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Results of extremely low birth weight infants randomized to receive extra enteral calcium supply

William F. Carroll, MD^{1,2}, Jorge Fabres, MD, MSPH^{1,3}, Tim R. Nagy, PhD⁴, Marcela Frazier, OD⁵, Claire Roane, RN, MSN¹, Frank Pohlandt, MD, MS⁶, Waldemar A. Carlo, MD¹, and Ulrich H. Thome, MD^{1,7,*}

¹ Division of Neonatology, Department of Pediatrics, University of Alabama at Birmingham, Birmingham AL 35233-7335, USA

² Miami Children's Hospital, Miami, Florida, USA

³ Departamento de Pediatria, P. Universidad Catolica de Chile, Santiago, Chile

⁴ Department of Nutrition Sciences, University of Alabama at Birmingham, Birmingham AL 35233-7335, USA

⁵ School of Optometry, University of Alabama at Birmingham, Birmingham AL 35233-7335, USA

⁶ Section of Neonatology and Pediatric Critical Care, University Children's Hospital, 89070 Ulm, Germany

⁷ Division of Neonatology, University Center for Women and Children, Liebigstrasse 20a, 04103 Leipzig, Germany

Abstract

Background—Bone mineral deficiency continues to occur in extremely low birth weight (ELBW) infants despite formulas enriched in Ca and P.

Objective—This study tested whether extra enteral Ca supplementation increases bone mineral content (BMC) and prevents dolichocephalic head flattening and myopia in ELBW infants.

Study design—Infants 401–1000 birth weight receiving enteral feeds were randomized to receive feeds supplemented with Ca-gluconate powder, or pure standard feeds. Main outcome measures were the excretion of Ca and P by weekly spot urine measurements, the degree of dolichocephalic deformation (fronto-occipital to biparietal diameter ratio, FOD/BPD) at 36 weeks postmenstrual age and the BMC (by dual-energy x-ray absorptiometry) at discharge. Cycloplegic refraction was measured at 18–22 months corrected age.

Results—Ninety-nine ELBW infants with a gestational age of 26 weeks (23–31) [Median (minimum-maximum)] were randomized at a postnatal age of 12 days (5–23) weighing 790g (440–1700). Urinary Ca excretion increased, P excretion decreased in the Ca supplemented group. Total body BMC was 89.9 ± 2.4 g (mean $\pm$ SE) in the supplemented group and 85.2 ± 2.6 g in the control group ($p = 0.19$). The FOD/BPD was 1.50 (1.13–1.69, mean $\pm$ SD) and 1.47 (1.18–1.64) in the supplemented and control groups, and the refraction 0.98 ± 1.23 and 1.40 ± 1.33 dpt. ($p = 0.68$), respectively in 64 ELBW infants (79% of survivors) at 2-year-follow-up.

Conclusions—Extra enteral Ca supplementation did not change BMC, head shape or refraction. The decreased P excretion may reflect P deficiency in infants receiving extra Ca, preventing improved bone mineral accretion.

*Corresponding author Tel. +49-341-97-26831, Fax +49-341-97-23579, uhthome@web.de.

Keywords

bone mineral content; bone mineral density; preterm infant; bioavailability; phosphorus

Introduction

Many ELBW infants develop bone mineral deficiency, manifest as 'rickets' or osteopenia of prematurity.^{1–6} With adequate nutrition, ELBW infants can achieve growth and mineral accretion rates up to those achieved *in utero*.^{7–10}

Both Ca and P must be simultaneously available in sufficient amounts in order to form bone mineral.⁹ Postnatal bone demineralization develops when the supply of Ca or P or both does not match the demand of the rapidly growing skeleton.^{11–14} Redistribution of existing bone mineral leads to a diffusely mineral deficient skeleton,¹⁵ which is prone to fractures. Furthermore, bone mineral deficiency has been linked to skull deformities.¹⁶ Presumably, forces of gravity act on the demineralized skull bones of an infant's head, which tends to be positioned sideways most of the time. Thereby, the head develops a dolichocephalic deformity, which may include deformation of the orbits and hence the eyeballs, possibly leading to myopia of prematurity.^{17,18}

Ca absorption depends on gestational and postnatal age and varies with the type of feedings used.^{19,20} Older studies reported bioavailabilities as low as 15–35%^{7,19} in formula fed infants while P was found to be absorbed by more than 50%. Therefore, an enteral supply of Ca and P in the molar ratio of Ca/P of 1.4 to 2.0²¹ may still result in relative or absolute Ca deficiency.^{9,10,22} This is in line with a decade of experience using urinalysis guided individualized Ca and P supplementation – a P supplement was rarely needed in ELBW infants fed fortifiers or formula.

