**ORIGINAL ARTICLE**

Vitamin C supplementation in very preterm infants: a randomised controlled trial

B A Darlow, H Buss, F McGill, L Fletcher, P Graham, C C Winterbourn


**Objective:** To determine whether regulating vitamin C (ascorbic acid: AA) intake to achieve higher or lower plasma concentrations was associated with improved clinical outcome.

**Design:** A double blind, randomised controlled trial.

**Setting:** Neonatal intensive care unit at Christchurch Women’s Hospital.

**Patients:** Infants with birth weight <1500 g or gestation <32 weeks, admitted to the unit within 48 hours of birth.

**Intervention:** Infants were randomised to one of three protocols with regard to AA supplementation for the first 28 days of life: group LL received low supplementation throughout; group LH received low until day 10 and then high; group HH received high throughout.

**Main outcome measures:** Primary outcome measures were oxygen requirement at 28 days and 36 weeks postmenstrual age, total days supplemental oxygen, and retinopathy of prematurity. AA concentrations were measured at study entry (day 2), and days 10, 21, and 28.

**Results:** A total of 119 infants were enrolled over 24 months (mean gestation 28.4 weeks; birth weight 1161 g). Six infants died, and these had significantly higher AA concentrations before randomisation than surviving infants (116 μmol/l [95% confidence interval 90 to 142] v 51 μmol/l [45 to 58], p = <0.0001). There were no significant differences in primary outcomes between the groups. However, the proportion of surviving infants with an oxygen requirement at 36 weeks postmenstrual age in group HH (19%) was half that in group LL (41%) (p = 0.06).

**Conclusions:** In a randomised controlled trial, no significant benefits or harmful effects were associated with treatment allocation to higher or lower AA supplementation throughout the first 28 days of life.

**METHODS**

Infants with birth weight <1500 g or gestation <32 weeks admitted to the neonatal intensive care unit at Christchurch Women’s Hospital were eligible for study. Signed, informed parental consent was obtained within 72 hours of birth. The study was approved by the Canterbury Ethics Committee.

This was a randomised, double blinded, placebo controlled trial. Infants were randomised by pharmacy through sealed envelopes to one of three protocols for AA supplementation for the first 28 days of life: group LL received low AA supplements throughout; group LH received low AA supplements until day 10, then high supplements until day 28; group HH received high AA supplements throughout.

**Abbreviations:** AA, ascorbic acid; CRIB, clinical risk index for babies; ROP, retinopathy of prematurity

**Vitamin C** (ascorbic acid: AA) is an important aqueous phase antioxidant in cells and plasma. However, at least in vitro, AA also has pro-oxidant activity, principally by reducing ferric iron to the ferrous form, which converts hydrogen peroxide into the more toxic hydroxyl radical (Fenton reaction). AA has a number of important metabolic functions and is actively transported across the placenta. AA concentrations in cord plasma are higher than the mother’s and, in term infants, plasma concentrations fall considerably over the first 24 hours of life. Preterm infants generally have higher cord AA concentrations than term infants, and concentrations then decline over a few days.

Most preterm infants receive AA as part of a multivitamin supplement, but there are few data on which to base optimum concentrations. One recommendation derives from the concentrations found in healthy breast fed term infants, with an adequate AA concentration stated as >34 μmol/l. Breast milk contains 3.5–5.5 mg AA per 100 ml so that an average infant having 150 ml/kg/day of milk will receive 5.2–8 mg/kg/day. The alternative view is that preterm infants should receive higher doses of AA, 25–31 mg/kg/day, to achieve concentrations closer to those in utero in the third trimester.

There have been few studies of the relation between AA concentration and morbidity in very preterm infants. Silvers et al reported that plasma AA concentrations within 2 hours of birth were significantly higher in infants who died compared with survivors. These researchers also observed that higher AA concentrations on day 2 were associated with a greater risk of developing bronchopulmonary dysplasia. In contrast, Moison et al reported lower plasma AA concentrations on day 10 in preterm infants who developed bronchopulmonary dysplasia compared with those who did not. In a pilot observational study of very low birthweight infants (birth weight <1500 g), we found an increased risk of retinopathy of prematurity (ROP) with higher plasma AA concentrations at day 7 and an increased risk of bronchopulmonary dysplasia with lower concentrations at 28 days.

