In brief, a 10% randomly selected subsample of parents whose babies were born in the last 6 months of the ALSPAC study were invited to attend a research clinic (Children in Focus, CIF). At age 30 months, parental consent was sought for a venous blood sample, and was given by 81% of the 1135 children in the CIF group. The sample was drawn into lead-free tubes from 653 (71%) of

children attending the clinic. However, 69 samples were insufficient, leaving 582 samples for analysis. Analysis was by atomic absorption spectrometry (Southampton General Hospital, UK) with appropriate quality controls.

Balance variables

Clinic measures Full details of the balance outcomes including details of the measurements and validity have previously been published.[23] In brief, at age 7 years the heel-to-toe walking test of the Movement Assessment Battery for Children[24] was conducted with the total number of successful steps out of a maximum of 15 recorded (n=5402). At age 10 years a range of tests were used to assess balance: (1) walking along a beam, heel-to-toe, eyes open; (2) heel-to-toe balance on a beam, eyes closed; (3) standing on one leg, eyes closed. Each child had two attempts at beam-walking; for tests of static balance, children only had a second attempt if they failed to achieve the maximum score on the first attempt.[23] These tests were based on standard clinical tests to assess balance in children and have significant commonality with the balance subtest of the both editions of the Bruininks–Oseretsky Test of Motor Proficiency.[25] [26] The measures are also in common use when testing balance informally in the paediatric clinic.

Questionnaire items The primary caregiver (usually the mother) received a series of postal self-completion questionnaires. The questionnaires are available from the study website (http://www.bris.ac.uk/alspac/researchers/data-access/data-dictionary/). When their child was aged 18, 30, 42 and 81 months, the parent completing the questionnaire was asked to indicate 'Yes, can do well'/'Has only done once or twice'/'Has not yet started' in response to the statement 'He/She can balance on one foot for at least 1 second'. When their child was aged

10 years, the parent was asked to indicate 'Very well'/'Just OK'/'Can almost'/'Not at all' in response to the following questions: How well can your child stand on one leg in a stable position (e.g. when putting on trousers, skirt)?; How well can your child ride a bike (without stabilisers)?; How well can your child walk in the dark?

Confounding variables

Information on passive smoking exposure during the week and at weekends was obtained from questionnaires at 77 and 103 months. Information on traffic levels, type of accommodation, lowest level of accommodation and maternal education were obtained at from questionnaires completed by the mother during pregnancy. Dietary Ca and Fe intake at 7 years and 10 years were derived from food frequency questionnaires as previously described in detail [27].

Statistical analysis

Statistical analysis was carried out with IBM SPSS Statistics 21. Balance measures were derived as previously described.[23] In brief, for the heel-to-toe test at age 7 years, the number of steps (maximum 15) was categorised into 0–5, 6–10 and 11–15 steps for categorical associations, and 1–14 versus 15 steps for regression analyses. For the measure of dynamic balance at 10 years (beam-walking test), the mean of two attempts was categorised into quartiles. For measures of static balance at age 10 years (heel to toe balance on a beam with eyes closed/standing on one leg eyes closed), the sum of the score (s) from both attempts was calculated. Children who scored the maximum of 20 on the first attempt and so did not have a second attempt were given a final score of 40. The final scores were put into four categories

(0–9, 10–19, 20–39 and 40). All of the four static balance tests with eyes closed were summed to create a static balance eyes closed variable (SBEC).

Blood lead and cadmium levels were put into two categories (<5, ≥5 µg/dl for lead and <1, ≥1 µg/l for Cd). These categories were chosen in accordance with the levels of concern of the US Centers for Disease Control, the US Association of Occupation and Environmental Clinics and the American College of Obstetricians and Gynaecologists for Pb,[28-31] and the German Federal Environmental Agency for Cd.[32] Blood levels were also categorised into quartiles.

Chi square tests were used to compare categorical variables. Unadjusted and adjusted logistic regression analyses were used to investigate the association of blood levels with balance variables.

RESULTS

As previously reported, the mean child blood lead level was 4.22±3.12 µg/dl (n=582)[3 22]; the mean prenatal blood lead level was 3.67±1.47 µg/dl (n=4285) and the mean prenatal cadmium level was 0.58±0.63 µg/l (n=4286).[33 34]

Associations of measures of balance with prenatal lead and cadmium levels and with child lead levels

Associations with in utero exposure to lead and cadmium

There was no evidence of any association between the results of the heel-to-toe test at age 7 years and maternal lead or cadmium level during pregnancy (p=0.861 and p=0.112, respectively) (Table 1). Similarly, at age 10 years there were no associations between

dynamic balance (beam walking) or static balance (SBEC) and maternal blood lead or cadmium levels in pregnancy (all p>0.4) (Table 1).

In logistic regression models adjusted for sex, passive smoking, and calcium and iron intake, there was no evidence of any association between maternal blood lead or cadmium levels and measure of balance in the child at 7 and 10 years (all p>0.1) (Table 2). When the models were repeated with quintiles of maternal blood lead or cadmium level rather than a dichotomous variable, there was also no evidence of any associations (all p>0.01 with the exception of maternal blood lead for the odds of static balance dysfunction at 10 years where there was a protective effect (p for trend 0.038) (Supplementary Table 1).

Associations with child lead level

There was no evidence of any association between the results of the heel-to-toe test at age 7 years and child lead level (p for trend=0.146) (Table 3). Similarly, at age 10 years there were no associations between dynamic balance (beam walking) or static balance (SBEC) and child blood lead levels (p for trend=0.798 and p=0.918, respectively) (Table 3). In logistic regression models adjusted for sex, passive smoking, and calcium and iron intake, there was no evidence of any association between child blood levels at 30 months and measure of balance at 7 and 10 years (Table 4; all p for trend >0.3). When the models were repeated with quintiles of child blood lead rather than a dichotomous variable, there was also no evidence of any associations (all p for trend >0.1) with the exception of static balance where there was a weakly protective effect (p for trend=0.038 (Supplementary Table 1).

Table 1 Associations of in utero lead and cadmium exposure with measures of balance in the child at 7 and 10 years in ALSPAC

	Age (years)	Category	Maternal Pb (µg/dl)			Maternal Cd (µg/l)		
			<5	≥5	P value for trend	<1	≥1	P value for trend
Heel to toe test	7	0–5 steps	520 (27.1)	82 (26.5)	0.861	520 (26.6)	81 (29.1)	0.112
		6–10 steps	273 (14.2)	45 (14.5)		270 (13.8)	49 (17.6)	
		11–15 steps	1128 (58.7)	183 (59.0)		1164 (59.6)	148 (53.2)	
Beam walking (dynamic balance)	10	Q1	431 (23.4)	70 (24.1)	0.450	439 (23.3)	62 (25.3)	0.897
		Q2	461 (25.0)	80 (27.6)		484 (25.6)	57 (23.3)	
		Q3	456 (24.8)	66 (22.8)		463 (24.5)	59 (24.1)	
		Q4	494 (26.8)	74 (25.5)		502 (26.6)	67 (27.3)	
Static balance eyes closed score (SBEC) (static balance)	10	Q1	460 (24.4)	74 (25.8)	0.558	471 (25.4)	63 (25.8)	0.842
		Q2	459 (25.4)	58 (20.2)		455 (24.6)	62 (25.4)	
		Q3	430 (23.8)	83 (28.9)		457 (24.7)	57 (23.4)	
		Q4	459 (25.4)	72 (25.1)		469 (25.3)	62 (25.4)	

Values are n (%).

Q, quartile.

Table 2 Associations of in utero lead and cadmium exposure with balance measures in the child at 7 and 10 years old in ALSPAC

	Age (years)	Prenatal lead exposure			Prenatal cadmium exposure		
		OR of balance dysfunction (95% CI)		P value	OR of balance dysfunction (95% CI)		P value
Heel to toe test	7	Unadjusted	1.02 (0.97, 1.08)	0.503	Unadjusted	0.82 (0.70, 0.95)	0.010
		Adjusted [a]	1.02 (0.96, 1.08)	0.555	Adjusted [a]	0.99 (0.80, 1.22)	0.904
Dynamic balance	10	Unadjusted	1.01 (0.95, 1.08)	0.790	Unadjusted	1.00 (0.84, 1.21)	0.946
		Adjusted [b]	1.02 (0.95, 1.09)	0.692	Adjusted [b]	1.20 (0.95, 1.52)	0.135
Static balance	10	Unadjusted	0.98 (0.92, 1.05)	0.569	Unadjusted	1.06 (0.88, 1.28)	0.523
		Adjusted [b]	0.98 (0.92, 1.06)	0.661	Adjusted [b]	1.00 (0.79, 1.26)	0.995

Logistic regression showing odds ratio of balance dysfunction (95% CI).

[a]Adjusted for: sex, passive smoking at 77 months old (weekdays and weekends), and Ca and Fe intake at 7 years.

[b]Adjusted for: sex, passive smoking at 103 months old (weekdays and weekends), and Ca and Fe intake at 10 years.

Table 3 Associations of child lead level at 30 months with measures of balance at 7 and 10 years in ALSPAC

Test	Age (years)	Category	Child Pb (µg/dl)		P value for trend
			<5	≥5	
Heel to toe test	7	0–5 steps	82 (25.8)	34 (30.9)	0.146
		6–10 steps	52 (16.4)	22 (20.0)	
		11–15 steps	185 (57.9)	54 (49.1)	
Beam walking (dynamic balance)	10	Q1	74 (24.0)	24 (23.5)	0.798
		Q2	82 (26.6)	30 (29.4)	
		Q3	72 (23.4)	23 (22.5)	
		Q4	80 (26.0)	25 (24.5)	
Static balance eyes closed score (SBEC) (static balance)	10	Q1	80 (26.5)	22 (22.0)	0.918
		Q2	70 (23.2)	30 (30.0)	
		Q3	77 (25.5)	24 (24.0)	
		Q4	75 (24.8)	24 (24.0)	

Values are n (%).

Q, quartile.

Table 4 Child blood lead level at 30 months and balance measures at 7 and 10 years old in ALSPAC

	Age (years)	OR of balance dysfunction (95% CI)		P value
Heel to toe test	7	Unadjusted	0.99 (0.93, 1.05)	0.618
		Adjusted [a]	0.99 (0.93, 1.06)	0.778
Dynamic balance	10	Unadjusted	1.03 (0.96, 1.11)	0.422
		Adjusted [b]	1.01 (0.93, 1.09)	0.814
Static balance	10	Unadjusted	1.04 (0.96, 1.12)	0.345
		Adjusted [b]	1.03 (0.94, 1.12)	0.540

Logistic regression analysis comparing results for children with blood lead level ≥5 vs <5 µg/dl.

[a]Adjusted for: sex, passive smoking at 77 months old (weekdays and weekends), and Ca and Fe intake at 7 years.

[b]Adjusted for: sex, passive smoking at 103 months old (weekdays and weekends), and Ca and Fe intake at 10 years.

Associations with questionnaire items at 18–81 months and at 10 years

There was no evidence of any associations between child blood lead level at 30 months and the ability to stand on one foot for at least 1 second at 30, 42 or 81 months (all p>0.6; Supplementary Table 2). There was no evidence of any associations between maternal blood lead level during pregnancy and the ability of the child to stand on one foot for at least 1 second at 18, 30, 42 or 81 months (all p>0.3) (Supplementary Table 2). There was no evidence of any associations between maternal blood cadmium level during pregnancy and the ability of the child to stand on one foot for at least 1 second at 42, or 81 months (all p>0.4), but there were associations at 18 and 30 months (p<0.001 and p=0.003, respectively; maternal cadmium ≥1 µg/l was associated with being more likely to be able to stand on one foot well) (Supplementary Table 2).