This randomized trial was conducted to test the hypothesis that extra enteral Ca supplementation alone would increase bone mineral content, decrease dolichocephalic deformation and increase the proportion of spot urine samples showing simultaneous excretion of Ca and P, and, as primary hypothesis, reduce the incidence of myopia of prematurity.¹⁷

Methods

Eligible patients and randomization

The institutional review board of the University of Alabama at Birmingham approved the study, and written informed consent was obtained in all cases. ELBW infants with a birth weight of 401–1000 g and postnatal age less than 14 days were eligible, unless they had major congenital malformations, chromosomal aberrations, bowel perforation or necrotizing enterocolitis stage 2A or greater diagnosed before randomization. The infants were randomized to receive standard feedings (fortified human milk or formula) or standard feedings supplemented with Ca gluconate. Randomization was stratified for infants with 401–750 g and 751–1000 g birth weight according to balanced block schemes with variable block sizes (2–6) using sealed opaque envelopes.

Infant nutrition and care

Total parenteral nutrition was started within 48 hours of birth and generally contained 1–2 mmol/kg/d Ca and 1 mmol/kg/d P. Enteral feeds were generally started on day of life 1–3. Feedings were advanced to at least 150 ml/kg/d unless there were contraindications. Supine and prone positioning were not governed by a specific policy and were generally alternated

as soon as umbilical lines had been removed. There was no nursing protocol to influence head shape.

Study intervention

Human milk was preferred when available and was fortified with Enfamil human milk fortifier (1 pack Enfamil per 25 ml breast milk, Mead Johnson, West Palm Beach, FL), once enteral feedings amounted to 100 ml/kg/d or more. Otherwise, a 24 cal/oz preterm formula, Similac Special Care 24[®] (Ross Laboratories, Columbus OH), was used. Theoretically, most Ca (80%) contained in fortified human milk originated from the fortifier, and thus variations of human milk Ca content were low. USP grade Ca-gluconate powder (Sigma Chemical Company Cat # C-8231, St. Louis, MO) was added to the standard feedings of ELBW infants randomized to extra enteral Ca supplementation as soon as they tolerated 100 ml/kg/d feeds. The Ca dose was estimated to meet fetal Ca accretion when enteral Ca bioavailability was 50% (even lower values were reported^{7,19}) and growth approximated fetal weight gain.^{7,23} ELBW infants with a current body weight below 1000 g received 2.6 mmol (1.12 g) Ca-gluconate (equivalent to 104 mg Ca) added to 100 ml of feeds and infants above 1000 g body weight received 1.3 mmol (0.56 g). Total Ca and P contents in the feeds are given in Table 1. The Ca dose was higher than in a previous randomized trial.²⁴ See online-only appendix for further Ca dosing information (. Stocks of Ca gluconate powder were tested monthly for bacterial growth on blood agar plates, and were always negative. Extra enteral Ca supplementation was continued until discharge, but interrupted when feeding intolerance (intake less than 100 ml/kg/d) occurred or the infant developed acute abdominal disease symptoms.