In this study, we aimed to determine in a randomised, controlled trial whether regulating AA intake to achieve higher or lower plasma concentrations is associated with improved clinical outcome. We hypothesised that maintaining a lower plasma AA concentration (target 35–50 μmol/l) in the first week of life and a higher concentration (target 90 μmol/l) in weeks 3–4 would be accompanied by least morbidity.
AA supplements

Standard parenteral multivitamin supplementation was 1 ml/kg/day Soluvit N (Kabi Pharmacia AB, Stockholm, Sweden) plus 4 ml/kg/day Vitlipid N (Kabi Pharmacia AB), both added to Intralipid (Kabi Pharmacia AB), which provided 10 mg/kg/day AA. While requiring parenteral nutrition, infants randomised to low AA received this standard multivitamin regimen, and infants randomised to a high regimen received the standard regimen plus an extra 20 mg/kg/day AA. The combination of Soluvit and Vitlipid was chosen because this regimen delivers comparable amounts of all vitamins to that delivered by 2 ml/kg/day MVI-Pediatric (Rhone-Poulenc Rorer, Montreal, Canada), except that the AA content is lower (table 1).

Our policy is to encourage enteral feeding with mother’s own breast milk (assumed to contain 4.0 mg/100 ml AA). Formula fed infants received S26 LBW formula (Wyeth, Auckland, New Zealand), which contains 11 mg/100 ml AA. S26 LBW powder (5 g/100 ml, containing 3.3 mg AA) was used to fortify breast milk when required. Orally fed infants also received Vitadol C (Nutricia, Auckland, New Zealand), which contains 33 mg AA in 10 drops (0.3 ml). Table 2 shows feeding regimens for infants fed 150 ml/kg/day. Infants randomised to high AA (group HH and group LH from day 11 onwards) also received an additional 20 mg/kg/day AA as a clear oral solution (10 mg/ml). Infants randomised to low AA (group LL and group LH to day 10) received sterile water as placebo. Both AA solution and placebo were dispensed as 2 ml/kg from a separate named bottle for each infant, marked “vitamin C or placebo”.

Measurement of AA and protein carbonyls

Plasma AA concentrations were measured at study entry (usually day 2) and at day 10 (range 8–12), 21, and 28, on heparinised blood (0.4 ml) collected at the time of routine sampling. Samples were stored at −80°C for no more than 30 minutes before the plasma was separated and frozen at −80°C. AA, in the reduced form, was measured by high performance liquid chromatography (HPLC) using a C18 column with electrochemical detection. The detection limit was 1 μg/l, and the intra-assay coefficient of variation for plasma containing 60 μM AA was 3.9%. Protein carbonyls were measured on selected day 28 samples by an enzyme linked immunosorbet assay (ELISA) method that involves derivatisation with 2,4-dinitrophenylhydrazine, using a commercial kit (Zenith Technology, Dunedin, New Zealand).

Outcome measures

Clinicians and laboratory staff remained blinded to treatment allocation throughout the trial. Perinatal and neonatal data, including the clinical risk index for babies (CRIB) score, were collected as part of an ongoing clinical audit. Primary outcomes were oxygen requirement at 28 days and 36 weeks postmenstrual age, total days supplemental oxygen, and ROP in infants eligible for screening. Supplemental oxygen was generally administered to infants unable to maintain a saturation of 95% or more in room air. ROP was assessed by an experienced paediatric ophthalmologist and reported using international criteria. Screening for ROP is routinely undertaken in New Zealand for infants of <1250 g birth weight or <31 weeks gestation, and outside these limits at physician discretion.

Power calculations and statistical analysis

Power calculations were conducted, using Monte Carlo simulation based on our preliminary study, for logistic regressions of major outcomes on AA concentrations, with adjustment for gestational age. They suggested that 120 patients would provide at least 80% power to detect a 40% reduction in risk for major outcomes—that is, a relative risk of 0.6—assuming significance testing at the 5% level.

Data were analysed in two ways. Firstly, conventional between group analysis by intention to treat was performed using z tests, Fisher’s exact test, or non-parametric analysis of variance (Kruskal-Wallis) as appropriate. Adjustment for covariate imbalance between the three treatment groups was achieved using a logistic regression model. Secondly, the association between AA concentrations, treated as a continuous variable, and binary outcomes was investigated with logistic regression models. Logistic regression models were used to estimate relative risks of morbidity (ROP, oxygen requirement at 28 days, and at 36 weeks postmenstrual age) comparing risk at optimal AA concentrations at different time points with risk at higher or lower concentrations.

