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Article title: Aluminium exposure from intravenous feeding solutions and later bone health: 15 year follow-up of a randomised trial in preterm infants
Year of publication: 2009
Citation: Fewtrell, M.S. et al (2009). 'Aluminium exposure from intravenous feeding solutions and later bone health: 15 year follow-up of a randomised trial in preterm infants'. Pediatrics, 124 (5) 1372-1379.
Link to published version: http://dx.doi.org/10.1542/peds.2009-0783
DOI: 10.1542/peds.2009-0783

Aluminum exposure from intravenous feeding solutions and later bone health: 15 year follow-up of a randomised trial in preterm infants

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Key words: preterm infant, bone health, parenteral nutrition, aluminum

Funding: Research grant from UK Medical Research Council

Word count

Abstract 247

Main text 2684

Tables 3, Figures 2, Refs 19

Abstract

Background: Aluminum has known neurotoxicity and may impair short-term bone health. In a randomised trial we showed reduced neurodevelopmental scores in preterm infants previously exposed to aluminum from parenteral nutrition solutions. Here, in the same cohort, we test the hypothesis that neonatal aluminum exposure also adversely affects long-term bone health, as indicated by reduced bone mass.

Methods: Bone area (BA) and bone mineral content (BMC) of lumbar spine, hip and whole body were measured with Dual X-ray Absorptiometry (DXA) in 13-15yr olds who were born preterm and randomly assigned standard or aluminum-depleted parenteral nutrition (PN) solutions during the neonatal period.

Results: 59 subjects (32% of survivors) were followed. Those randomised to standard PN solution had lower lumbar spine BMC; apparently explained by a concomitant decrease in bone size. In non-randomised analyses, subjects exposed to neonatal aluminum intakes above the median (55mcg/kg) had lower hip BMC (by 7.6% (95% CI 0.21 to 2.38; p=0.02)), independent of bone (or body) size.

Conclusion: Neonates exposed to parenteral aluminum may have reduced lumbar spine and hip bone mass during adolescence, potential risk factors for later osteoporosis and hip fracture. These findings need confirmation in larger, more detailed studies. Nevertheless, given our previous finding of adverse developmental outcome in these subjects, and the sizeable number of contemporary infants undergoing intensive neonatal care who are still exposed to aluminum via parenteral feeding solutions, the potential adverse long term consequences of early aluminum exposure now deserve renewed attention.

Aluminum is the most common metallic element in the earth's crust, but has no known biological role. It accumulates in the body when protective gastrointestinal mechanisms are bypassed, renal function is impaired, or exposure is high – all of which apply frequently to sick or preterm infants. Recognised clinical manifestations of aluminum toxicity, for instance from older renal dialysis solutions, included progressive dementia, anaemia and bone disease.

Parenteral feeding solutions used in infants are contaminated with aluminum[2,3], mostly from calcium gluconate solutions stored in glass vials, where complex-forming anions dissolve aluminum from the glass during autoclaving. When fed parenterally, infants retain up to 78% of the aluminum[4], with high serum, urine and tissue levels[1]. Increased aluminum concentrations have been observed post mortem in the brain of a parentally fed preterm infant[5].

Given the known toxicity of aluminum and the increasing survival of high risk neonates requiring parenteral nutrition, we elected to explore whether early exposure to intravenous aluminum has adverse long-term effects on health. Assigning infants to high levels of aluminum exposure would have been unethical However, because standard parenteral nutrition solutions contain significant aluminum, it was ethical for us to conduct a randomised trial comparing these with corresponding solutions specially sourced for low aluminum content. Our trial, conducted in preterm infants, showed those exposed for >10 days to standard solutions had impaired neurologic development at 18 months post-term[6]. Bone health was not assessed at that stage. However, in rats, pigs,

dogs and adult humans excess aluminum accumulates at the mineralization front and is associated with reduced bone formation[7]. Uraemic adults, and those on TPN have low bone formation, with patchy osteomalacia[7]. Sedman[8] found bone aluminum concentrations were 10 times higher in preterm infants fed parenterally for more than 3 weeks than in controls. None of these studies tested whether early aluminum exposure might influence *long-term* bone health; and, notably, result in reduced bone mass, believed to be a key predictor of osteoporosis and fracture risk. In this study, therefore, we have used our trial to test experimentally the hypothesis that neonatal exposure to aluminum in standard parenteral nutrition solutions results in reduced bone mass during adolescence.