Outcome assessment

Fronto-occipital diameter (FOD) and biparietal diameter (BPD) (above the ears on the infant's skull) were measured with a pelvimeter attached above the ears on the infant's skull at randomization and at 36 weeks postmenstrual age.¹⁶ Dolichocephalic deformation was assessed by the FOD/BDP ratio (FOD/BPD). Spot urine (2 ml) samples were collected weekly for analysis of Ca and P content. The samples were stored frozen and analyzed at the Department of Clinical Chemistry, University of Ulm, Germany.⁹ As additional blood sampling was not part of the protocol, serum Ca and P concentrations as well as alkaline phosphatase were obtained when ordered by the clinical team, measured at UAB's Clinical Chemistry department using the facility's standard procedures and quality controls. Whole body and femur bone mineral content was measured by a randomization-masked investigator at the time of discharge using dual-energy X-ray absorptiometry (DXA, GE-Lunar-Prodigy, Madison, WI). Scanning was performed without sedation and repeated in case of movement artifacts. All subjects were scanned and data analyzed using the small animal module of the enCORE™ 2002 software (version 6.10.029). Both whole body and femur BMC were determined using the customized region of interest tool. All scans and analyses were conducted by the same technician. SDS scores were calculated according to Cole et al.²⁵.

Refractive error was measured at 18–22 months corrected age by the same investigator who was masked to the patients' group assignment. Refractive error was determined by cycloplegic retinoscopy performed 30 minutes after instillation of 1% cyclopentolate hydrochloride in each eye. A speculum was not used, but in some cases, it was necessary to hold the lids lightly to allow better viewing without deforming the globe. Retinoscopy bars and a manual streak retinoscope were used to measure the refractive error in the principal meridians. The spherical power was measured as the power in the least minus meridian and the cylinder power was measured as the power in the most minus meridian. The spherical refraction equivalent reported in this work is derived by adding one half of the difference

between these values to the sphere component, thus yielding the mean value of the lowest and highest meridian. The dioptric equivalent of the investigator's working distance (1.5 D, 66cm) was subtracted from all the measurements. Eyes with a spherical equivalent ≤ -1 were counted as myopic, those with a spherical equivalent ≥ 1 hyperopic. Cylindrical refraction angles of 90 ± 45 degrees were considered as astigmatism against the rule, all others as astigmatism with the rule.

Statistical data analysis

Refraction measured at follow-up was the primary outcome. Demonstrating a 1 dpt refraction difference with $SD = 1.44$,^{26–28} $\alpha = 0.05$ and power = 0.8 required 68 patients, and 28 additional patients were planned to compensate for deaths or losses of follow up. Further predefined outcome criteria of the in-hospital phase reported here were BMC, FOD/BPD, the percentage of urine samples containing at least 1 mmol/l Ca and 1 mmol/l P, feeding tolerance (defined as the cumulative amount of enteral feeds from achieving 100 ml/kg until 36 weeks PMA), survival and the incidence of NEC. Student's t, Mann-Whitney U and Fisher's exact tests were used as appropriate. DXA results were evaluated by an analysis of covariance (ANCOVA) with correction for the patients' weight at measurement. SAS software version 9.2 was used (SAS Institute, Cary, NC).

Results

Enrollment took place from Feb 2002 until June 2004 and was hampered by many parents living far away from the neonatal unit and visiting infrequently. The trial profile is shown in Fig. 1. The study groups were similar in their base-line characteristics (Table 2).

Total body and femoral bone mineral content were measured at a median postmenstrual age of 39 1/7 weeks (36 3/7–45 2/7) and were similar in both study groups at discharge (Table 3). Weight gained, head circumference and skull shape as measured by FOD/BPD were also similar in both study groups at 36 weeks postmenstrual age (Table 4). Furthermore, FOD/BPD did not change substantially from randomization till 36 weeks postmenstrual age (Tables 1 and 3). SDS scores were also not different. Survival, incidence of necrotizing enterocolitis and incidence of retinopathy of prematurity were similar in both study groups. The number of days per patient of furosemide treatment was not different between the randomized study groups (Table 4). Clinically evident fractures were not observed.

Refraction measurements at 18–22 months corrected age were performed in 64 patients (79% of 81 infants surviving to 36 weeks postmenstrual age). The most common reasons for missing the refraction exam was decline by the parents. Spherical and cylindrical refraction errors occurred with similar incidence and magnitude in both study groups (Table 5). Furthermore, there was no association between FOD/BPD and myopia detectable (not shown).