RESULTS

A total of 119 infants, 40 in both LL and LH groups and 39 in the HH group, and 90% of those eligible, were enrolled over 24 months. The mean (SD) gestational age was 28.4

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Comparison of vitamin doses (per kg per day) received by preterm infants given one of two standard multivitamin regimens during parenteral nutrition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C (mg)</td>
</tr>
<tr>
<td>RDA (1/kg/day)</td>
<td>1.5-25</td>
</tr>
<tr>
<td>MVP* (2 ml/kg/day)</td>
<td>32</td>
</tr>
<tr>
<td>SolitVitlip†</td>
<td>10</td>
</tr>
</tbody>
</table>

*Rhone-Poulenc Rorer, Montreal, Canada.
†Soluvit as 1.0 ml/kg/day, Vitlipid as 4 ml/kg/day.
RDA, Recommended daily allowance.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Calculated average daily amounts of vitamins C, A, D, and E received by infants randomised to a low ascorbic acid (AA) regimen and fed 150 mg/kg/day. Infants randomised to high AA received an additional 20 mg/kg/day vitamin C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C (mg)</td>
</tr>
<tr>
<td>EBM+5 drops/kg Vitadol C*</td>
<td>22.5</td>
</tr>
<tr>
<td>S26 LBW formula=3 drops/kg Vitadol C</td>
<td>26.4</td>
</tr>
<tr>
<td>EBM+5 g/100 ml S26+3 drops/kg Vitadol C</td>
<td>20.4</td>
</tr>
</tbody>
</table>

*Nutricia, Auckland, New Zealand.
EBM, Expressed breast milk.
We selected two groups of day 28 samples, those with AA concentrations $\leq$50 µmol/l ($n = 30$; mean (SD) 31 (12) µmol/l) and those with AA concentrations $\geq$80 µmol/l ($n = 27$; 103 (29) µmol/l). The low AA samples had a mean protein carbonyl concentration of 0.133 (0.071) nmol/mg compared with 0.126 (0.059) nmol/mg for the high AA samples; these values were not significantly different.

**DISCUSSION**

We hypothesised that having lower AA concentrations in the first week of life (target 35–50 µmol/l) and higher concentrations in weeks 3–4 (target 90 µmol/l) would decrease morbidity (chronic lung disease and ROP) in very low birthweight infants. Therefore we designed the study with three groups, one aiming to keep AA concentrations low throughout (LL), one with high concentrations throughout (HH), and a group crossing over from low initially to high at day 10 (LH). Although we achieved significantly different AA concentrations at days 10, 21, and 28 between the groups in the direction expected, there were no significant differences in primary outcomes. The results did show trends towards better respiratory outcome being associated with higher AA intake, and it is a possibility that by distributing infants in

![Figure 1](http://fn.bmj.com/)