There was no evidence of any association of elevated child lead level, or in utero lead or cadmium exposure, with ability to stand on one leg or to walk in the dark at 10 years (all p>0.09, chi-square test) (Supplementary Table 3). However, prenatal lead level ≥5 µg/dl was associated with not being able to ride a bike without stabilisers (p=0.007), whereas child lead level ≥5 µg/dl and prenatal cadmium ≥1 µg/l were weakly associated with being able to ride a bike without stabilisers very well (p=0.050 and p=0.075 respectively). When these associations were modelled in a logistic regression analysis adjusted for variables that could affect availability of a bicycle and being able to ride a bicycle locally (traffic level on the home street, type of accommodation, lowest level of accommodation), and maternal education, there was very weak evidence for an association of child lead level being associated with being able to ride a bike well without stabilisers (OR in unadjusted model 2.79 (95% CI 0.096, 8.10), p=0.059; OR in adjusted model OR 2.57 (95% CI 0.87, 7.62), p=0.089). The association was stronger for prenatal lead level in the unadjusted model, but the effect was attenuated with adjustment (OR in unadjusted model 0.59 (95% CI 0.40, 0.87),

p=0.007; OR in adjusted model 0.73 (95% CI 0.47, 1.14), p=0.167); for prenatal cadmium level again the weak effect was attenuated by adjustment (unadjusted OR 1.69 (95% CI 0.94, 3.01, p=0.078); adjusted OR 1.44 (95% CI 0.75, 2.75, p=0.274).

DISCUSSION

We did not find any evidence of an association of prenatal exposure to lead or cadmium, or lead levels in childhood, on balance ability (static and dynamic) in children. Counterintuitively, there was a suggestion that higher child lead levels and in utero lead exposure were associated with the ability to ride a bike without stabilisers at age 10 years, but these effects were negated when the associations were adjusted for variables that included the lowest level of accommodation and traffic levels outside the home. This is the first study, to our knowledge, reporting on the associations between in utero exposure to lead and cadmium and balance ability of the child, and adds to the few studies on child lead levels and balance ability.

Postural balance is controlled by a complex interaction of sensorimotor processes, including visual, proprioception and the vestibular system. In our study the measure of static balance with eyes closed eliminated vision and minimised proprioceptive inputs, thereby enabling the assessment of vestibular information as the primary input. Use of clinical measures similar to these is commonplace in epidemiological studies (e.g. [35-38]), as the tests are in common clinical usage and require little or no specialist equipment. However, although the vestibular dominant condition of tests of standing balance (reduced base of support with eyes closed) has been shown to correlate well with more expensive systems such as computerised dynamic posturography (considered to be the 'gold standard' method for assessing balance function) in adults,[39] there are questions about whether such a correlation exists in children.[40] It has even

been suggested that computerised measures using a force-platform and clinical tests such as those used by ALSPAC give complementary rather than concurrent information.[41] A cautious approach should therefore be taken if seeking to compare studies using these two different types of outcome measure.

Most studies on lead levels and balance in children have included children with relatively high lead levels (means in each study of 11.6 to 20.7 µg/dl) and measured balance with a force platform system,[10-13] and have found negative associations. There are several reason for our results being in contrast to these studies. First, the mean child blood lead level in our study (4.22 µg/dl) was lower than reported in these earlier studies, and may have been too low either to have been sufficient to cause balance dysfunction. Alternatively, the effect on balance might have been too small to be detectable with our tests, although a study in Inuit children had levels that were more comparable to the present study (mean 5.4 µg/dl) showed a significant association with sway oscillations.[14] Second, we used a series of assessments based on clinical tests to measure balance rather than a force platform or measurement of sway oscillations and this may account for differences in the findings. As discussed earlier, clinical and force platform measures may be giving complementary rather than concurrent information: whereas posturography is a measure of the motor and sensory strategies used to control balance, clinical tests evaluate the results of that balance control.[41] Measuring sway oscillations using a force platform will also be more sensitive than the ALSPAC measures, which measured time before a procedural fault such as touching the floor with either foot or lifting a foot off the beam. Third, early exposure to lead and/or cadmium could damage the vestibular system, but the plasticity of the balance system might compensate for this so that there is no functionally measurable effect. This is in accordance with measures of balance in the present study indirectly assessing the vestibular system. This could also account for effects being reported in adults, in whom plasticity is less effective for overall balance

compensation, but not in children. It is also possible that vestibular system (to include peripheral (ear) and central (brain) components of the vestibular pathway) dysfunction caused by in utero exposure to lead or cadmium may not be apparent in childhood but may manifest in later life. Finally, it is also possible that studies showing non-significant results have tended not to have been published.

To our knowledge, there are no studies that have examined the effect of in utero exposure to lead or cadmium on balance ability in the child. Our results provide preliminary evidence for lack of effect, but this requires confirmation on other cohorts.

The strengths of the study are that: (1) it is a population-based study; (2) the data were collection prospectively; and (3) the numbers included in the study were large compared with several other studies. There are several limitations to the study. First, measures of blood lead and cadmium do not necessarily reflect lifetime exposure. Bone lead, which makes up more than 95% of the body lead, can be measured by K x-ray fluorescence,[42] but this is expensive, technically demanding and may not always be ethically permissible in children. This limitation is of less consequence for in utero exposure as the maternal blood level largely determines the fetal blood level. Second, there may be confounders we were unable to account for. Third, we were unable to control for lead and cadmium separately in the models because of multicollinearity. Finally, the balance measures used by ALSPAC had low test–retest reliability,[23] which is a common problem with measures of childhood balance.[43-45] The measure of ability to balance on one foot for 1 s, a test which was originally developed by Chamberlain and Davey [46] in 1976, has been particularly criticised for having poor test validity as it is difficult to discriminate a failed attempt from a successful attempt.[8] It should ideally form part of a battery of clinical measures, perhaps with a longer duration of standing.

These combined effects may have led to random misclassification in our balance assessments and dilution of the estimates of the effects of lead and cadmium exposure.

CONCLUSIONS

We did not find any evidence of an association of prenatal exposure to lead or cadmium with balance ability in children. In contrast to previous studies, we did not find any association of child blood lead with balance ability in children. This may reflect variation in the methods used to assess balance in different studies, or may be related to the lower mean lead level in the children in the present study than in previous studies. Further work in other cohorts is needed to confirm the results.

Contributions: CMT and RH conceived the study; CMT carried out the data analysis; CMT and RH drafted the paper; JG, AE and AH participated in the design of the study; all authors helped to draft the manuscript, and read and approved the final manuscript.

Data Sharing: Further details of ALSPAC are available at www.bris.ac.uk/alspac. This website includes details of how to access data.

Ethics approval: Ethics approval for the study was obtained from the ALSPAC Ethics and Law Committee and the Local Research Ethics Committees.

Competing interests: The authors declare that there are no competing interests

Acknowledgements: We are extremely grateful to all the families who took part in this study, the midwives for their help in recruiting them, and the whole ALSPAC team, which includes interviewers, computer and laboratory technicians, clerical workers, research scientists, volunteers, managers, receptionists and nurses.

Funding: The UK Medical Research Council and the Wellcome Trust (Grant ref: 102215/2/13/2) and the University of Bristol currently provide core support for ALSPAC. This publication is the work of the authors who will serve as guarantors for the contents of this paper. CMT was supported by a Wellcome Trust Career Re-entry Fellowship (Grant ref: 104077/Z/14/Z). The publication is the work of the authors who will serve as guarantors for the contents of the paper.

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Supplementary Table 1 Associations of responses to questionnaire items related to balance at 7–10 years old in ALSPAC

Quartile	Prenatal exposure				Child Pb[c]	
	Maternal Pb[a]		Maternal Cd[b]			
	OR of balance dysfunction (95% CI)	P value for trend	OR of balance dysfunction (95% CI)	P value for trend	OR of balance dysfunction (95% CI)	P value for trend
Heel to toe test at 7 years[d]						
1	1.00 (Ref)	0.441	(Ref)	0.189	(Ref)	0.203
2	0.94 (0.72, 1.22)		1.17 (0.91, 1.52)		1.44 (0.83, 2.52)	
3	1.16 (0.90, 1.51)		0.90 (0.69, 1.18)		0.88 (0.49, 1.57)	
4	1.11 (0.85, 1.44)		0.94 (0.66, 1.33)		0.85 (0.47, 1.53)	
Dynamic balance at 10 years[e]						
1	(Ref)	0.148	(Ref)	0.332	(Ref)	0.629
2	1.34 (0.99, 1.81)		0.85 (0.65, 1.12)		0.80 (0.41, 1.56)	
3	1.09 (0.81, 1.46)		0.95 (0.71, 1.26)		1.08 (0.54, 2.14)	
4	1.09 (0.81, 1.46)		1.10 (0.76, 1.59)		0.86 (0.43, 1.72)	
Static balance at 10 years[e]						
1	(Ref)	0.038	(Ref)	0.605	(Ref)	0.297
2	0.68 (0.50, 0.92)		1.11 (0.84, 1.48)		0.71 (0.36, 1.39)	
3	0.85 (0.62, 1.16)		1.11 (0.83, 1.49)		1.20 (0.59, 2.47)	
4	0.89 (0.65, 1.23)		0.97 (0.67, 1.41)		0.87 (0.427, 1.78)	

[a]Maternal Pb quartiles: Q1 0.20–2.66, Q2 2.67–3.40, Q3 3.41–4.33, Q4 4.34–19.14 µg/dl.
[b]Maternal Cd quartiles: Q1 0.14–0.14, Q2 0.20–0.29, Q3 0.30–0.72, Q4 0.73–6.30 µg/l.
[c]Child Pb quartiles: Q1 0.83–2.18, Q2 2.28–3.32, Q3 3.42–5.18, Q4 5.28–27.56 µg/dl.
[d]Adjusted for: sex, passive smoking at 77 months old (weekdays and weekends), and Ca and Fe intake at 7 years.
[e]Adjusted for: sex, passive smoking at 103 months old (weekdays and weekends), and Ca and Fe intake at 10 years.

Supplementary Table 2 Associations of responses to questionnaire items related to balance at 18–81 months old in ALSPAC

	Age (months)	Category	Prenatal exposure						Child Pb (µg/dl)		
			Maternal Pb (µg/dl)			Maternal Cd (µg/l)					
			<5	≥5	P value (chi square)	<1	≥1	P value (chi square)	<5	>5	P value (chi square)
Stand on 1 foot for 1 s	18	Yes, can do well	1397 (50.8%)	237 (51.9%)	0.680	1350 (49.5%)	280 (58.6%)	<0.001	-	-	-
		Only done 1–2 times/Not yet started	1352 (49.2%)	220 (48.4%)		1376 (50.5%)	198 (41.4%)		-	-	
Stand on 1 foot for 1 s	30	Yes, can do well	1853 (70.6%)	313 (71.5%)	0.711	1811 (69.7%)	352 (76.5%)	0.003	270 (69.6%)	95 (70.4%)	0.865
		Only done 1–2 times/Not yet started	772 (29.4%)	125 (28.5%)		789 (30.3%)	108 (23.5%)		118 (30.4%	40 (29.6%)	
Stand on 1 foot for 4 s	42	Yes, does well	1862 (72.4%)	290 (70.2%)	0.366	1836 (71.9%)	316 (73.7%)	0.442	288 (73.8%)	104 (73.8%)	0.934
		Yes, not very well/Not yet done	711 (27.6%)	123 (29.8%)		719 (28.1%)	113 (26.3%)		102 (26.2%)	37 (26.2%)	
Stand on 1 foot for 8 s	81	Yes, can do well	2052 (95.0%)	339 (95.8%)	0.537	2077 (95.1%)	314 (94.9%)	0.826	329 (94.5%)	106 (93.0%)	0.538
		Yes, but not well/Has not yet done/ Unable to try/Not had a chance	108 (5.0%)	15 (4.2%)		106 (4.9%)	17 (5.1%)		19 (5.5%)	8 (7.0%)	

Values are n (%).