Approximately 88% of urine samples of the control group contained P, whereas Ca was present in 67%. Therefore most infants in the control group had a sufficient P supply for their actual bone mineralization processes, and Ca was the critical component. Less than 50% of samples contained both Ca and P simultaneously (Fig. 2). In the Ca-supplemented group, more than 90% of urine samples contained Ca, but less than 50% of samples contained P and 20% contained both Ca and P simultaneously. The proportions of urine samples containing Ca, P, or both were significantly different between the study groups ($p < 0.02$).

Urinary Ca concentrations were higher in supplemented infants compared to controls (Table 6), and weakly correlated to serum Ca concentrations ($r=0.41$, $p<0.0001$). Urinary P

concentrations were significantly lower in supplemented infants compared to controls. Serum Ca concentrations were measured in 201 of 1128 total patient weeks and were higher in infants randomized to supplementation compared to control infants (Table 6). The highest values were observed during weeks when feeds were tolerated and thus supplements were actually given. Serum P concentrations were measured in 308 of 1128 total patient weeks and were unrelated to extra enteral Ca supplementation. Weeks of measurements were evenly distributed throughout the hospital stay. Serum Ca concentrations were inversely correlated to serum P concentrations ($r = 0.2$, $p < 0.05$). Furthermore, although P supply followed unit standards and feeds were supplemented with P by the manufacturers, serum P concentrations were largely below the threshold for tubular reabsorption and thus suboptimal²⁹.

Feeding tolerance was also similar in both groups (Table 7). Feeds were started, 100 ml/kg of feeds were achieved, and parenteral nutrition was discontinued, at similar ages in both groups. No difference in the type of feeds or the proportion of breast milk was found between groups. Extra enteral Ca supplementation did not interfere with feeding tolerance as there was a similar cumulative feeding amount in both groups, and no Ca soap bezoars occurred. However, feeding tolerance interfered with extra enteral Ca supplementation, because Ca was to be given only when feeding tolerance was good. The infants randomized to extra Ca achieved feeds > 100 ml/kg on 2336 of 3719 (63%) patient days, in the control group on 1994 of 3390 (59%) patient days. As a result, infants in the Ca group actually received the Ca supplement on a total of 1924 (52% of total) patient days; therefore, the major reason for not giving Ca was feeds less than 100 ml/kg/d, in accordance with the protocol.

Discussion

In this randomized trial, extra enteral Ca supplementation did not increase bone mineral content or affect the head shape. Given these results, the absence of differences in eye refraction errors between study groups is consistent with our hypothesis.¹⁷ The urinalyses, however, were distinctly different, highlighting the peculiar balance between Ca and P supply. Control infants excreted Ca in 67% but P in 88% of their urine samples, indicating that Ca deficiency was more abundant than P deficiency, and P supply was sufficient in the vast majority of infants. In the group receiving extra Ca, however, urinary P levels were decreased and many infants ceased to excrete P, indicating that P became the limiting nutrient in the infants supplemented with extra Ca despite a high P content in the feeds.

Since a significant increase in BMC was not detected in Ca supplemented infants, the apparent P deficiency may not be explained by a higher bone mineral formation in supplemented infants consuming all supplied P. Furthermore, weight gain was not affected by the supplement. An alternative sequence may have led to increased fecal losses of P. In this particular study, the P was provided mostly as inorganic phosphate,^{30,31} whose bioavailability may be impaired by precipitation as calcium phosphate when Ca salts are added.³² Phosphorus has a poor bioavailability out of Ca triphosphate³³. We speculate that P absorption may depend on the chemical nature of the actual P supplement, and that organically bound P has a higher bioavailability than inorganic P at high Ca supply.⁸ European formulas usually contain organically bound P (such as glycerophosphate) which may explain why such a reduced bioavailability of P has not been observed in previous studies of Ca supplementation, which were conducted in Europe.^{9,10,22} Interestingly, the natural P in breast milk is also organically bound to casein micelles and would thus be optimized for intestinal absorption in the presence of Ca.³⁴ Added inorganic phosphate, however, would not benefit from that mechanism.