Methods

Study subjects were adolescents previously randomised to aluminum-depleted versus standard parenteral nutrition (PN) solutions during the neonatal period. Details are given elsewhere[6] but summarised below.

Randomised trial

227 preterm infants (gestation < 34 weeks, birthweight < 1850g) were recruited from neonatal intensive care units in Cambridge and Norwich, UK. Infants were eligible for the study if there was a clinical decision to initiate intravenous feeding. Infants were randomly assigned according to a multiple random permuted-block method to receive either standard (S) aluminum-depleted (AD) PN solution. Investigators and staff were blind to the assignments. The study was approved by the research ethics committee, and

parental consent obtained. PN was introduced (typically on postnatal day 2 or 3) and stopped at the discretion of NICU medical staff. The composition of the two solutions (Table 1) was identical except that the AD solution contained less aluminum and more chloride, reflecting use of calcium chloride rather than calcium gluconate. Employing a mixed sodium-potassium phosphate solution instead of potassium acid phosphate further reduced aluminium and minimised the increase in chloride.

Data were collected on the neonatal course of each infant, including detailed records of intravenous fluids and PN, enteral feeds and clinical events. The aluminum content of the parenteral solutions was measured by graphite-furnace atomic-absorption spectrometry (see Bishop[6] for details). Total aluminum exposure from PN nutrition, expressed as mcg/kg, was calculated for each infant from the daily volume of each parenteral fluid.

Follow-up study

Subjects were invited for follow-up at age 13-15 years, to examine long-term effects of the intervention on (i) bone health; and (ii) cognitive and neurological outcomes (to be reported separately). Children with neurological impairment or a previous Bailey score of <85 were excluded. The study was approved by the Great Ormond Street Hospital Research Ethics Committee. Subjects visited our research centre with a parent. Written informed consent was obtained from a parent and written assent from the subject. Weight was measured using digital scales and height using a portable stadiometer. A food frequency questionnaire quantified current calcium intake (Calquest[9]); a simple questionnaire determined hours of weight-bearing activity/week; and parents rated the

child's activity level compared to his peers (rated 1-5: 1 = much less active; 5 = much more active). A general medical history and fracture history were taken.

Bone densitometry

Dual X-ray absorptiometry (DXA; Lunar Prodigy, GE, USA) was used to measure Bone Mineral Content (BMC), Bone Area (BA) and Bone Mineral Density (BMD) at the lumbar spine (L2-4), hips and whole body. Subjects wore light indoor clothing after removing metal objects. Total radiation exposure was below daily background levels (approx 7 microSv/day in the UK). As recommended by the International Society for Clinical Densitometry[10] we used 'total body less head' values for whole body scans.

Statistics

Groups were compared using t-test or chi-square test. Some variables were transformed to ensure normal distribution. The target sample size of 64 per group at follow-up would allow a difference of 0.5SD to be detected at 80% power and 5% significance.

Bone mass was adjusted for size in three ways: (i) Bone Mineral Apparent Density (BMAD) of the lumbar spine, calculated as $BMC/BA^{1.5}$ BMAD Z scores were calculated for age, sex and ethnic group using UK machine-specific reference data[11]; (ii) for whole body bone mass, a two-stage procedure was used. The indices $lean/height^{3}$ and $BMC/lean^{0.7}$ were calculated with the power relationships required to remove any residual association with height determined using log-log regression; (iii) multiple regression was used firstly to examine the effect of PN solution assignment on later bone

mass at skeletal sites after adjusting for age, sex, pubertal stage and body size (weight and height); and secondly, to adjust for potential confounding factors, including current physical activity and calcium intake. Continuous variables were transformed to natural logarithms for regression analyses, allowing coefficients to be expressed as percentages (sympercents[12]).

Relationships between neonatal aluminum exposure and later bone mass were also examined in a non-randomised manner, using total neonatal aluminum exposure from TPN as both a continuous and dichotomous variable. Multiple regression was used with backward elimination of non-significant variables ($p>0.05$), adjusting for potential confounders including PN duration and factors related to neonatal illness severity.

Results

Comparison of randomised groups

59 subjects from the original cohort (26% of those randomised; 32% of survivors; 33% of those eligible for follow-up) completed the bone health protocol (Figure 1). Subjects followed had significantly higher birth-weight SD score than those not seen, but there were no other baseline differences (Table 2).