Supplementary Table 3 Associations of responses to questionnaire items related to balance at 10 years old in ALSPAC

Test	Category	Prenatal exposure						Child Pb (µg/dl)		
		Maternal Pb (µg/dl)			Maternal Cd (µg/l)					
		<5	≥5	P value	<1	≥1	P value	<5	≥5	P value
Stand on one leg	Very well	1411 (76.1%)	219 (74.7%)	0.623	1455 (76.5%)	177 (71.7%)	0.094	236 (76.1%)	79 (76.0%)	0.972
	Just OK/ Can almost/ Not at all	444 (23.9%)	74 (25.3%)		447 (23.5%)	70 (28.3%)		75 (23.9%)	25 (24.0%)	
Ride a bike without stabilisers	Very well	1715 (92.5%)	259 (87.8%)	0.007	1741 (91.4%)	234 (94.7%)	0.075	278 (90.0%)	100 (96.2%)	0.050
	Just OK/ Can almost/ Not at all	140 (7.5%)	36 (12.2%)		163 (8.6%)	13 (5.3%)		31 (10.0%)	4 (3.8%)	
Walk in dark	Very well	1367 (75.2%)	222 (76.6%)	0.628	1410 (75.6%)	180 (74.1%)	0.603	225 (75.3%)	78 (75.7%)	0.923
	Just OK/ Can almost/ Not at all	450 (24.8%)	68 (23.4%)		455 (24.4%)	63 (25.9%)		74 (24.7%)	25 (24.6%)	

Chi square test.

Balance ability in 7- and 10-year-old children: associations with prenatal lead and cadmium exposure and with blood lead levels in children in a prospective birth cohort study

Caroline M Taylor, Rachel Humphriss, Amanda Hall, Jean Golding, Alan M Emond

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Line no.
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2 and 23
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	18-38
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	49-76
Objectives	3	State specific objectives, including any prespecified hypotheses	77-79
Methods			
Study design	4	Present key elements of study design early in the paper	82-86
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	89-153
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	82-96
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	99-153
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	99-153
Bias	9	Describe any efforts to address potential sources of bias	94-95
Study size	10	Explain how the study size was arrived at	89-96
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	156-172
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	156-175
		(*b*) Describe any methods used to examine subgroups and interactions	

	(*c*) Explain how missing data were addressed
	(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy
	(*e*) Describe any sensitivity analyses

Continued on next page

Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Table 1-4
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	89-96
		(b) Indicate number of participants with missing data for each variable of interest	
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	Tables 1-4
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	185-208
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
Discussion			
Key results	18	Summarise key results with reference to study objectives	268-276
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	322-336
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	339-344
Generalisability	21	Discuss the generalisability (external validity) of the study results	343
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	355-360

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Balance ability in 7- and 10-year-old children: associations with prenatal lead and cadmium exposure and with blood lead levels in children in a prospective birth cohort study

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2015-009635.R1
Article Type:	Research
Date Submitted by the Author:	12-Oct-2015
Complete List of Authors:	Taylor, Caroline; University of Bristol, Centre for Child and Adolescent Health Humphriss, Rachel; University Hospitals Bristol NHS Foundation Trust, Children's Hearing Centre; University of Bristol, School of Social and Community Medicine Hall, Amanda; University of Bristol, Centre for Hearing and Balance Studies Golding, Jean; University of Bristol, Centre for Child and Adolescent Health Emond, Alan; University of Bristol, Centre for Child and Adolescent Health; University of Bristol, School of Social and Community Medicine
Primary Subject Heading:	Epidemiology
Secondary Subject Heading:	Epidemiology, Paediatrics
Keywords:	

Balance ability in 7- and 10-year-old children: associations with prenatal lead and cadmium exposure and with blood lead levels in children in a prospective birth cohort study

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Keywords: Lead, Cadmium, Vestibular function, Balance, Children, Pregnancy, ALSPAC

Word count: (excluding title page, abstract, references, figures and tables): 3560

Abstract

Objectives Most studies reporting evidence of adverse effects of lead and cadmium on balance have been conducted in high-exposure groups or have included adults. The effects of prenatal exposure have not been well studied, nor have the effects directly in children. The aim of the study was to identify the associations of lead (in utero and in childhood) and cadmium (in utero) exposure with balance ability in 7- and 10-year-old children.

Design Prospective birth cohort study

Participants Maternal blood lead (n=4284) and cadmium (n=4286) levels were measured by inductively coupled plasma mass spectrometry in women enrolled in the Avon Longitudinal Study of Parents and Children (ALSPAC) during pregnancy. Child lead levels were measured in a subsample of 582 of ALSPAC children at age 30 months.

Main outcome measures Children completed a heel-to-toe walking test at 7 years. At 10 years the children underwent clinical tests of static and dynamic balance. Statistical analysis included logistic regression modelling comparing categories of ≥5 vs <5 µg/dl for lead and ≥1 vs <1 µg/l for cadmium with SPSS v19.

Results Balance at age 7 years was not associated with elevated in utero lead or cadmium exposure (adjusted OR for balance dysfunction: Pb 1.01 (95% CI 0.95, 1.01), n=1732; Cd 0.95 (0.77, 1.20), n=1734), or with elevated child blood lead level at age 30 months (adjusted OR 0.98 (0.92, 1.05), n=354). Similarly, neither measures of static nor dynamic balance at age 10 years were associated with in utero lead or cadmium exposure, or child lead level.

Conclusions These findings do not provide any evidence of an association of prenatal exposure to lead or cadmium, or lead levels in childhood, on balance ability in children. Confirmation in other cohorts is needed.

Word count: 281

Strengths and limitations of the study

- Data were collected prospectively in a population-based study
- The number of participants was large compared with several comparable studies
- Measures of Pb and Cd do not necessarily reflect lifetime exposure
- Balance measures have a poor test–retest reliability

INTRODUCTION

Balance, or postural stability, is defined as the ability to keep the centre of gravity over the base of support.[1] The maintenance of balance underpins the ability to carry out nearly all daily activities. Balance impairment in adults is also a major cause of falls and of fall-related injuries, such as hip fracture, which can cause isolation and make it difficult to live independently. The control of balance is complex and is dependent on sensory inputs from the vestibular and visual systems, neural processing centres in the central nervous system, and motor inputs from the proprioceptive centre. Functional damage or deficits in any of these systems can lead to balance dysfunction, which can be associated with low self-esteem, anxiety and loss of confidence in children.[2]

Lead and cadmium are toxic metals: the effects of lead on neurocognitive and behavioural functions in children are well documented,[3-5] but those of cadmium are clear.[6-8] Lead passes freely through the placenta so that ratio of fetal to maternal blood lead is about 0.8, although the placenta can act as a partial barrier to cadmium.[9] The fetus is particularly vulnerable to the effects of these metals because of high rates of cell division and development. The development of the inner ear and vestibular function spans the whole of the period of gestation (for example, the membranous labyrinth is complete by week 7 with development of the bony labyrinth from week 9 to 23; the vestibular apparatus is in an adult-like form by week 25, and is active by week 32; vestibular ganglions develop from week 12 and reach maturity at week 39, and so on[10]). Thus, prenatal exposure to lead and cadmium may have adverse effects on the development of the inner ear, and hence on vestibular function and balance ability in later childhood.

It was noted in the 1980s that children who survived acute lead encephalopathy had ataxia and experienced difficulties in maintaining postural balance.[11] This led to a series of studies in children with somewhat more moderate levels of lead exposure (5.0 to 20.7 µg/dl) showing that the child's lead level was associated with balance dysfunction and sway oscillation.[12-16] To our knowledge, there are no reports of the effect of cadmium on balance ability in children. However, a recent study of lead and cadmium levels in adults in the US National Health and Nutrition Examination Survey (NHANES) found preliminary evidence of an association of lead and cadmium with balance and vestibular function.[17] In addition, altered postural balance response has been reported in adult workers occupationally exposed to lead.[18-20] and cadmium.[21] These results require confirmation in other cohorts and particularly in children.

The aims of our study were to investigate the associations of in utero exposure to lead and cadmium, and lead levels in children, on balance in childhood using data obtained from the Avon Longitudinal Study of Parents and Children (ALSPAC).

Methods

We first modelled associations of in utero exposure to lead and cadmium, using maternal blood levels during pregnancy, with clinical measures of balance (dynamic and static) at 7 and 10 years. We also investigated associations with questionnaire items related to balance repeated at 30 months, 42 and 81 months, and further items at 10 years. We also modelled the associations of child levels of lead with the balance variables.

The ALSPAC study

The study sample was derived from the ALSPAC study, a population-based study investigating environmental and genetic influences on the health, behaviour and development of children. All pregnant women in the former Avon Health Authority with an expected delivery date between 1 April 1991 and 31 December 1992 were eligible for the study; 14,541 pregnant women were initially enrolled, resulting in a cohort of 14,062 live births.[22] The social and demographic characteristics of this cohort were similar to those found in UK national census surveys. [23] Further details of ALSPAC are available at www.bris.ac.uk/alspac.

Collection, storage and analysis of blood samples

Maternal blood samples Whole blood samples were collected in acid-washed vacutainers (Becton and Dickinson, Oxford, UK) by midwives as early as possible in pregnancy. The median gestational age at the time of blood sampling was 11 weeks. The interquartile range was 9–13 weeks, and 93% of the samples were collected at <18 weeks gestation. Whole blood samples were stored in the original tube at 4ºC at the collection site before being transferred to the central Bristol laboratory within 1–4 days. Samples were at ambient temperature during transfer (up to 3 h). They were then stored at 4ºC until analysis. Samples were analysed for lead using inductively coupled plasma mass spectrometry in standard mode by R. Jones at the Centers for Disease Control (CDC), Bethesda, MD, USA (CDC Method 3009.1). Quality control was monitored as outlined in Golding, et al. [24] The analyses were completed on 4284 samples for Pb and 4286 for Cd. One sample had a Pb level below the limit of detection (0.29 µg/dl); 1119 samples were below the lower limit of detection for Cd

(0.20 µg/l). These samples were assigned a value of 0.7 times the lower limit of detection (LOD/√2).[25 26]

Child blood samples Details of the selection of the subsample of children and analysis of the blood samples have been reported previously in detail.[3 27] In brief, a 10% randomly selected subsample of parents whose babies were born in the last 6 months of the ALSPAC study were invited to attend a research clinic (Children in Focus, CIF). At age 30 months, parental consent was sought for a venous blood sample, and was given by 81% of the 1135 children in the CIF group. The sample was drawn into lead-free tubes from 653 (71%) of children attending the clinic. However, 69 samples were insufficient, leaving 582 samples for analysis. Analysis was by atomic absorption spectrometry (Southampton General Hospital, UK) with appropriate quality controls.

Balance variables

Clinic measures Full details of the balance outcomes including details of the measurements and validity have previously been published.[28] In brief, at age 7 years the heel-to-toe walking test of the Movement Assessment Battery for Children[29] was conducted with the total number of successful steps out of a maximum of 15 recorded. At age 10 years a range of tests were used to assess balance: (1) walking along a beam, heel-to-toe, eyes open; (2) heel-to-toe balance on a beam, eyes closed; (3) standing on one leg, eyes closed. Each child had two attempts at beam-walking; for tests of static balance, children only had a second attempt if they failed to achieve the maximum score on the first attempt.[28] These tests were based on standard clinical tests to assess balance in children and have significant commonality with the balance subtest of the both editions of the Bruininks–Oseretsky Test of Motor Proficiency.[30]

[31] The measures are also in common use when testing balance informally in the paediatric clinic.

Questionnaire items The primary caregiver (usually the mother) received a series of postal self-completion questionnaires. The questionnaires are available from the study website (http://www.bris.ac.uk/alspac/researchers/data-access/data-dictionary/). When their child was aged 18, 30, 42 and 81 months, the parent completing the questionnaire was asked to indicate 'Yes, can do well'/'Has only done once or twice'/'Has not yet started' in response to the statement 'He/She can balance on one foot for at least 1 second'. When their child was aged 10 years, the parent was asked to indicate 'Very well'/'Just OK'/'Can almost'/'Not at all' in response to the following questions: How well can your child stand on one leg in a stable position (e.g. when putting on trousers, skirt)?; How well can your child ride a bike (without stabilisers)?; How well can your child walk in the dark?