Ca absorption of preterm infants is highly variable and may be less than 50%.^{7,19,20} This variability may expose infants to Ca deficiency and thus prevent optimal bone mineralization even when following current recommendations or using current commercial infant formulas. It was expected that absolute Ca absorption would increase, since a linear relationship between Ca supply and absorption has been demonstrated.³⁵ To meet the Ca demand for intrauterine growth and Ca accretion rates even in patients with low Ca absorption, the Ca dose in the treated group was 2–2.5fold higher than in the most recent European recommendations,³⁶ thus accepting an oversupply in patients with slower growth or better absorption. In the control group, the Ca dose was 1.5–1.75fold higher than in these recommendations.³⁶ Calculations using skeletal growth, necessary P retention, published P bioavailability and the high P content of commercial formulas and fortifiers indicated that additional P supplementation would not be necessary. The P dose was already 1.3–2fold higher than in the European recommendations.³⁶ This was corroborated by extensive clinical experience from a decade of urinalysis-guided feed supplementation that an extra P supplement is rarely necessary when formula or human milk fortifiers are used.^{9,10} Therefore, individualized supplement adjustments were not planned to simplify study conduct and blinding. Urinalyses were performed solely to aid in the interpretation of the results. Further studies are necessary to determine whether an urinalysis guided additional P supplement (organic or inorganic) might improve bone mineral accretion, or if an organically bound P supplement (individualized or flat) is necessary to ensure sufficient bioavailability of P in the presence of generous Ca supplementation.^{8,33}

Newborn infants have similar FOD/BPD across all gestational ages,^{16,37} but increasing values, indicating dolichocephalic deformation, were described for older preterm infants.^{16,37,38} Two factors have been considered to affect head shape: bone mineral deficiency¹⁶ and positioning.³⁹ Apparently, extremely mineral deficient bone is too soft to counter the forces of gravity working on the conventionally sideways positioned heads. From preliminary data, some authors have suggested a protection against myopia of prematurity when bone mineral deficiency and dolichocephalic deformation can be avoided by supplementing with Ca and P.^{17,18} In this study, infants were slightly dolichocephalic at study entry in both groups, as the FOD/BPD values were higher (1.44 ± 0.11 and 1.43 ± 0.09 , Table 2) than the previously reported 1.27 ± 0.073 SD.¹⁶ The FOD/BPD ratio did not change during the study in either group, which may be interpreted as sufficient bone mineralization to prevent further dolichocephalic deformation in most infants. Alternatively, staff in the neonatal unit might have used alternative head positioning, as this was not controlled.

We anticipated measuring the refractions of 68 infants, but only 64 were actually measured because of several parents declining the refraction examination during their follow-up visit. However, the data shows no trend towards a refraction difference between the study groups, which makes it highly unlikely that a significant difference might have been detected with 4 more measurements.

The incidence of myopia in preterm born infants varies among the publications.^{40–46} In this study, the incidence of myopia was rather low in comparison to 30–50 years ago when many very low birth weight infants developed myopia and dolichocephalic modification of their head shape.^{1–6} Most infants in this study were normopic or slightly hyperopic at follow-up, which is normal for this age group. Astigmatism was predominantly with the rule, which is also physiologic but different from a preterm population published previously.⁴¹ Furthermore the study groups did not differ in their refraction results. According to our hypothesis, refraction errors are increased in the presence of head deformities. As Ca and P supply appeared to be sufficient in either group to prevent bone demineralization and skull deformities, there were no skull deformities which could cause refraction errors. The FOD/

BPD values of the 4 myopic infants at follow-up were between 1.25 and 1.38, showing an even less dolichocephalic head shape than the mean of the study group, therefore, the myopia in these infants cannot be attributed to the head shape and, since it was so rare, not even to prematurity.

In general, BMC values increased with body weight and therefore with postnatal age^{24,47–53} but varied with the demographics of the studied infants. A similar DXA setup as ours has been successfully used in Gambian newborns,⁵² resulting in the expected BMC values for the Gambian population. In our study, total body BMC results were slightly higher than in a recent report on term infants measured with a different equipment, while femur BMC values were lower,⁵¹ but all within the published range. Furthermore, our values were highly correlated to body weight, as expected. Differences in instrument calibration and image processing may play a part, but do not invalidate our conclusion that there was no difference in BMC between our study groups. Overall, the constant head shapes and the absence of any fractures indicate that bone mineralization was at least sufficient to prevent these side effects, although this finding has to be interpreted with caution because fractures were not systematically sought.