Neonatal data for those followed-up (Table 3) showed the randomised groups were well-matched for birth-weight, gestation, days in the trial and days of iv feeding. There were no differences in neonatal peak plasma calcium, minimum phosphate or maximum alkaline phosphatase (ALP) (data not shown). Median (25th,75th centile) peak ALP

concentrations were 609 (502,751) and 606 (438,705) IU/l in groups AD and S respectively with maximum values of 982 and 1087IU/l. Total neonatal aluminum exposure from PN expressed in mcg/kg was, by design, significantly higher in subjects who received standard feeding solutions. The proportion of human milk in the diet did not differ between groups. All infants required ventilatory support; with no group differences in duration or time spent in 30% oxygen. Socio-economic and educational indices did not differ between groups.

At follow-up, there were no group differences in gender distribution, pubertal stages, age or anthropometric variables, although there was a trend towards greater weight, weight SDS and BMI in AD subjects (Table 4). Seven group S and 6 group AD subjects reported a current or previous history of asthma. Two group S and 5 group AD subjects were currently receiving no treatment, 5 group S and 1 group AD subjects were using bronchodilators, and one group S subject was also receiving inhaled corticosteroids. No other significant medical conditions were reported in either group.

AD subjects had significantly higher LSBMC and LSBA; with a similar though non-significant trend in WBBMC, WBBA, WBBMD, WBBMD Z score, LSBMDZ, Hip BMC and Hip BA (Table 4).

Size-adjusted bone mass

We explored whether the increase in LSBMC was due to a concomitant increase in bone size in the AD group. Supporting this, we found no difference between groups in (i):

LSBMC, after adjusting for height, weight and LSBA (LSBMC 2.7% lower in group S: 95% CI -8.9 to 3.6); and (ii) lumbar spine BMAD Z scores. There were no group differences in WBBMC and hip BMC adjusted for height, weight and BA (WBBMC 1.6% lower (95% CI -4.5 to 1.4) and Hip BMC 2.5% lower (-8.5 to 3.5)) in group S: nor in lean/height and WBBMC/lean ratios.

Neonatal aluminum exposure and bone mass: non-randomised analyses

Calculated neonatal aluminum exposure from parenteral nutrition varied with both the type of solution and duration of parenteral feeding. Values for the exposure of infants (μg/kg) by randomised group (Figure 2) showed overlap, with values in 24 infants falling into a common range. Mean (SD), median (25th, 75th centiles), minimum and maximum concentrations in the two groups were 3.0 (0.8), 28 (17,46), 4, 152 μ/kg for the AD group and 21.3 (7.2), 280 (91,417), 19, 840 μ/kg for group S ($p<0.001$ for all).

The total aluminum exposure from PN as a continuous variable was not a significant predictor of adjusted BMC at any site, after adjusting for relevant neonatal variables (birthweight, gestation, days of ventilation, days of iv feeding) and follow-up variables (age, sex, weight, height, BA). However, to look for a 'threshold' effect, aluminum exposure was categorised as 'low' and 'high' using the median exposure (55mcg/kg) as a cut-off. Subjects with ‘high’ exposure had significantly lower hip BMC (by 7.6% (95% CI 0.21 to 2.38; $p=0.02$). The median value was chosen as the cut-off to ensure equal numbers in the two groups, especially considering the relatively small sample size. However, exploratory analyses using other cut-offs (not shown) suggested that there was

a significant relationship between aluminium intake and later hip BMC only once the intake exceeded 45 mcg/kg. The largest effect size was seen using a cut-off of 65μ/kg (adjusted hip BMC -9.6% (-15.8 to -3.3) lower in group S). Above this level the effect plateaued. This association was not present for any other skeletal site. For example, using the median exposure (55mcg/kg) as a cut-off, adjusted whole body BMC was 2.7% (-6.1 to 0.7) lower in group S and lumbar spine BMC was 3.0% (-9.8 to 3.9) lower.

Current calcium intake and physical activity did not predict size-adjusted bone mass (data not shown). Fracture rates were not influenced by (i) randomised group or (ii) whether aluminum exposure was below or above the median (24% and 23% for lower versus higher aluminum exposure group in both comparisons). No subject reported repeated fractures or unusual fragility fractures suggestive of poor bone health.

Discussion

Our study produced two principle findings suggesting that brief exposure to aluminum from standard PN solutions used in the neonatal period may impair long term bone mineralisation. Firstly, subjects born preterm and randomised to an aluminium-depleted parenteral nutrition solution had significantly higher whole body BMC and BA, and higher lumbar spine BMC, BA and BMD during adolescence. After adjusting for current body and bone size, these differences between groups were no longer significant, suggesting that the higher bone mass reflects greater skeletal size in the AD group. Secondly, in non-randomised analyses relating neonatal aluminium exposure to later bone outcomes, we found that hip BMC was reduced in subjects with aluminium exposure

above the median (>55mcg/kg) than in those with lower exposure. In contrast to the effect on whole body and lumbar spine bone mass seen in the randomised comparison, the higher hip BMC associated with 'low' aluminium exposure was not apparently related to greater bone size. These findings have potential relevance for later osteoporosis and fracture risk.