Confounding variables

Information on passive smoking exposure during the week and at weekends was obtained from questionnaires at 77 and 103 months. Information on traffic levels, type of accommodation, lowest level of accommodation and maternal education were obtained at from questionnaires completed by the mother during pregnancy. Dietary Ca and Fe intake at 7 years and 10 years were derived from food frequency questionnaires as previously described in detail. [32]

Statistical analysis

Statistical analysis was carried out with IBM SPSS Statistics 21. Balance measures were derived as previously described.[28] In brief, for the heel-to-toe test at age 7 years, the number of steps (maximum 15) was categorised into 0–5, 6–10 and 11–15 steps for categorical associations, and 1–14 (failed to complete in 20 s) versus 15 steps (successfully completed in 20 s) for regression analyses. For the measure of dynamic balance at 10 years (beam-walking test), the mean of two attempts was categorised into quartiles. For measures of static balance at age 10 years (heel to toe balance on a beam with eyes closed/standing on one leg eyes closed), the sum of the score (s) from both attempts was calculated. Children who scored the maximum of 20 on the first attempt and so did not have a second attempt were given a final score of 40. The final scores were put into four categories (0–9, 10–19, 20–39 and 40). All of the four static balance tests with eyes closed were summed to create a static balance eyes closed variable (SBEC).

Blood lead and cadmium levels were put into two categories (<5, ≥5 µg/dl for lead and <1, ≥1 µg/l for Cd). These categories were chosen in accordance with the levels of concern of the US Centers for Disease Control, the US Association of Occupational and Environmental Clinics and the American College of Obstetricians and Gynecologists for Pb,[33-36] and the German Federal Environmental Agency for Cd.[37] Blood levels were also categorised into quartiles.

Chi square tests were used to compare categorical variables. Unadjusted and adjusted logistic regression analyses were used to investigate the association of blood levels with balance variables.

RESULTS

As previously reported, the mean child blood lead level was 4.22±3.12 µg/dl (n=582)[3 27]; the mean prenatal blood lead level was 3.67±1.47 µg/dl (n=4284) and the mean prenatal cadmium level was 0.58±0.63 µg/l (n=4286).[38 39] Mothers who consented to provide a blood sample were better educated and older than mothers who did not.[38] Children who had lead levels measured were from families where the mother was better educated and more likely to be a homeowner, and was there was a better home environment with fewer adversities.[3]

Associations of measures of balance with prenatal lead and cadmium levels and with child lead levels

Associations with in utero exposure to lead and cadmium

There was no evidence of any association between the results of the heel-to-toe test at age 7 years and maternal lead or cadmium level during pregnancy (p=0.441 and p=0.189, respectively) (Table 1). Similarly, at age 10 years there were no associations between dynamic balance (beam walking) or static balance (SBEC) and maternal blood lead or cadmium levels in pregnancy (all p>0.1) (Table 1).

In logistic regression models adjusted for sex, passive smoking, and calcium and iron intake, there was no evidence of any association between maternal blood lead or cadmium levels and measure of balance in the child at 7 and 10 years (all p>0.1) (Table 2). When the models were repeated with quintiles of maternal blood lead or cadmium level rather than a dichotomous variable, there was also no evidence of any associations (all p>0.1 with the exception of maternal blood lead for the odds of static balance dysfunction at 10 years where there was a protective effect (p for trend 0.038) (Supplementary Table 1).

Associations with child lead level

There was no evidence of any association between the results of the heel-to-toe test at age 7 years and child lead level (p for trend=0.146) (Table 3). Similarly, at age 10 years there were no associations between dynamic balance (beam walking) or static balance (SBEC) and child blood lead levels (p for trend=0.798 and p=0.918, respectively) (Table 3). In logistic regression models adjusted for sex, passive smoking, and calcium and iron intake, there was no evidence of any association between child blood levels at 30 months and measure of balance at 7 and 10 years (Table 4; all p for trend >0.3). When the models were repeated with quintiles of child blood lead rather than a dichotomous variable, there was also no evidence of any associations (all p for trend >0.1) with the exception of static balance where there was a weakly protective effect (p for trend=0.038 (Supplementary Table 1).

Table 1 Associations of in utero lead and cadmium exposure with measures of balance in the child at 7 and 10 years in ALSPAC

	Age (years)	Category	Maternal Pb (µg/dl)			Maternal Cd (µg/l)		
			<5	≥5	P value (chi square)	<1	≥1	P value (chi square)
Heel to toe test	7	0–5 steps	520 (27.1)	82 (26.5)	0.861	521 (26.6)	81 (29.1)	0.112
		6–10 steps	273 (14.2)	45 (14.5)		270 (13.8)	49 (17.6)	
		11–15 steps	1128 (58.7)	183 (59.0)		1164 (59.6)	148 (53.2)	
Beam walking (dynamic balance)	10	Q1	431 (23.4)	70 (24.1)	0.450	439 (23.3)	62 (25.3)	0.897
		Q2	461 (25.0)	80 (27.6)		484 (25.6)	57 (23.3)	
		Q3	456 (24.8)	66 (22.8)		463 (24.5)	59 (24.1)	
		Q4	494 (26.8)	74 (25.5)		503 (26.6)	67 (27.3)	
Static balance eyes closed score (SBEC) (static balance)	10	Q1	460 (24.4)	74 (25.8)	0.558	472 (25.5)	63 (25.8)	0.842
		Q2	459 (25.4)	58 (20.2)		455 (24.6)	62 (25.4)	
		Q3	430 (23.8)	83 (28.9)		457 (24.7)	57 (23.4)	
		Q4	459 (25.4)	72 (25.1)		469 (25.3)	62 (25.4)	

Values are n (%).

Q, quartile.

Table 2 Associations of in utero lead and cadmium exposure with balance measures in the child at 7 and 10 years old in ALSPAC

	Age (years)	Prenatal lead exposure				Prenatal cadmium exposure			
			OR of balance dysfunction (95% CI)	P value	n		OR of balance dysfunction (95% CI)	P value	n
Heel to toe test	7	Unadjusted	1.01 (0.96, 1.07)	0.503	2231	Unadjusted	0.81 (0.70, 0.94)	0.010	2233
		Adjusted [a]	1.01 (0.95, 1.01)	0.555	1732	Adjusted [a]	0.95 (0.77, 1.20)	0.904	1734
Dynamic balance	10	Unadjusted	1.01 (0.95, 1.08)	0.790	2132	Unadjusted	1.00 (0.84, 1.21)	0.946	2134
		Adjusted [b]	1.02 (0.95, 1.09)	0.692	1761	Adjusted [b]	1.20 (0.95, 1.52)	0.135	1763
Static balance	10	Unadjusted	0.98 (0.92, 1.05)	0.569	2095	Unadjusted	1.06 (0.88, 1.28)	0.523	2097
		Adjusted [b]	0.98 (0.92, 1.06)	0.661	1734	Adjusted [b]	1.00 (0.79, 1.26)	0.995	1736

Logistic regression showing odds ratio of balance dysfunction (95% CI).
[a]Adjusted for: sex, passive smoking at 77 months old (weekdays and weekends), and Ca and Fe intake at 7 years.
[b]Adjusted for: sex, passive smoking at 103 months old (weekdays and weekends), and Ca and Fe intake at 10 years.

Table 3 Associations of child lead level at 30 months with measures of balance at 7 and 10 years in ALSPAC

Test	Age (years)	Category	Child Pb (µg/dl)		P value (chi square)
			<5	≥5	
Heel to toe test	7	0–5 steps	82 (25.8)	34 (30.9)	0.146
		6–10 steps	52 (16.4)	22 (20.0)	
		11–15 steps	184 (57.9)	54 (49.1)	
Beam walking (dynamic balance)	10	Q1	74 (24.0)	24 (23.5)	0.798
		Q2	68 (22.1)	19 (18.6)	
		Q3	86 (27.9)	34 (33.3)	
		Q4	80 (26.0)	25 (24.5)	
Static balance eyes closed score (SBEC) (static balance)	10	Q1	80 (26.5)	22 (22.0)	0.918
		Q2	70 (23.2)	30 (30.0)	
		Q3	77 (25.5)	24 (24.0)	
		Q4	75 (24.8)	24 (24.0)	

Values are n (%).

Q, quartile.

Table 4 Child blood lead level at 30 months and balance measures at 7 and 10 years old in ALSPAC

	Age (years)	OR of balance dysfunction (95% CI)		P value	n
Heel to toe test	7	Unadjusted	0.98 (0.92, 1.04)	0.618	428
		Adjusted [a]	0.98 (0.92, 1.05)	0.778	354
Dynamic balance	10	Unadjusted	1.03 (0.96, 1.11)	0.422	410
		Adjusted [b]	1.01 (0.93, 1.09)	0.814	363
Static balance	10	Unadjusted	1.04 (0.96, 1.12)	0.345	402
		Adjusted [b]	1.03 (0.94, 1.12)	0.540	357

Logistic regression analysis comparing results for children with blood lead level ≥5 vs <5 µg/dl.
[a]Adjusted for: sex, passive smoking at 77 months old (weekdays and weekends), and Ca and Fe intake at 7 years.
[b]Adjusted for: sex, passive smoking at 103 months old (weekdays and weekends), and Ca and Fe intake at 10 years.

Associations with questionnaire items at 18–81 months and at 10 years

There was no evidence of any associations between child blood lead level at 30 months and the ability to stand on one foot for at least 1 second at 30, 42 or 81 months (all p>0.6; Supplementary Table 2). There was no evidence of any associations between maternal blood lead level during pregnancy and the ability of the child to stand on one foot for at least 1 second at 18, 30, 42 or 81 months (all p>0.3) (Supplementary Table 2). There was no evidence of any associations between maternal blood cadmium level during pregnancy and the ability of the child to stand on one foot for at least 1 second at 42, or 81 months (all p>0.4), but there were associations at 18 and 30 months (p<0.001 and p=0.003, respectively; maternal cadmium ≥1 µg/l was associated with being more likely to be able to stand on one foot well) (Supplementary Table 2).

There was no evidence of any association of elevated child lead level, or in utero lead or cadmium exposure, with ability to stand on one leg or to walk in the dark at 10 years (all p>0.09, chi-square test) (Supplementary Table 3). However, prenatal lead level ≥5 µg/dl was associated with not being able to ride a bike without stabilisers (p=0.007), whereas child lead level ≥5 µg/dl and prenatal cadmium ≥1 µg/l were weakly associated with being able to ride a bike without stabilisers very well (p=0.050 and p=0.075 respectively). When these associations were modelled in a logistic regression analysis adjusted for variables that could affect availability of a bicycle and being able to ride a bicycle locally (traffic level on the home street, type of accommodation, lowest level of accommodation), and maternal education, there was very weak evidence for an association of child lead level being associated with being able to ride a bike well without stabilisers (OR in unadjusted model 2.79 (95% CI 0.096, 8.10), p=0.059, n=413; OR in adjusted model OR 2.57 (95% CI 0.87, 7.62), p=0.089, n=387). The association was stronger for prenatal lead level in the unadjusted model, but the effect was attenuated with adjustment (OR in unadjusted model 0.59 (95% CI

0.40, 0.87), p=0.007, n=2150; OR in adjusted model 0.73 (95% CI 0.47, 1.14), p=0.167 , n=1904); for prenatal cadmium level again the weak effect was attenuated by adjustment (unadjusted OR 1.69 (95% CI 0.94, 3.01, p=0.078, n=2151; adjusted OR 1.44 (95% CI 0.75, 2.75, p=0.274, n=1905).

DISCUSSION

We did not find any evidence of an association of prenatal exposure to lead or cadmium, or lead levels in childhood, on balance ability (static and dynamic) in children. Counterintuitively, there was a suggestion that higher child lead levels and in utero lead exposure were associated with the ability to ride a bike without stabilisers at age 10 years, but these effects were negated when the associations were adjusted for variables that included the lowest level of accommodation and traffic levels outside the home. This is the first study, to our knowledge, reporting on the associations between in utero exposure to lead and cadmium and balance ability of the child, and adds to the few studies on child lead levels and balance ability.