Infants in both treated and control groups received at least 1.5fold more Ca and P than recommended by the European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) committee on Nutrition (3–3.5 mmol Ca and 1.9–2.9 mmol P per kg per day).³⁶ The compositions of modern standard preterm infant formulas and human milk fortifiers appear to exceed these recommendations already. Whether the lower amounts recommended by the ESPGHAN committee also support bone mineralization sufficiently to prevent dolichocephalic head deformation, cannot be determined from our data.

No difference in the incidence of complications between the study groups, most notably renal and gastrointestinal complications, were seen in this study as well as in previous studies of Ca and P supplementation.^{9,54} Intestinal obstruction, Ca soap bezoars, the milk curd syndrome^{55,56} or renal function impairment were not observed. Thus, Ca supplementation appeared to be safe. Furthermore, feeding tolerance was similar in both study groups and thus not influenced by the supplement. Feeding intolerance was frequent in our study group, which is common in the enrolled patients,^{9,10} and cannot be excluded experimentally in a clinical trial. It is very common to withdraw feeds quickly for any abdominal symptoms in order to prevent NEC. Feeding intolerance affected patients equally, independent of the randomization.

In this randomized trial of extra enteral Ca supplementation in ELBW infants, weight gain was within the reported range for preterm infants^{57,58}. Furthermore, sufficient bone mineralization was achieved with both standard feeds and Ca supplemented feeds to prevent severe dolichocephalic deformation. Therefore, our hypothesis that dolichocephalic deformation is associated with myopia and against the rule astigmatism cannot be proven or ruled out. Furthermore, the incidences of myopia and against the rule astigmatisms were reassuringly low and not different from the normal population.³⁶ In the infants receiving extra Ca supplements, urinary excretion of P was reduced, indicating that bioavailability of P had deteriorated and P had become the limiting mineral of bone mineral formation. We suggest that future recommendations regarding the composition of formulas for preterm infants may account for the chemical nature of P compounds and their bioavailability in the presence of Ca. If a calcium phosphate precipitation was indeed the reason for the decreased P supply in Ca supplemented infants, formulas containing inorganic P as used in this study cannot be further improved by adding extra Ca.

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Abbreviations

Ca	calcium
BMC	bone mineral content
BPD	biparietal diameter
DXA	dual energy x-ray absorptiometry
FOD	fronto-occipital diameter
NEC	necrotizing enterocolitis
P	phosphorus
PCL	posterior chamber length
ELBW	extremely low birth weight
VLBW	very low birth weight

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Appendix

Amount of extra Ca supplementation

As optimal growth of preterm infants matches fetal growth⁷, optimal Ca and P accretion were predicted using the largest available study of fetal weight gain, including 563480 singletons²³, and fetal whole body analyses^{59–62} indicating a Ca content increasing by 0.203 mmol/g and a P content increasing by 0.152 mmol/g fetal body weight. Fetuses under 1000g gained 20–24 g/kg/day²³, which translated into a Ca retention of 4.1–4.8 mmol/kg/d, and a P retention of 3.1–3.6 mmol/kg/d. Fetuses and infants above 1000 g gained 16–19 g/kg/d²³, which translated into a Ca retention of 3.2–3.8 mmol/kg/d, and a P retention of 2.4–2.8 mmol/kg/d under optimal conditions. Any Ca and P supply short of these amounts may lead to mineral deficient bone.

Standard feeding mixtures at the University of Alabama at Birmingham were **Breast milk** with Enfamil human milk fortifier 1pk/25 ml, containing approximately 3.5 mmol Ca and 2.5 mmol P (partly inorganic) per 100 ml, and **Similac Special Care 24**, containing 3.6 mmol Ca and 2.6 mmol P (as potassium phosphate) per 100ml. Therefore, fortified human milk contained almost the same amounts of Ca and P as Similac Special Care 24, thus eliminating the need for separate Ca supplementation schemes.