Short-term adverse effects of aluminum on bone health have been shown in animals and adult humans[7], but no study has previously investigated whether such effects persist beyond the period of exposure. Our work in other areas shows neonatal influences may have lasting effects on bone health indices. Thus, we showed so-called 'metabolic bone disease of prematurity', due to early calcium and phosphorus insufficiency, is linked to stunting of linear growth later in childhood[13]. Recently, we found an association between greater intakes of human milk intake during the neonatal period and higher whole body BMC and BA in young adults born preterm[14]. These observations suggest early interventions may affect later skeletal size and mineralisation; and add plausibility to our findings here.

Our findings have contemporary relevance. In practice, despite greater recognition of aluminum toxicity, little progress has been made on reducing exposure. Poole[3] recently concluded that meeting current FDA recommendations to limit aluminum exposure to <5mcg/kg/day is impossible in patients weighing <50kg using currently available PN products; and calculated aluminum exposure in infants <3kg was 30.3-59.9mcg/kg/day –

indeed, somewhat higher than the calculated exposure of infants receiving standard PN solution in our trial.

The mechanism for long-term effects of aluminum on bone health is unclear. A direct toxic effect seems unlikely since bone tissue will have been replaced more than once by age 13-15 years. Possibly, aluminum exposure might "program" the responsiveness of bone such that, for example, subjects exposed to more aluminum form less bone for a given level of mechanical stimulus. This could explain the apparent site-specific effects of aluminum exposure. Alternatively, aluminum might have neurotoxic effects, affecting central mechanisms controlling bone mass. Indeed, bone remodelling is partly controlled by the central nervous system. In animals, several neuropeptides affect bone formation via the hypothalamus, with signal transmission to bone cells via the sympathetic nervous system[15]. Plausibly, then, the effects observed here might be another facet of early aluminium neurotoxicity rather than reflecting a direct effect on bone. If so, our study may have under-estimated the effect of aluminium exposure, since by design our protocol excluded subjects with known neurological impairment or with a Bayley score less than 85.

Whilst the effects of high aluminium exposure on BMC of the lumbar spine appeared related primarily to reduced lumbar spine bone size (bone area), effects on hip BMC were apparently unrelated to any corresponding stunting of hip bone growth. It is well recognised that interventions may have differential effects at different skeletal sites. For example, exercise typically affects only loaded bones[16], whilst leptin has different effects

on the trabecular and appendicular skeleton, perhaps through differential influences on trabecular and cortical bone[17]. Such differential effects cannot be studied by DXA, used here, which provides no information on bone geometry or structure - likely determinants of bone strength and fracture risk.. Hence we suggest future explanatory studies require additional techniques such as hip structural analysis or pQCT.

Study limitations

The major limitation of our study relates to the inevitable cohort attrition over 15 years since study initiation. We could only test 33% of eligible subjects (32% of survivors); a follow-up rate typical of that reported in a variety of other recent long-term cohort studies[18]. We recently discussed the implications of cohort attrition for data analysis and interpretation, and emphasised the importance of explicitly considering effects on study power, bias and generalisability[18]. Our original planned sample size (128 subjects) would detect a difference of 0.5SD between groups at 80% power and 5% significance; but with around 60 subjects here, we had the power to detect a difference of 0.7SD and might have missed smaller, though biologically relevant effects. Regarding selection bias, subjects followed here tended to be those with higher birth-weight SD scores; nevertheless, if adverse effects of aluminum exposure were seen in larger infants, the effects on smaller, more vulnerable infants might be at least as large, if not greater.

Secondly, we did not quantify all possible sources of parenteral aluminum, for instance from occasional albumin infusions. This would not be expected to influence the bone

outcome differences seen between randomised groups, but we cannot exclude an effect in the non-randomised analyses.