Postural balance is controlled by a complex interaction of sensorimotor processes, including visual, proprioception and the vestibular system. In our study the measure of static balance with eyes closed eliminated vision and minimised proprioceptive inputs, thereby enabling the assessment of vestibular information as the primary input. Use of clinical measures similar to these is commonplace in epidemiological studies (e.g. [40-43]), as the tests are in common clinical usage and require little or no specialist equipment. However, although the vestibular dominant condition of tests of standing balance (reduced base of support with eyes closed) has been shown to correlate well with more expensive systems such as computerised dynamic posturography (considered to be the 'gold standard' method for assessing balance function) in

adults,[44] there are questions about whether such a correlation exists in children.[45] It has even been suggested that computerised measures using a force-platform and clinical tests such as those used by ALSPAC give complementary rather than concurrent information.[46] A cautious approach should therefore be taken if seeking to compare studies using these two different types of outcome measure.

Most studies on lead levels and balance in children have included children with relatively high lead levels (means in each study of 11.6 to 20.7 µg/dl) and measured balance with a force platform system,[12-15] and have found negative associations. There are several reasons for our results being in contrast to these studies. First, the mean child blood lead level in our study (4.22 µg/dl) was lower than reported in these earlier studies, and may have been too low either to have been sufficient to cause balance dysfunction. Alternatively, the effect on balance might have been too small to be detectable with our tests, although a study in Inuit children had levels that were more comparable to the present study (mean 5.4 µg/dl) showed a significant association with sway oscillations.[16] Second, we used a series of assessments based on clinical tests to measure balance rather than a force platform or measurement of sway oscillations and this may account for differences in the findings. As discussed earlier, clinical and force platform measures may be giving complementary rather than concurrent information: whereas posturography is a measure of the motor and sensory strategies used to control balance, clinical tests evaluate the results of that balance control.[46] Measuring sway oscillations using a force platform will also be more sensitive than the ALSPAC measures, which measured time before a procedural fault such as touching the floor with either foot or lifting a foot off the beam. Third, early exposure to lead and/or cadmium could damage the vestibular system, but the plasticity of the balance system might compensate for this so that there is no functionally measurable effect. This is in accordance with measures of balance in the present study indirectly assessing the vestibular system. This could also account for

effects being reported in adults, in whom plasticity is less effective for overall balance compensation, but not in children. It is also possible that vestibular system (to include peripheral (ear) and central (brain) components of the vestibular pathway) dysfunction caused by in utero exposure to lead or cadmium may not be apparent in childhood but may manifest in later life. Finally, it is also possible that studies showing non-significant results have tended not to have been published.

To our knowledge, there are no studies that have examined the effect of in utero exposure to lead or cadmium on balance ability in the child. Our results provide preliminary evidence for lack of effect, but this requires confirmation on other cohorts.

The strengths of the study are that: (1) it is a population-based study; (2) the data were collection prospectively; and (3) the numbers included in the study were large compared with several other studies. There are several limitations to the study. First, measures of blood lead and cadmium do not necessarily reflect lifetime exposure. Bone lead, which makes up more than 95% of the body lead, can be measured by K x-ray fluorescence,[47] but this is expensive, technically demanding and may not always be ethically permissible in children. This limitation is of less consequence for in utero exposure as the maternal blood level largely determines the fetal blood level. Second, there was a high proportion of blood cadmium levels below the limit of detection, which may make the results less reliable than for lead. Third, there may be confounders we were unable to account for. Fourth, we were unable to control for lead and cadmium separately in the models because of multicollinearity. Fifth, the apparently protective effect of Finally, the balance measures used by ALSPAC had low test–retest reliability,[28] which is a common problem with measures of childhood balance.[48-50] The measure of ability to balance on one foot for 1 s, a test which was originally developed by Chamberlain and Davey [51] in 1976, has been particularly criticised for having poor test

validity as it is difficult to discriminate a failed attempt from a successful attempt.[10] It should ideally form part of a battery of clinical measures, perhaps with a longer duration of standing. These combined effects may have led to random misclassification in our balance assessments and dilution of the estimates of the effects of lead and cadmium exposure.

CONCLUSIONS

We did not find any evidence of an association of prenatal exposure to lead or cadmium with balance ability in children. In contrast to previous studies, we did not find any association of child blood lead with balance ability in children. This may reflect variation in the methods used to assess balance in different studies, or may be related to the lower mean lead level in the children in the present study than in previous studies. Further work in other cohorts is needed to confirm the results.

Data sharing statement: No additional data available.

Contributors statement: CMT conceived the study and undertook data analysis in conjunction with RH, AH and JG. CMT took the lead in writing the manuscript with critical revisions and additions from RH, AH, JG and AME. All authors contributed to and approved the final version of the manuscript.

Ethics approval: Ethics approval for the study was obtained from the ALSPAC Ethics and Law Committee and the Local Research Ethics Committees.

Competing interests: The authors declare that there are no competing interests

Acknowledgements: We are extremely grateful to all the families who took part in this study, the midwives for their help in recruiting them, and the whole ALSPAC team, which includes interviewers, computer and laboratory technicians, clerical workers, research scientists, volunteers, managers, receptionists and nurses.

Funding: The UK Medical Research Council and the Wellcome Trust (Grant ref: 102215/2/13/2) and the University of Bristol currently provide core support for ALSPAC. This publication is the work of the authors who will serve as guarantors for the contents of this paper. CMT was supported by a Wellcome Trust Career Re-entry Fellowship (Grant ref: 104077/Z/14/Z). The publication is the work of the authors who will serve as guarantors for the contents of the paper.

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Balance ability in 7- and 10-year-old children: associations with prenatal lead and cadmium exposure and with blood lead levels in children in a prospective birth cohort study

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Supplementary Table 1 Associations of quintiles of in utero lead and cadmium exposures and of child lead with measures of balance in the child at 7 and 10 years in ALSPAC

Quartile	Prenatal exposure				Child Pb[c]	
	Maternal Pb[a]		Maternal Cd[b]			
	OR of balance dysfunction (95% CI)	P value for trend	OR of balance dysfunction (95% CI)	P value for trend	OR of balance dysfunction (95% CI)	P value for trend
Heel to toe test at 7 years[d]						
1	1.00 (Ref)	0.349 (n=1732)	(Ref)	0.133 (n=1457)	(Ref)	0.234 (n=354)
2	0.94 (0.72, 1.22)		1.17 (0.91, 1.53)		1.47 (0.82, 2.63)	
3	1.17 (0.90, 1.53)		0.91 (0.69, 1.20)		0.96 (0.52, 1.78)	
4	1.18 (0.82, 1.41)		0.90 (0.62, 1.27)		0.78 (0.52, 1.42)	
Dynamic balance at 10 years[e]						
1	(Ref)	0.981 (n=1761)	(Ref)	0.433 (n=1763)	(Ref)	0.290 (n=363)
2	1.34 (0.99, 1.81)		0.85 (0.65, 1.12)		0.80 (0.41, 1.56)	
3	1.09 (0.81, 1.46)		0.95 (0.71, 1.26)		1.08 (0.54, 2.14)	
4	1.09 (0.81, 1.46)		1.10 (0.76, 1.59)		0.86 (0.43, 1.72)	
Static balance at 10 years[e]						
1	(Ref)	0.811 (n=1734)	(Ref)	0.851 (n=1736)	(Ref)	0.891 (n=357)
2	0.68 (0.50, 0.92)		1.11 (0.84, 1.48)		0.71 (0.36, 1.39)	
3	0.85 (0.62, 1.16)		1.11 (0.83, 1.49)		1.20 (0.59, 2.47)	
4	0.89 (0.65, 1.23)		0.97 (0.67, 1.41)		0.87 (0.43, 1.78)	

[a]Maternal Pb quartiles: Q1 0.20–2.66, Q2 2.67–3.40, Q3 3.41–4.33, Q4 4.34–19.14 µg/dl.
[b]Maternal Cd quartiles: Q1 0.14–0.14, Q2 0.20–0.29, Q3 0.30–0.72, Q4 0.73–6.30 µg/l.
[c]Child Pb quartiles: Q1 0.83–2.18, Q2 2.28–3.32, Q3 3.42–5.18, Q4 5.28–27.56 µg/dl.
[d]Adjusted for: sex, passive smoking at 77 months old (weekdays and weekends), and Ca and Fe intake at 7 years.
[e]Adjusted for: sex, passive smoking at 103 months old (weekdays and weekends), and Ca and Fe intake at 10 years.

Supplementary Table 2 Associations of responses to questionnaire items related to balance at 18–81 months old in ALSPAC

	Age (months)	Category	Prenatal exposure						Child Pb (µg/dl)		
			Maternal Pb (µg/dl)			Maternal Cd (µg/l)					
			<5	≥5	P value (chi square)	<1	≥1	P value (chi square)	<5	>5	P value (chi square)
Stand on 1 foot for 1 s	18	Yes, can do well	1397 (50.8%)	237 (51.9%)	0.680	1353 (49.5%)	280 (58.6%)	<0.001	-	-	-
		Only done 1–2 times/Not yet started	1352 (49.2%)	220 (48.4%)		1376 (50.5%)	198 (41.4%)		-	-	
Stand on 1 foot for 1 s	30	Yes, can do well	1853 (70.6%)	313 (71.5%)	0.711	1815 (69.7%)	352 (76.5%)	0.001	270 (69.6%)	95 (70.4%)	0.865
		Only done 1–2 times/Not yet started	772 (29.4%)	125 (28.5%)		789 (30.3%)	108 (23.5%)		118 (30.4%)	40 (29.6%)	
Stand on 1 foot for 4 s	42	Yes, does well	1862 (72.4%)	290 (70.2%)	0.366	1838 (71.9%)	316 (73.7%)	0.447	288 (73.8%)	204 (73.8%)	0.984
		Yes, not very well/Not yet done	711 (27.6%)	123 (29.8%)		720 (28.1%)	113 (26.3%)		102 (26.2%)	37 (26.2%)	
Stand on 1 foot for 8 s	81	Yes, can do well	2052 (95.0%)	339 (95.8%)	0.537	2079 (95.1%)	314 (94.9%)	0.823	329 (94.5%)	106 (93.0%)	0.538
		Yes, but not well/Has not yet done/ Unable to try/Not had a chance	108 (5.0%)	15 (4.2%)		106 (4.9%)	17 (5.1%)		19 (5.5%)	8 (7.0%)	

Values are n (%).