**Figure 1** Plasma ascorbic acid (AA) concentrations (mean (SD)) for the group who received low AA supplementation throughout (LL), the group who received low AA until day 10 and then high (LH), and the group who received high AA throughout (HH) at the different time points.
| Study groups: LL, received low ascorbic acid (AA) supplementation throughout; LH, received low AA until day 10 and then high; HH, received high AA throughout. |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Table 4** Plasma ascorbic acid concentrations (μmol/l) and clinical outcomes by study group | **LL** | **LH** | **HH** | **p Value** |
| **N** | **Mean (95% CI)** | **N** | **Mean (95% CI)** | **N** | **Mean (95% CI)** | **p Value** |
| Ascorbic acid day 2 | 38 | 53.9 (44.1 to 63.4) | 39 | 51.2 (40.2 to 62.1) | 39 | 58.3 (45.1 to 71.6) | 0.69† |
| Ascorbic acid day 10 | 32 | 38.5 (28.9 to 48.1) | 29 | 35.9 (28.4 to 43.4) | 26 | 69.7 (49.6 to 89.8) | 0.001† |
| Ascorbic acid day 21 | 33 | 47.7 (39.5 to 55.8) | 25 | 55.8 (44.7 to 66.9) | 32 | 70.6 (57.5 to 83.7) | 0.006† |
| Ascorbic acid day 28 | 33 | 51.5 (42.0 to 61.0) | 31 | 59.4 (50.0 to 68.9) | 29 | 78.6 (63.4 to 93.8) | 0.0003† |
| **N** | **Mean (95% CI)** | **N** | **Mean (95% CI)** | **N** | **Mean (95% CI)** | **p Value** |
| Days of ventilation | 31 | 10.3 (4.8 to 15.7) | 27 | 9.2 (3.5 to 15.0) | 25 | 7.5 (2.7 to 12.2) | 0.98† |
| Days of oxygen therapy | 40 | 29 (18 to 40) | 40 | 25 (12 to 38) | 39 | 28 (14 to 41) | 0.08† |
| Days parenteral nutrition | 32 | 11.7 (7.5 to 16.0) | 29 | 9.9 (5.5 to 14.4) | 30 | 7.7 (5.6 to 9.7) | 0.32† |
| Age (days) at full oral feeding | 40 | 10.6 (8.2 to 13.0) | 40 | 88.8 (58 to 11.8) | 39 | 8.2 (6.2 to 10.2) | 0.12† |
| **N** | **% (95% CI)** | **N** | **% (95% CI)** | **N** | **% (95% CI)** | **p Value** |
| Oxygen at 28 days | 39 | 51 (36 to 67) | 38 | 37 (22 to 52) | 36 | 36 (20 to 52) | 0.32† |
| Oxygen at 36 weeks PMA | 39 | 41 (26 to 57) | 38 | 29 (15 to 43) | 36 | 19 (7 to 32) | 0.12† |
| Death or oxygen at 28 days | 40 | 53 (37 to 68) | 40 | 20 (25 to 55) | 39 | 41 (26 to 57) | 0.46† |
| Death or oxygen at 36 weeks PMA | 40 | 43 (27 to 58) | 40 | 33 (18 to 47) | 39 | 26 (12 to 39) | 0.28† |
| Postnatal steroids | 40 | 15 (4 to 26) | 40 | 10.0 (1 to 19) | 39 | 5.1 (2 to 12) | 0.39* |
|ROP* | 31 | 39 (22 to 56) | 30 | 37 (19 to 54) | 28 | 40 (21 to 59) | 0.97† |
| Necrotising enterocolitis | 40 | 2.5 (0 to 7) | 40 | 7.5 (0 to 16) | 39 | 2.6 (0 to 8) | 0.62† |

**Study groups:** LL, received low ascorbic acid (AA) supplementation throughout; LH, received low AA until day 10 and then high; HH, received high AA throughout.

*Screening for retinopathy of prematurity (ROP) confided to infants <31 weeks gestation or <1250 g birth weight.

†Kruskal-Wallis test.

‡χ² tests with 2 degrees of freedom.

*Fisher’s exact test.

PMA, postmenstrual age.
three groups, the study lacked sufficient power to detect a difference. Comparing only the LL and HH groups, the mean requirement for oxygen at 36 weeks was twice as high in the low intake group. Most would consider this difference clinically significant and it approached statistical significance (p = 0.06), as did comparisons across the three groups with the data corrected for gestation and CRIB score (table 5, p = 0.06). We must be cautious in attributing any advantage to the high dose AA, as there was no relation between AA concentrations at any time point and outcome measures. However, there was no evidence that the high dose AA was harmful, and the results suggest that supplementing very preterm infants at this higher AA concentration may be beneficial.

One possible limitation is that AA concentrations in the high groups did not reach our target range. This target was based on our preliminary observational study where the mean AA concentration at 28 days for infants without bronchopulmonary dysplasia was 93 μmol/l. During that study, infants who were fed parenterally received 25 mg/kg/day AA over 24 hours, and some high AA concentrations may have resulted from the infant’s demise per se.

In conclusion, in a blinded randomised controlled trial, we concluded that such pro-oxidant activity was at best unproven under physiological conditions. In very low birthweight infants, the situation is more complex, as they are born with low transferrin concentrations, and tend to have high iron status through repeated transfusions. Non-transferrin bound iron has been detected in plasma of preterm infants by several groups. However, there was no evidence of increased lipid or protein oxidation associated with the presence of this iron and high AA concentrations. Together with our observation that protein oxidation was no different for high and low plasma AA concentrations, these results suggest that a pro-oxidant role of ascorbate at the doses we used is unlikely.