The long-term clinical significance of the observed effects of early aluminum exposure on bone mass at 13-15 years cannot currently be quantified; albeit our subjects were only 5-8 years from attaining peak bone mass, considered a powerful predictor of outcome. The estimated effect was sizeable: hip bone mass was 7.6% lower when aluminum exposure was above the median; and in those randomised to standard parenteral nutrition solutions, lumbar spine BMC was 0.7SD lower - around 14% of population variance, if normally distributed. Of potential relevance here, we note that Hernandez[19] suggests the strongest predictor of osteoporosis risk is peak bone mass, estimating a 10% increase would delay the onset of osteoporosis by 10 years.

Conclusion

Neonates exposed to parenteral aluminum may have reduced lumbar spine and hip bone mass during adolescence, potential risk factors for later osteoporosis and hip fracture. Our randomised trial with long-term follow-up is, to our knowledge, the only one in this area. Our findings must be interpreted in the context of the relatively small sample size and multiple comparisons performed, and our findings should be confirmed on a larger sample and with additional tools to investigate bone indices; yet, we recognise such studies require many years to undertake, and reappraisal of current practice is now needed. At 18 month follow-up, before significant cohort attrition, subjects from this cohort exposed to higher aluminum intakes had reduced developmental scores, with an

estimated loss of one developmental quotient point for each day of standard parenteral nutrition. Aluminum has no known biological purpose, and its potential hazards when given unphysiologically, by the parenteral route, are well recognised in other contexts. Given our new findings, we suggest it would be prudent, even with existing knowledge, to further consider reducing aluminum in modern PN solutions. This is complex[20], and may involve one or more of three generic approaches: (i) changing (with research and product filing if required) existing PN components to alternatives with lower aluminum, such as organic phosphorus sources; (ii) use of new methodologies for aluminum removal from PN products (such as calcium salts); and (iii) repackaging of PN components (such as mineral salts) in plastic vials to reduce contamination from glass (which may require new product development and testing). Whilst these obstacles have inhibited progress, increasing safety concerns should now lead to re-evaluation of aluminum exposure in current parenteral nutrition, given to many thousands of preterm and high risk infants each year.

Acknowledgements

We thank Catherine Wilson who performed and analysed the DXA scans, Professor Tim Cole for helpful statistical advice, Dahlia Haroun for practical help with the study, and the children and parents who participated.

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Table 1. Composition and aluminum content of the standard and aluminum-depleted intravenous feeding solutions.

Solution	Standard		Low aluminum	
	Volume (ml)	Al content (μ)	Volume (ml)	Al content (μ)
Vamin infant	50	1.5	50	1.5
Intralipid 20%	15	0.1	15	0.1
Vitalipid	1	0.3	1	0.3
Solivito	1	<0.1	1	<0.1
Neotrace	1.6	1.2	1.6	1.2
Potassium acid phosphate	1.3	2.8	-	-
Polyfusor phosphate	-	-	14.4	0.3
Calcium gluconate	8.0	38.8	-	-
Calcium chloride	-	-	2.1	0.5
Dextrose,sodium, potassium	102	<0.1	102	<0.1
Total aluminum intake at 180ml/kg/day	45μg/kg/day		4.0-4.5μg/kg/day	

Vamin infant contained essential amino acids without added electrolytes. Intralipid 20% was a fat emulsion containing 20g of fatty acids per dl. Vitalipid contained fat-soluble vitamins, and Solivito contained water-soluble vitamins. Neotrace was an in-house preparation containing copper and zinc only. Vamin infant, Intralipid 20%, Vitalipid and Solivito were manufactured by Kabi Vitrum.

Table 2. Neonatal data for those seen or not seen at the current follow-up (mean (SD) unless stated).

	Seen at 15 years n=59	Not seen at 15 years n=168	p
Birthweight (g)	1270 (295)	1204 (311)	0.2
Birthweight SD score	-0.10 (0.99)	-0.51 (1.2)	0.02*
Gestation (weeks)	28.9 (2.0)	29.0 (2.4)	0.8
Male (n(%))	27 (46)	91 (54)	0.3
Singleton (n(%))	42 (71)	125 (74)	0.9
Days in study	41 (18)	38 (23)	0.4
Days of iv	15 (9)	14 (11)	0.5
Days to reach full enteral feeds	15 (9)	14 (6)	0.3
Days of ventilation median (25th,75th centiles)	5 (3,8)	4 (2,9)	0.2

*p<0.05

Table 3. Neonatal data for subjects seen at follow-up according to original randomised group (mean(SD)) unless stated.