Supplementary Table 3 Associations of responses to questionnaire items related to balance at 10 years old in ALSPAC

Test	Category	Prenatal exposure						Child Pb (µg/dl)		
		Maternal Pb (µg/dl)			Maternal Cd (µg/l)					
		<5	≥5	P value (chi square)	<1	≥1	P value (chi square)	<5	≥5	P value (chi square)
Stand on one leg	Very well	1411 (76.1%)	219 (74.7%)	0.623	1455 (76.5%)	177 (71.7%)	0.09	236 (76.1%)	79 (76.0%)	0.972
	Just OK/ Can almost/ Not at all	444 (23.9%)	74 (25.3%)		448 (23.5%)	70 (28.3%)		74 (23.9%)	25 (24.0%)	
Ride a bike without stabilisers	Very well	1715 (92.5%)	259 (87.8%)	0.007	1742 (91.4%)	234 (94.7%)	0.07	278 (90.0%)	100 (96.2%)	0.050
	Just OK/ Can almost/ Not at all	140 (7.5%)	36 (12.2%)		163 (8.6%)	13 (5.3%)		31 (10.0%)	4 (3.8%)	
Walk in dark	Very well	1367 (75.2%)	222 (76.6%)	0.628	1411 (75.6%)	180 (74.1%)	0.39	225 (75.3%)	78 (75.7%)	0.923
	Just OK/ Can almost/ Not at all	450 (24.8%)	68 (23.4%)		455 (24.4%)	63 (25.9%)		74 (24.7%)	25 (24.6%)	

Balance ability in 7- and 10-year-old children: associations with prenatal lead and cadmium exposure and with blood lead levels in children in a prospective birth cohort study

Caroline M Taylor, Rachel Humphriss, Amanda Hall, Jean Golding, Alan M Emond

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Line no.
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2 and 23
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	18-38
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	49-76
Objectives	3	State specific objectives, including any prespecified hypotheses	77-79
Methods			
Study design	4	Present key elements of study design early in the paper	82-86
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	89-153
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	82-96
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	99-153
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	99-153
Bias	9	Describe any efforts to address potential sources of bias	94-95
Study size	10	Explain how the study size was arrived at	89-96
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	156-172
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	156-175
		(*b*) Describe any methods used to examine subgroups and interactions	

	(*c*) Explain how missing data were addressed
	(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy
	(*e*) Describe any sensitivity analyses

Continued on next page

Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Table 1-4
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	89-96
		(b) Indicate number of participants with missing data for each variable of interest	
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	Tables 1-4
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	185-208
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
Discussion			
Key results	18	Summarise key results with reference to study objectives	268-276
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	322-336
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	339-344
Generalisability	21	Discuss the generalisability (external validity) of the study results	343
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	355-360

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Balance ability in 7- and 10-year-old children: associations with prenatal lead and cadmium exposure and with blood lead levels in children in a prospective birth cohort study

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2015-009635.R2
Article Type:	Research
Date Submitted by the Author:	10-Nov-2015
Complete List of Authors:	Taylor, Caroline; University of Bristol, Centre for Child and Adolescent Health Humphriss, Rachel; University Hospitals Bristol NHS Foundation Trust, Children's Hearing Centre; University of Bristol, School of Social and Community Medicine Hall, Amanda; University of Bristol, Centre for Hearing and Balance Studies Golding, Jean; University of Bristol, Centre for Child and Adolescent Health Emond, Alan; University of Bristol, Centre for Child and Adolescent Health; University of Bristol, School of Social and Community Medicine
Primary Subject Heading:	Epidemiology
Secondary Subject Heading:	Epidemiology, Paediatrics
Keywords:	Lead, Cadmium, Vestibular function, Balance, Pregnancy, ALSPAC

Balance ability in 7- and 10-year-old children: associations with prenatal lead and cadmium exposure and with blood lead levels in children in a prospective birth cohort study

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Keywords: Lead, Cadmium, Vestibular function, Balance, Children, Pregnancy, ALSPAC

Word count: (excluding title page, abstract, references, figures and tables): 3560

Abstract

Objectives Most studies reporting evidence of adverse effects of lead and cadmium on balance have been conducted in high-exposure groups or have included adults. The effects of prenatal exposure have not been well studied, nor have the effects directly in children. The aim of the study was to identify the associations of lead (in utero and in childhood) and cadmium (in utero) exposure with balance ability in 7- and 10-year-old children.

Design Prospective birth cohort study

Participants Maternal blood lead (n=4284) and cadmium (n=4286) levels were measured by inductively coupled plasma mass spectrometry in women enrolled in the Avon Longitudinal Study of Parents and Children (ALSPAC) during pregnancy. Child lead levels were measured in a subsample of 582 of ALSPAC children at age 30 months.

Main outcome measures Children completed a heel-to-toe walking test at 7 years. At 10 years the children underwent clinical tests of static and dynamic balance. Statistical analysis included logistic regression modelling comparing categories of ≥5 vs <5 µg/dl for lead and ≥1 vs <1 µg/l for cadmium with SPSS v19.

Results Balance at age 7 years was not associated with elevated in utero lead or cadmium exposure (adjusted OR for balance dysfunction: Pb 1.01 (95% CI 0.95, 1.01), n=1732; Cd 0.95 (0.77, 1.20), n=1734), or with elevated child blood lead level at age 30 months (adjusted OR 0.98 (0.92, 1.05), n=354). Similarly, neither measures of static nor dynamic balance at age 10 years were associated with in utero lead or cadmium exposure, or child lead level.

Conclusions These findings do not provide any evidence of an association of prenatal exposure to lead or cadmium, or lead levels in childhood, on balance ability in children. Confirmation in other cohorts is needed.

Word count: 281

Strengths and limitations of the study

- Data were collected prospectively in a population-based study
- The number of participants was large compared with several comparable studies
- Measures of Pb and Cd do not necessarily reflect lifetime exposure
- Balance measures have a poor test–retest reliability

INTRODUCTION

Balance, or postural stability, is defined as the ability to keep the centre of gravity over the base of support.[1] The maintenance of balance underpins the ability to carry out nearly all daily activities. Balance impairment in adults is also a major cause of falls and of fall-related injuries, such as hip fracture, which can cause isolation and make it difficult to live independently. The control of balance is complex and is dependent on sensory inputs from the vestibular and visual systems, neural processing centres in the central nervous system, and motor inputs from the proprioceptive centre. Functional damage or deficits in any of these systems can lead to balance dysfunction, which can be associated with low self-esteem, anxiety and loss of confidence in children.[2]

Lead and cadmium are toxic metals: the effects of lead on neurocognitive and behavioural functions in children are well documented,[3-5] but those of cadmium are less clear.[6-8] Lead passes freely through the placenta so that ratio of fetal to maternal blood lead is about 0.8, although the placenta can act as a partial barrier to cadmium.[9] The fetus is particularly vulnerable to the effects of these metals because of high rates of cell division and development. The development of the inner ear and vestibular function spans the whole of the period of gestation (for example, the membranous labyrinth is complete by week 7 with development of the bony labyrinth from week 9 to 23; the vestibular apparatus is in an adult-like form by week 25, and is active by week 32; vestibular ganglions develop from week 12 and reach maturity at week 39, and so on[10]). Thus, prenatal exposure to lead and cadmium may have adverse effects on the development of the inner ear, and hence on vestibular function and balance ability in later childhood.

It was noted in the 1980s that children who survived acute lead encephalopathy had ataxia and experienced difficulties in maintaining postural balance.[11] This led to a series of studies in children with somewhat more moderate levels of lead exposure (5.0 to 20.7 µg/dl) showing that the child's lead level was associated with balance dysfunction and sway oscillation.[12-16] To our knowledge, there are no reports of the effect of cadmium on balance ability in children. However, a recent study of lead and cadmium levels in adults in the US National Health and Nutrition Examination Survey (NHANES) found preliminary evidence of an association of lead and cadmium with balance and vestibular function.[17] In addition, altered postural balance response has been reported in adult workers occupationally exposed to lead.[18-20] and cadmium.[21] These results require confirmation in other cohorts and particularly in children.

The aims of our study were to investigate the associations of in utero exposure to lead and cadmium, and lead levels in children, on balance in childhood using data obtained from the Avon Longitudinal Study of Parents and Children (ALSPAC).

Methods

We first modelled associations of in utero exposure to lead and cadmium, using maternal blood levels during pregnancy, with clinical measures of balance (dynamic and static) at 7 and 10 years. We also investigated associations with questionnaire items related to balance repeated at 30 months, 42 and 81 months, and further items at 10 years. We also modelled the associations of child levels of lead with the balance variables.

The ALSPAC study

The study sample was derived from the ALSPAC study, a population-based study investigating environmental and genetic influences on the health, behaviour and development of children. All pregnant women in the former Avon Health Authority with an expected delivery date between 1 April 1991 and 31 December 1992 were eligible for the study; 14,541 pregnant women were initially enrolled, resulting in a cohort of 14,062 live births.[22] The social and demographic characteristics of this cohort were similar to those found in UK national census surveys. [23] Further details of ALSPAC are available at www.bris.ac.uk/alspac.

Collection, storage and analysis of blood samples

Maternal blood samples Whole blood samples were collected in trace-element free vacutainers (Becton and Dickinson, Oxford, UK) by midwives as early as possible in pregnancy. The median gestational age at the time of blood sampling was 11 weeks. The interquartile range was 9–13 weeks, and 93% of the samples were collected at <18 weeks gestation. Whole blood samples were stored in the original tube at 4ºC at the collection site before being transferred to the central Bristol laboratory within 1–4 days. Samples were at ambient temperature during transfer (up to 3 h). They were then stored at 4ºC until analysis. Samples were analysed for lead and cadmiumusing inductively coupled plasma mass spectrometry in standard mode by R. Jones at the Centers for Disease Control (CDC), Bethesda, MD, USA (CDC Method 3009.1). Quality control was monitored as outlined in Golding, et al. [24] The analyses were completed on 4284 samples for lead and 4286 for cadmium. One sample had a lead level below the limit of detection (0.29 µg/dl); 1119

samples were below the lower limit of detection for cadmium (0.20 µg/l). These samples were assigned a value of 0.7 times the lower limit of detection (LOD/√2).[25 26]

Child blood samples Details of the selection of the subsample of children and analysis of the blood samples have been reported previously in detail.[3 27] In brief, a 10% randomly selected subsample of parents whose babies were born in the last 6 months of the ALSPAC study were invited to attend a research clinic (Children in Focus, CIF). At age 30 months, parental consent was sought for a venous blood sample, and was given by 81% of the 1135 children in the CIF group. The sample was drawn into lead-free tubes from 653 (71%) of children attending the clinic. However, 69 samples were insufficient, leaving 582 samples for analysis. Analysis was by atomic absorption spectrometry (Southampton General Hospital, UK) with appropriate quality controls.

Balance variables

Clinic measures Full details of the balance outcomes including details of the measurements and validity have previously been published.[28] In brief, at age 7 years the heel-to-toe walking test of the Movement Assessment Battery for Children[29] was conducted with the total number of successful steps out of a maximum of 15 recorded. At age 10 years a range of tests were used to assess balance: (1) walking along a beam, heel-to-toe, eyes open; (2) heel-to-toe balance on a beam, eyes closed; (3) standing on one leg, eyes closed. Each child had two attempts at beam-walking; for tests of static balance, children only had a second attempt if they failed to achieve the maximum score on the first attempt.[28] These tests were based on standard clinical tests to assess balance in children and have significant commonality with the balance subtest of the both editions of the Bruininks–Oseretsky Test of Motor Proficiency.[30]

[31] The measures are also in common use when testing balance informally in the paediatric clinic.

Questionnaire items The primary caregiver (usually the mother) received a series of postal self-completion questionnaires. The questionnaires are available from the study website (http://www.bris.ac.uk/alspac/researchers/data-access/data-dictionary/). When their child was aged 18, 30, 42 and 81 months, the parent completing the questionnaire was asked to indicate 'Yes, can do well'/'Has only done once or twice'/'Has not yet started' in response to the statement 'He/She can balance on one foot for at least 1 second'. When their child was aged 10 years, the parent was asked to indicate 'Very well'/'Just OK'/'Can almost'/'Not at all' in response to the following questions: How well can your child stand on one leg in a stable position (e.g. when putting on trousers, skirt)?; How well can your child ride a bike (without stabilisers)?; How well can your child walk in the dark?

Confounding variables

Information on passive smoking exposure during the week and at weekends was obtained from questionnaires at 77 and 103 months. Information on traffic levels, type of accommodation, lowest level of accommodation and maternal education were obtained at from questionnaires completed by the mother during pregnancy. Dietary Ca and Fe intake at 7 years and 10 years were derived from food frequency questionnaires as previously described in detail. [32]

Statistical analysis

Statistical analysis was carried out with IBM SPSS Statistics 21. Balance measures were derived as previously described.[28] In brief, for the heel-to-toe test at age 7 years, the number of steps (maximum 15) was categorised into 0–5, 6–10 and 11–15 steps for categorical associations, and 1–14 (failed to complete in 20 s) versus 15 steps (successfully completed in 20 s) for regression analyses. For the measure of dynamic balance at 10 years (beam-walking test), the mean of two attempts was categorised into quartiles. For measures of static balance at age 10 years (heel to toe balance on a beam with eyes closed/standing on one leg eyes closed), the sum of the score (s) from both attempts was calculated. Children who scored the maximum of 20 on the first attempt and so did not have a second attempt were given a final score of 40. The final scores were put into four categories (0–9, 10–19, 20–39 and 40). All of the four static balance tests with eyes closed were summed to create a static balance eyes closed variable (SBEC).