Below 1000g actual weight

Considering a daily accretion of 4.5 mmol/kg Ca and 3.2 mmol/kg P and bioavailabilities for Ca and P of 50% and 80%, respectively^{7,20}, enteral daily intakes of 9 mmol/kg Ca and 4 mmol/kg P were desired. To achieve this with 150 ml/kg enteral feedings, the feeding mixture had to contain 6 mmol Ca and 2.6 mmol P per 100 ml. Therefore, feedings for supplemented infants below 1000 g actual weight were supplemented with additional 2.6 mmol Ca per 100 ml. The P demand was already met by the standard feeding mixtures. The molar ratio of Ca/P was 2.3.

Above 1000g actual weight

Considering a daily accretion of 3.6 mmol/kg Ca and 2.7 mmol/kg P and bioavailabilities for Ca and P of 50% and 80%, respectively^{7,20}, enteral daily intakes of 7.2 mmol/kg Ca and 3.4 mmol/kg P were desired. To achieve this with 150 ml/kg enteral feedings, the feeding mixture had to contain 4.8 mmol Ca and 2.3 mmol P per 100 ml. Therefore, feedings for supplemented infants above 1000 g actual weight were supplemented with additional 1.3

mmol Ca per 100 ml. The P demand was met by the standard feeding mixtures. The molar ratio of Ca/P was 2.1.

Fig. 1.

The trial profile. Dual energy x-ray absorptiometry (DXA) scans were unreadable in 3 patients because of movement artifacts. DXA scanning was not possible in 11 patients because they were discharged from another hospital and did not return. These infants did not differ from the rest in any demographic characteristic. NEC = necrotizing enterocolitis.

Fig. 2. Ca and P in spot urine samples. Median values of each patient's percentage of urine samples containing more than 1 mmol/l Ca, P or both are shown. Error bars represent 25th–75th percentile. Asterisks indicate values significantly different by a Mann-Whitney U test ($p < 0.02$).

Table 1

Ca and P content in mmol and mg per 100 ml feeds

	Ca supplemented				Control			
	FHM		SSC24		FHM		SSC24	
	mmol	mg	mmol	mg	mmol	mg	mmol	mg
Ca content, birth weight <1000g	6.1	244	6.2	248	3.5	140	3.6	144
Ca content, birth weight ≥1000g	4.8	192	4.9	196	3.5	140	3.6	144
P content	2.5	78	2.6	81	2.5	78	2.6	81

FHM = Fortified human milk (1 pack Enfamil human milk fortifier per 25 ml breast milk)

SSC24 = Similac Special Care 24

Table 2

Demographic data

	Extra Ca (n=46)	Control (n=47)	P
Gestational age (weeks)	26.0 ± 1.6	26.3 ± 1.7	0.43
Birth weight (g)	762 ± 123	765 ± 139	0.86
Male *	20 (43)	26 (55)	0.30
Race (Black/White/Other) *	28/16/2	24/21/2	0.52
SGA ‡	15 (33)	14 (30)	0.83
Age at randomization †	12 (5–18)	13 (6–23)	0.30
Weight at randomization (g)	772 ± 132	810 ± 206	0.36
Head circumference at randomization (cm)	24.0 ± 2.2	23.9 ± 2.4	0.89
FOD/BPD at randomization	1.44 ± 0.11	1.43 ± 0.09	0.95

* Number (%), Fisher's Exact test

† Median (Min-Max), Mann Whiney U test;

All others: Mean ± SD

‡ SGA defined as birth weight < 10th percentile

Table 3

DXA data at discharge

	Extra Ca (n = 35)	Control (n = 32)	P
Weight (g) *	2623 ± 675	2781 ± 902	0.61
SDS for weight	-1.7 ± 1.1	-1.4 ± 1.2	0.27
Whole body BMC (g ± SE) †	89.9 ± 2.4	85.2 ± 2.6	0.19
Femur BMC (g ± SE) †	1.96 ± 0.08	1.98 ± 0.09	0.92