	Low aluminum n=33	Standard n=26	p
Birthweight (g)	1290 (281)	1244 (316)	0.6
Gestation (weeks)	29.0 (1.9)	28.8 (2.1)	0.6
Boys (n(%))	17 (52)	11 (42)	0.6
Singleton (N(%))	21 (68)	17 (77)	0.6
Days in trial	42 (16)	41 (20)	0.7
Days of iv feeding	12.5 (8.8)	13.2 (9.2)	0.8
Days to reach enteral full feeds	14 (6)	15 (6)	0.6
Total aluminum exposure from PN (μg/kg)	39.1 (35.6)	280 0 (212.8)	<0.001
Daily aluminum exposure from PN (μg/kg/day)	3.0 (0.83)	21.3 (7.2)	<0.001
Received human milk (n(%))	27 (82)	16 (62)	0.14
% of enteral intake as human milk median (25th/75th centile)	58 (24,99)	55 (23,99)	0.7
Days of ventilation median (25th/75th centile)	5 (3,8)	5 (3,7)	0.9
Days in >30% O2 median (25th/75th centile)	6 (5,27)	8 (4,41)	0.4

Table 4. Anthropometry and bone densitometry data at follow-up according to original randomised group

		Low-Al	Standard	p
Age at follow-up (yrs)		15.29 (0.76)	15.15 (0.76)	0.5
Pubertal stage (n (%))				
	3	10 (33)	3 (12)	0.2
(breast/genital	4	9 (30)	10 (39)	
development)	5	10 (33)	12 (46)	
	missing	1 (3)	1 (4)	
Reached menarche (n(%))		16 (100)	14 (93)	
Weight (kg)		63.18 (15.84)	57.38 (14.02)	0.15
Weight SDS		0.57 (1.29)	0.15 (1.14)	0.2
Height (cm)		163.6 (8.3)	162.2 (7.4)	0.5
Height SDS		-0.40 (1.03)	-0.42 (0.70)	0.9
Head circumference (cm)		55.2 (5.2)	55.3 (1.8)	0.9
HC SDS		-0.48 (3.7)	-0.22 (1.03)	0.7
BMI (kg/m2)		25.6 (13.2)	21.8 (4.2)	0.2
BMI SDS		1.07 (1.50)	0.50 (1.17)	0.1
MUAC (cm)		26.9 (6.0)	26.6 (4.3)	0.8

Waist circumference (cm)	75.0 (12.9)	73.9 (11.1)	0.7
Bone densitometry data			
Whole body BMC less head (g)	1909 (355)	1739 (339)	0.07
Whole body BA less head (cm2)	1870 (225)	1769 (215)	0.09
Whole body BMD less head (g/cm2)	1.014 (0.079)	0.976 (0.083)	0.08
Whole body BMD Z score	0.26 (0.84)	-0.19 (0.94)	0.054
Hip BMC (g)	32.4 (5.8)	29.7 (5.7)	0.08
Hip BA (cm2)	31.1 (3.3)	29.8 (3.2)	0.1
Hip BMD (g/cm2)	1.04 (0.094)	0.992 (0.130)	0.15
Lumbar spine BMC (g)	44.9 (8.8)	39.8 (6.5)	0.02
Lumbar spine BA (cm2)	40.5 (5.4)	37.8 (3.7)	0.03
Lumbar spine BMD (g/cm2)	1.102 (0.119)	1.053 (0.149)	0.2
Lumbar spine BMD Z score	-0.23 (1.20)	-0.63 (1.28)	0.2
Lumbar spine BMAD Z score	0.046 (1.0)	-0.081 (1.22)	0.7
Fat mass (kg)	18.5 (10.5)	15.5 (10.1)	0.3
Lean mass (kg)	41.8 (9.2)	39.2 (6.9)	0.2
Lean/height**3	9.45 (1.27)	9.14 (0.98)	0.32
BMC-head/lean**0.7	0.20 (0.02)	0.19 (0.03)	0.1

Figure 1. Study plan

Infants	*Randomised groups*	
	Standard	**Low aluminum**
Total enrolled	112	115
Died in neonatal period	14	13
Lost to follow-up by 18 mo	8	7
Seen at 18 mo	90	92
Eligible for 15 yr follow-up	**85**	**92**
Seen for follow-up	**26**	**33**
Declined or failed to attend	12	10
No reply to invitation	24	24
Untraceable	23	25
Not eligible for 15 yr follow-up	**13**	**10**
Previous Bayley score <85 or neurocognitive impairment, or no Bayley performed	10	8
Received no TPN	3	2

Figure 2. Calculated aluminum exposure from parenteral nutrition during the neonatal period according to randomized group. Each symbol represents a single subject.