Blood lead and cadmium levels were put into two categories (<5, ≥5 µg/dl for lead and <1, ≥1 µg/l for Cd). These categories were chosen in accordance with the levels of concern of the US Centers for Disease Control, the US Association of Occupational and Environmental Clinics and the American College of Obstetricians and Gynecologists for Pb,[33-36] and the German Federal Environmental Agency for Cd.[37] Blood levels were also categorised into quartiles.

Chi square tests were used to compare categorical variables. Unadjusted and adjusted logistic regression analyses were used to investigate the association of blood levels with balance variables.

RESULTS

As previously reported, the mean child blood lead level was 4.22±3.12 µg/dl (n=582)[3 27]; the mean prenatal blood lead level was 3.67±1.47 µg/dl (n=4284) and the mean prenatal cadmium level was 0.58±0.63 µg/l (n=4286).[38 39] Mothers who consented to provide a blood sample were better educated and older than mothers who did not.[38] Children who had lead levels measured were from families where the mother was better educated and more likely to be a homeowner, and was there was a better home environment with fewer adversities.[3]

Associations of measures of balance with prenatal lead and cadmium levels and with child lead levels

Associations with in utero exposure to lead and cadmium

There was no evidence of any association between the results of the heel-to-toe test at age 7 years and maternal lead or cadmium level during pregnancy (p=0.441 and p=0.189, respectively) (Table 1). Similarly, at age 10 years there were no associations between dynamic balance (beam walking) or static balance (SBEC) and maternal blood lead or cadmium levels in pregnancy (all p>0.1) (Table 1).

In logistic regression models adjusted for sex, passive smoking, and calcium and iron intake, there was no evidence of any association between maternal blood lead or cadmium levels and measure of balance in the child at 7 and 10 years (all p>0.1) (Table 2). When the models were repeated with quintiles of maternal blood lead or cadmium level rather than a dichotomous variable, there was also no evidence of any associations (all p>0.1 with the exception of maternal blood lead for the odds of static balance dysfunction at 10 years where there was a protective effect (p for trend 0.038) (Supplementary Table 1).

Associations with child lead level

There was no evidence of any association between the results of the heel-to-toe test at age 7 years and child lead level (p for trend=0.146) (Table 3). Similarly, at age 10 years there were no associations between dynamic balance (beam walking) or static balance (SBEC) and child blood lead levels (p for trend=0.798 and p=0.918, respectively) (Table 3). In logistic regression models adjusted for sex, passive smoking, and calcium and iron intake, there was no evidence of any association between child blood levels at 30 months and measure of balance at 7 and 10 years (Table 4; all p for trend >0.3). When the models were repeated with quintiles of child blood lead rather than a dichotomous variable, there was also no evidence of any associations (all p for trend >0.1) with the exception of static balance where there was a weakly protective effect (p for trend=0.038 (Supplementary Table 1).

Table 1 Associations of in utero lead and cadmium exposure with measures of balance in the child at 7 and 10 years in ALSPAC

	Age (years)	Category	Maternal Pb (µg/dl)			Maternal Cd (µg/l)		
			<5	≥5	P value (chi square)	<1	≥1	P value (chi square)
Heel to toe test	7	0–5 steps	520 (27.1)	82 (26.5)	0.861	521 (26.6)	81 (29.1)	0.112
		6–10 steps	273 (14.2)	45 (14.5)		270 (13.8)	49 (17.6)	
		11–15 steps	1128 (58.7)	183 (59.0)		1164 (59.6)	148 (53.2)	
Beam walking (dynamic balance)	10	Q1	431 (23.4)	70 (24.1)	0.450	439 (23.3)	62 (25.3)	0.897
		Q2	461 (25.0)	80 (27.6)		484 (25.6)	57 (23.3)	
		Q3	456 (24.8)	66 (22.8)		463 (24.5)	59 (24.1)	
		Q4	494 (26.8)	74 (25.5)		503 (26.6)	67 (27.3)	
Static balance eyes closed score (SBEC) (static balance)	10	Q1	460 (24.4)	74 (25.8)	0.558	472 (25.5)	63 (25.8)	0.842
		Q2	459 (25.4)	58 (20.2)		455 (24.6)	62 (25.4)	
		Q3	430 (23.8)	83 (28.9)		457 (24.7)	57 (23.4)	
		Q4	459 (25.4)	72 (25.1)		469 (25.3)	62 (25.4)	

Values are n (%).

Q, quartile.

Table 2 Associations of in utero lead and cadmium exposure with balance measures in the child at 7 and 10 years old in ALSPAC

	Age (years)	Prenatal lead exposure				Prenatal cadmium exposure			
			OR of balance dysfunction (95% CI)	P value	n		OR of balance dysfunction (95% CI)	P value	n
Heel to toe test	7	Unadjusted	1.01 (0.96, 1.07)	0.503	2231	Unadjusted	0.81 (0.70, 0.94)	0.010	2233
		Adjusted [a]	1.01 (0.95, 1.01)	0.555	1732	Adjusted [a]	0.95 (0.77, 1.20)	0.904	1734
Dynamic balance	10	Unadjusted	1.01 (0.95, 1.08)	0.790	2132	Unadjusted	1.00 (0.84, 1.21)	0.946	2134
		Adjusted [b]	1.02 (0.95, 1.09)	0.692	1761	Adjusted [b]	1.20 (0.95, 1.52)	0.135	1763
Static balance	10	Unadjusted	0.98 (0.92, 1.05)	0.569	2095	Unadjusted	1.06 (0.88, 1.28)	0.523	2097
		Adjusted [b]	0.98 (0.92, 1.06)	0.661	1734	Adjusted [b]	1.00 (0.79, 1.26)	0.995	1736

Logistic regression showing odds ratio of balance dysfunction (95% CI).
[a]Adjusted for: sex, passive smoking at 77 months old (weekdays and weekends), and Ca and Fe intake at 7 years.
[b]Adjusted for: sex, passive smoking at 103 months old (weekdays and weekends), and Ca and Fe intake at 10 years.

Table 3 Associations of child lead level at 30 months with measures of balance at 7 and 10 years in ALSPAC

Test	Age (years)	Category	Child Pb (µg/dl)		P value (chi square)
			<5	≥5	
Heel to toe test	7	0–5 steps	82 (25.8)	34 (30.9)	0.146
		6–10 steps	52 (16.4)	22 (20.0)	
		11–15 steps	184 (57.9)	54 (49.1)	
Beam walking (dynamic balance)	10	Q1	74 (24.0)	24 (23.5)	0.798
		Q2	68 (22.1)	19 (18.6)	
		Q3	86 (27.9)	34 (33.3)	
		Q4	80 (26.0)	25 (24.5)	
Static balance eyes closed score (SBEC) (static balance)	10	Q1	80 (26.5)	22 (22.0)	0.918
		Q2	70 (23.2)	30 (30.0)	
		Q3	77 (25.5)	24 (24.0)	
		Q4	75 (24.8)	24 (24.0)	

Values are n (%).

Q, quartile.

Table 4 Child blood lead level at 30 months and balance measures at 7 and 10 years old in ALSPAC

	Age (years)	OR of balance dysfunction (95% CI)		P value	n
Heel to toe test	7	Unadjusted	0.98 (0.92, 1.04)	0.618	428
		Adjusted [a]	0.98 (0.92, 1.05)	0.778	354
Dynamic balance	10	Unadjusted	1.03 (0.96, 1.11)	0.422	410
		Adjusted [b]	1.01 (0.93, 1.09)	0.814	363
Static balance	10	Unadjusted	1.04 (0.96, 1.12)	0.345	402
		Adjusted [b]	1.03 (0.94, 1.12)	0.540	357

Logistic regression analysis comparing results for children with blood lead level ≥5 vs <5 µg/dl.
[a]Adjusted for: sex, passive smoking at 77 months old (weekdays and weekends), and Ca and Fe intake at 7 years.
[b]Adjusted for: sex, passive smoking at 103 months old (weekdays and weekends), and Ca and Fe intake at 10 years.

Associations with questionnaire items at 18–81 months and at 10 years

There was no evidence of any associations between child blood lead level at 30 months and the ability to stand on one foot for at least 1 second at 30, 42 or 81 months (all p>0.6; Supplementary Table 2). There was no evidence of any associations between maternal blood lead level during pregnancy and the ability of the child to stand on one foot for at least 1 second at 18, 30, 42 or 81 months (all p>0.3) (Supplementary Table 2). There was no evidence of any associations between maternal blood cadmium level during pregnancy and the ability of the child to stand on one foot for at least 1 second at 42, or 81 months (all p>0.4), but there were associations at 18 and 30 months (p<0.001 and p=0.003, respectively; maternal cadmium ≥1 µg/l was associated with being more likely to be able to stand on one foot well) (Supplementary Table 2).

There was no evidence of any association of elevated child lead level, or in utero lead or cadmium exposure, with ability to stand on one leg or to walk in the dark at 10 years (all p>0.09, chi-square test) (Supplementary Table 3). However, prenatal lead level ≥5 µg/dl was associated with not being able to ride a bike without stabilisers (p=0.007), whereas child lead level ≥5 µg/dl and prenatal cadmium ≥1 µg/l were weakly associated with being able to ride a bike without stabilisers very well (p=0.050 and p=0.075 respectively). When these associations were modelled in a logistic regression analysis adjusted for variables that could affect availability of a bicycle and being able to ride a bicycle locally (traffic level on the home street, type of accommodation, lowest level of accommodation), and maternal education, there was very weak evidence for an association of child lead level being associated with being able to ride a bike well without stabilisers (OR in unadjusted model 2.79 (95% CI 0.096, 8.10), p=0.059, n=413; OR in adjusted model OR 2.57 (95% CI 0.87, 7.62), p=0.089, n=387). The association was stronger for prenatal lead level in the unadjusted model, but the effect was attenuated with adjustment (OR in unadjusted model 0.59 (95% CI

0.40, 0.87), p=0.007, n=2150; OR in adjusted model 0.73 (95% CI 0.47, 1.14), p=0.167 , n=1904); for prenatal cadmium level again the weak effect was attenuated by adjustment (unadjusted OR 1.69 (95% CI 0.94, 3.01, p=0.078, n=2151; adjusted OR 1.44 (95% CI 0.75, 2.75, p=0.274, n=1905).

DISCUSSION

We did not find any evidence of an association of prenatal exposure to lead or cadmium, or lead levels in childhood, on balance ability (static and dynamic) in children. Counterintuitively, there was a suggestion that higher child lead levels and in utero lead exposure were associated with the ability to ride a bike without stabilisers at age 10 years, but these effects were negated when the associations were adjusted for variables that included the lowest level of accommodation and traffic levels outside the home. This is the first study, to our knowledge, reporting on the associations between in utero exposure to lead and cadmium and balance ability of the child, and adds to the few studies on child lead levels and balance ability.

Postural balance is controlled by a complex interaction of sensorimotor processes, including visual, proprioception and the vestibular system. In our study the measure of static balance with eyes closed eliminated vision and minimised proprioceptive inputs, thereby enabling the assessment of vestibular information as the primary input. Use of clinical measures similar to these is commonplace in epidemiological studies (e.g. [40-43]), as the tests are in common clinical usage and require little or no specialist equipment. However, although the vestibular dominant condition of tests of standing balance (reduced base of support with eyes closed) has been shown to correlate well with more expensive systems such as computerised dynamic posturography (considered to be the 'gold standard' method for assessing balance function) in

adults,[44] there are questions about whether such a correlation exists in children.[45] It has even been suggested that computerised measures using a force-platform and clinical tests such as those used by ALSPAC give complementary rather than concurrent information.[46] A cautious approach should therefore be taken if seeking to compare studies using these two different types of outcome measure.