* Mean ± SD,

† Mean corrected for weight ± SE, Analysis of covariance

Table 4

Outcome data at 36 weeks postmenstrual age

	Extra Ca (n=46)	Control (n=47)	P
Alive	41 (89)	40 (85)	0.76
Weight *	1884 ± 318	1988 ± 404	0.27
SDS for weight	-2.0 ± 1.0	-1.8 ± 0.97	0.29
Head circumference (cm) *	31.3 ± 1.4	30.9 ± 1.7	0.39
FOD/BPD ratio *	1.48 ± 0.10	1.47 ± 0.10	0.52
Bronchopulmonary dysplasia ‡	9 (22)	12 (30)	0.56
Necrotizing enterocolitis	3 (7)	2 (4)	0.68
Retinopathy of prematurity (any)	13 (28)	14 (30)	0.82
Retinopathy of prematurity (laser treatment)	1	1	1.0
Days on furosemide †	4 (0–60)	5 (0–48)	0.38

* Mean ± SD,

† Median (Min-Max), Mann Whiney U test;

All others: Number (%), Fisher's exact test

‡ Defined as requirement of oxygen or mechanical ventilation at 36 weeks postmenstrual age

Table 5

Refraction measurements at 18–22 months corrected age

	Extra Ca (n = 33)	Control (n = 31)	P
Spherical refraction*	0.90 ± 1.16	1.29 ± 1.34	0.33
Number with myopia (refr. ≤ -1 dpt.) in at least one eye [†]	2 (6)	2 (6)	1.0
Number with hyperopia (refr. ≥ 1 dpt.) in at least one eye [†]	17 (52)	20 (65)	0.21
Cylindrical refraction*	-0.23 ± 0.29	-0.23 ± 0.36	0.74
Number of infants without astigmatism [†]	16 (48)	16 (52)	0.94
Number of infants with astigmatism (cyl. < 0) [†]	17 (52)	15 (48)	0.94
with the rule astigmatism	13 (39)	11 (35)	
against the rule astigmatism	4 (12)	4 (13)	

* Mean ± SD of individual patient means, t-test

[†] Number (percent), Fisher's exact test

Table 6

Ca and P measurements

	Extra Ca (all Ca measurements after randomization)	Extra Ca (as measured while Ca was actually given) [†]	Control (all measurements after randomization)
Ca in serum (mmol/l)	2.57 ± 0.44 (109) *	2.83 ± 0.48 (44) *	2.35 ± 0.23 (76)
P in serum (mmol/l)	1.30 ± 0.32 (156)	1.25 ± 0.32 (20)	1.34 ± 0.34 (133)
Ca in urine (mmol/l)	3.40 * (1.10–7.55) (325)	3.72 * (1.22–8.64) (253)	1.42 (0.71–2.96) (310)
P in urine (mmol/l)	0.75 * (0.23–3.35) (325)	0.43 * (0.20–2.64) (253)	4.46 (1.71–7.83) (309)

Mean ± SD of weekly measurements is shown for serum values, and differences were tested with Student's t-test. For urinalysis values, median and 25th–75th percentile are given because of skewed distributions, and the Mann Whitney U test was used.

Numbers in parentheses show numbers of patient weeks with contributing measurements.

* Significantly different from control group values ($p < 0.005$)

[†] Ca was considered “given” when it was actually given on at least 4 of the preceding 7 days (per protocol analysis). Ca supplementation was only given when at least 100 ml/kg/d feeds were tolerated.

Conversion to mg/dl is possible using the following factors: Ca 4.0, P 3.1.

Table 7

Feeding summary

	Extra Ca (n=46)	Control (n=47)	P
Age at first TPN given	3 (2–4)	2 (1–4)	0.42
Age at first feeds given	3 (1–16)	4 (1–13)	0.41
Days on TPN	23 (3–113)	16 (5–105)	0.54
Age 100 ml/kg feeds achieved	18 (6–60)	17 (8–64)	0.42
Cumulative feeds (ml) since achieving 100 ml/kg	15700 (797–37444)	13606 (1475–33806)	0.14
Age Ca started	23 (11–71)	-	-
Days on Study Ca	49 (3–88)	-	-

Median (Min-Max), Mann Whitney U test

TPN = total parenteral nutrition