Table 5 Outcome rates standardised for gestational age and CRIB score

<table>
<thead>
<tr>
<th></th>
<th>LL</th>
<th>LH</th>
<th>HH</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxygen at 28 days</td>
<td>49 (31 to 67)</td>
<td>34 (18 to 55)</td>
<td>28 (7 to 35)</td>
<td>0.28</td>
</tr>
<tr>
<td>Oxygen at 36 weeks PMA</td>
<td>36 (22 to 55)</td>
<td>24 (11 to 42)</td>
<td>16 (21 to 59)</td>
<td>0.06</td>
</tr>
<tr>
<td>ROP</td>
<td>30 (14 to 53)</td>
<td>34 (15 to 59)</td>
<td>32 (15 to 57)</td>
<td>0.78</td>
</tr>
</tbody>
</table>

Values are % (95% confidence intervals). Predicted from a logistic regression model equation with gestational age set to 28 weeks and CRIB score set to 2.1, the approximate median values.

CRIB, Clinical risk index for babies; PMA, postmenstrual age; ROP, retinopathy of prematurity; LL, received low ascorbic acid (AA) supplementation throughout; LH, received low AA until day 10 and then high; HH, received high AA throughout.

Table 6 Odds ratios and 95% confidence intervals for oxygen requirement a 28 days and 36 weeks postgestational age, and retinopathy of prematurity (ROP), related to plasma ascorbic acid concentrations (30 unit increases) after adjustment for gestational age and CRIB score by logistic regression and at each time point

<table>
<thead>
<tr>
<th>Day</th>
<th>Oxygen at 28 days OR 95% CI</th>
<th>Oxygen at 36 weeks OR 95% CI</th>
<th>ROP OR 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0.685 (0.421 to 1.117)</td>
<td>0.840 (0.530 to 1.330)</td>
<td>0.859 (0.458 to 1.612)</td>
</tr>
<tr>
<td>10</td>
<td>0.982 (0.511 to 1.887)</td>
<td>0.890 (0.536 to 1.447)</td>
<td>1.162 (0.662 to 2.037)</td>
</tr>
<tr>
<td>21</td>
<td>1.693 (0.893 to 3.209)</td>
<td>0.866 (0.449 to 1.689)</td>
<td>1.211 (0.567 to 2.586)</td>
</tr>
<tr>
<td>28</td>
<td>1.124 (0.495 to 2.550)</td>
<td>1.067 (0.541 to 2.108)</td>
<td>0.650 (0.219 to 1.930)</td>
</tr>
</tbody>
</table>

From an earlier report, high AA concentrations in the first days of life were associated with an increased risk of dying. Whereas this report suggested that this may reflect harmful pro-oxidant effects of AA, other explanations are possible. Brain tissue is rich in ascorbic acid. Arad and Eyal compared no supplementation with high iron status through repeated transfusions; four had bilateral intraventricular haemorrhage, plus intracerebral haemorrhage in one case, and one had widespread changes compatible with “anoxic brain injury”. (Only two other infants had an intraventricular haemorrhage and one an intracerebral haemorrhage, their day 2 AA concentrations being 35, 87, and 59 μmol/l respectively.) Although these findings warrant further investigation, it seems probable that the high AA concentrations found in infants who died are more likely to be a marker of a significant cerebral insult than a contributor to the infant’s demise per se.

In conclusion, in a blinded randomised controlled trial, we were able to achieve higher or lower AA concentrations at 10, 21, and 28 days of age. Treatment allocation was not associated with significantly improved outcome, although there was a trend towards less respiratory morbidity for infants randomised to receive higher AA supplementation throughout. With no evidence for any harmful pro-oxidant
effects, we suggest it may be reasonable to provide at least this level of supplementation.

ACKNOWLEDGEMENTS
This work was supported by a project grant from the Health Research Council of New Zealand. F McGregor was the recipient of a Masonic Postgraduate Fellowship in Paediatrics and Child Health. We thank Nina Mogridge for help with data collection and Dug Yeo Han for help with statistical analysis. We are also very grateful to the nursing staff of the neonatal intensive care unit of Christchurch Women’s Hospital and to the parents and families involved for their support for the study.

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Competing interests: none declared

REFERENCES
32 Berger TM, Frei B. Pro- or antioxidant activity of vitamin C in preterm infants? [Correspondence]. Arch Dis Child Fetal Neonatal Ed 1995;72:F211.
Vitamin C supplementation in very preterm infants: a randomised controlled trial

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Arch Dis Child Fetal Neonatal Ed 2005 90: F117-F122
doi: 10.1136/adc.2004.056440

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