Most studies on lead levels and balance in children have included children with relatively high lead levels (means in each study of 11.6 to 20.7 µg/dl) and measured balance with a force platform system,[12-15] and have found negative associations. There are several reasons for our results being in contrast to these studies. First, the mean child blood lead level in our study (4.22 µg/dl) was lower than reported in these earlier studies, and may have been too low to have been sufficient to cause balance dysfunction. Alternatively, the effect on balance might have been too small to be detectable with our tests, although a study in Inuit children had levels that were more comparable to the present study (mean 5.4 µg/dl) showed a significant association with sway oscillations.[16] Second, we used a series of assessments based on clinical tests to measure balance rather than a force platform or measurement of sway oscillations and this may account for differences in the findings. As discussed earlier, clinical and force platform measures may be giving complementary rather than concurrent information: whereas posturography is a measure of the motor and sensory strategies used to control balance, clinical tests evaluate the results of that balance control.[46] Measuring sway oscillations using a force platform will also be more sensitive than the ALSPAC measures, which measured time before a procedural fault such as touching the floor with either foot or lifting a foot off the beam. Third, early exposure to lead and/or cadmium could damage the vestibular system, but the plasticity of the balance system might compensate for this so that there is no functionally measurable effect. This is in accordance with measures of balance in the present study indirectly assessing the vestibular system. This could also account for

effects being reported in adults, in whom plasticity is less effective for overall balance compensation, but not in children. It is also possible that vestibular system (to include peripheral (ear) and central (brain) components of the vestibular pathway) dysfunction caused by in utero exposure to lead or cadmium may not be apparent in childhood but may manifest in later life. Finally, it is also possible that studies showing non-significant results have tended not to have been published.

To our knowledge, there are no studies that have examined the effect of in utero exposure to lead or cadmium on balance ability in the child. Our results provide preliminary evidence for lack of effect, but this requires confirmation on other cohorts.

The strengths of the study are that: (1) it is a population-based study; (2) the data were collection prospectively; and (3) the numbers included in the study were large compared with several other studies. There are several limitations to the study. First, measures of blood lead and cadmium do not necessarily reflect lifetime exposure. Bone lead, which makes up more than 95% of the body lead, can be measured by K x-ray fluorescence,[47] but this is expensive, technically demanding and may not always be ethically permissible in children. This limitation is of less consequence for in utero exposure as the maternal blood level largely determines the fetal blood level. Second, there was a high proportion of blood cadmium levels below the limit of detection, which may make the results less reliable than for lead. Third, there may be confounders we were unable to account for. Fourth, we were unable to control for lead and cadmium separately in the models because of multicollinearity. Finally, the balance measures used by ALSPAC had low test–retest reliability,[28] which is a common problem with measures of childhood balance.[48-50] The measure of ability to balance on one foot for 1 s, a test which was originally developed by Chamberlain and Davey[51] in 1976, has been particularly criticised for having poor test validity as it is difficult to discriminate a

failed attempt from a successful attempt.[10] It should ideally form part of a battery of clinical measures, perhaps with a longer duration of standing. These combined effects may have led to random misclassification in our balance assessments and dilution of the estimates of the effects of lead and cadmium exposure.

CONCLUSIONS

We did not find any evidence of an association of prenatal exposure to lead or cadmium with balance ability in children. In contrast to previous studies, we did not find any association of child blood lead with balance ability in children. This may reflect variation in the methods used to assess balance in different studies, or may be related to the lower mean lead level in the children in the present study than in previous studies. Further work in other cohorts is needed to confirm the results.

Data sharing statement: No additional data available.

Contributors statement: CMT conceived the study and undertook data analysis in conjunction with RH, AH and JG. CMT took the lead in writing the manuscript with critical revisions and additions from RH, AH, JG and AME. All authors contributed to and approved the final version of the manuscript.

Ethics approval: Ethics approval for the study was obtained from the ALSPAC Ethics and Law Committee and the Local Research Ethics Committees.

Competing interests: The authors declare that there are no competing interests

Acknowledgements: We are extremely grateful to all the families who took part in this study, the midwives for their help in recruiting them, and the whole ALSPAC team, which includes interviewers, computer and laboratory technicians, clerical workers, research scientists, volunteers, managers, receptionists and nurses.

Funding: The UK Medical Research Council and the Wellcome Trust (Grant ref: 102215/2/13/2) and the University of Bristol currently provide core support for ALSPAC. This publication is the work of the authors who will serve as guarantors for the contents of this paper. CMT was supported by a Wellcome Trust Career Re-entry Fellowship (Grant ref: 104077/Z/14/Z).

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Balance ability in 7- and 10-year-old children: associations with prenatal lead and cadmium exposure and with blood lead levels in children in a prospective birth cohort study

Caroline M Taylor, Rachel Humphriss, Amanda Hall, Jean Golding, Alan M Emond

Supplementary Table 1 Associations of quintiles of in utero lead and cadmium exposures and of child lead with measures of balance in the child at 7 and 10 years in ALSPAC

Quartile	Prenatal exposure				Child Pb[c]	
	Maternal Pb[a]		Maternal Cd[b]			
	OR of balance dysfunction (95% CI)	P value for trend	OR of balance dysfunction (95% CI)	P value for trend	OR of balance dysfunction (95% CI)	P value for trend
Heel to toe test at 7 years[d]						
1	1.00 (Ref)	0.349 (n=1732)	(Ref)	0.133 (n=1457)	(Ref)	0.234 (n=354)
2	0.94 (0.72, 1.22)		1.17 (0.91, 1.53)		1.47 (0.82, 2.63)	
3	1.17 (0.90, 1.53)		0.91 (0.69, 1.20)		0.96 (0.52, 1.78)	
4	1.18 (0.82, 1.41)		0.90 (0.62, 1.27)		0.78 (0.52, 1.42)	
Dynamic balance at 10 years[e]						
1	(Ref)	0.981 (n=1761)	(Ref)	0.433 (n=1763)	(Ref)	0.290 (n=363)
2	1.34 (0.99, 1.81)		0.85 (0.65, 1.12)		0.80 (0.41, 1.56)	
3	1.09 (0.81, 1.46)		0.95 (0.71, 1.26)		1.08 (0.54, 2.14)	
4	1.09 (0.81, 1.46)		1.10 (0.76, 1.59)		0.86 (0.43, 1.72)	
Static balance at 10 years[e]						
1	(Ref)	0.811 (n=1734)	(Ref)	0.851 (n=1736)	(Ref)	0.891 (n=357)
2	0.68 (0.50, 0.92)		1.11 (0.84, 1.48)		0.71 (0.36, 1.39)	
3	0.85 (0.62, 1.16)		1.11 (0.83, 1.49)		1.20 (0.59, 2.47)	
4	0.89 (0.65, 1.23)		0.97 (0.67, 1.41)		0.87 (0.43, 1.78)	

[a]Maternal Pb quartiles: Q1 0.20–2.66, Q2 2.67–3.40, Q3 3.41–4.33, Q4 4.34–19.14 µg/dl.
[b]Maternal Cd quartiles: Q1 0.14–0.14, Q2 0.20–0.29, Q3 0.30–0.72, Q4 0.73–6.30 µg/l.
[c]Child Pb quartiles: Q1 0.83–2.18, Q2 2.28–3.32, Q3 3.42–5.18, Q4 5.28–27.56 µg/dl.
[d]Adjusted for: sex, passive smoking at 77 months old (weekdays and weekends), and Ca and Fe intake at 7 years.
[e]Adjusted for: sex, passive smoking at 103 months old (weekdays and weekends), and Ca and Fe intake at 10 years.

Supplementary Table 2 Associations of responses to questionnaire items related to balance at 18–81 months old in ALSPAC

	Age (months)	Category	Prenatal exposure						Child Pb (µg/dl)		
			Maternal Pb (µg/dl)			Maternal Cd (µg/l)					
			<5	≥5	P value (chi square)	<1	≥1	P value (chi square)	<5	>5	P value (chi square)
Stand on 1 foot for 1 s	18	Yes, can do well	1397 (50.8%)	237 (51.9%)	0.680	1353 (49.5%)	280 (58.6%)	<0.001	-	-	-
		Only done 1–2 times/Not yet started	1352 (49.2%)	220 (48.4%)		1376 (50.5%)	198 (41.4%)		-	-	
Stand on 1 foot for 1 s	30	Yes, can do well	1853 (70.6%)	313 (71.5%)	0.711	1815 (69.7%)	352 (76.5%)	0.001	270 (69.6%)	95 (70.4%)	0.865
		Only done 1–2 times/Not yet started	772 (29.4%)	125 (28.5%)		789 (30.3%)	108 (23.5%)		118 (30.4%)	40 (29.6%)	
Stand on 1 foot for 4 s	42	Yes, does well	1862 (72.4%)	290 (70.2%)	0.366	1838 (71.9%)	316 (73.7%)	0.447	288 (73.8%)	204 (73.8%)	0.984
		Yes, not very well/Not yet done	711 (27.6%)	123 (29.8%)		720 (28.1%)	113 (26.3%)		102 (26.2%)	37 (26.2%)	
Stand on 1 foot for 8 s	81	Yes, can do well	2052 (95.0%)	339 (95.8%)	0.537	2079 (95.1%)	314 (94.9%)	0.823	329 (94.5%)	106 (93.0%)	0.538
		Yes, but not well/Has not yet done/ Unable to try/Not had a chance	108 (5.0%)	15 (4.2%)		106 (4.9%)	17 (5.1%)		19 (5.5%)	8 (7.0%)	

Values are n (%).

Supplementary Table 3 Associations of responses to questionnaire items related to balance at 10 years old in ALSPAC

Test	Category	Prenatal exposure						Child Pb (µg/dl)		
		Maternal Pb (µg/dl)			Maternal Cd (µg/l)					
		<5	≥5	P value (chi square)	<1	≥1	P value (chi square)	<5	≥5	P value (chi square)
Stand on one leg	Very well	1411 (76.1%)	219 (74.7%)	0.623	1455 (76.5%)	177 (71.7%)	0.09	236 (76.1%)	79 (76.0%)	0.972
	Just OK/ Can almost/ Not at all	444 (23.9%)	74 (25.3%)		448 (23.5%)	70 (28.3%)		74 (23.9%)	25 (24.0%)	
Ride a bike without stabilisers	Very well	1715 (92.5%)	259 (87.8%)	0.007	1742 (91.4%)	234 (94.7%)	0.07	278 (90.0%)	100 (96.2%)	0.050
	Just OK/ Can almost/ Not at all	140 (7.5%)	36 (12.2%)		163 (8.6%)	13 (5.3%)		31 (10.0%)	4 (3.8%)	
Walk in dark	Very well	1367 (75.2%)	222 (76.6%)	0.628	1411 (75.6%)	180 (74.1%)	0.39	225 (75.3%)	78 (75.7%)	0.923
	Just OK/ Can almost/ Not at all	450 (24.8%)	68 (23.4%)		455 (24.4%)	63 (25.9%)		74 (24.7%)	25 (24.6%)	

Balance ability in 7- and 10-year-old children: associations with prenatal lead and cadmium exposure and with blood lead levels in children in a prospective birth cohort study

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STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Line no.
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2 and 23
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	18-38
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	49-76
Objectives	3	State specific objectives, including any prespecified hypotheses	77-79
Methods			
Study design	4	Present key elements of study design early in the paper	82-86
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	89-153
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	82-96
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	99-153
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	99-153
Bias	9	Describe any efforts to address potential sources of bias	94-95
Study size	10	Explain how the study size was arrived at	89-96
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	156-172
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	156-175
		(*b*) Describe any methods used to examine subgroups and interactions	

	(*c*) Explain how missing data were addressed
	(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy
	(*e*) Describe any sensitivity analyses

Continued on next page

Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Table 1-4
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	89-96
		(b) Indicate number of participants with missing data for each variable of interest	
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	Tables 1-4
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	185-208
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
Discussion			
Key results	18	Summarise key results with reference to study objectives	268-276
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	322-336
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	339-344
Generalisability	21	Discuss the generalisability (external validity) of the study results	343
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	355-360

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.