

Supplementation with antioxidants and folic acid for children with Down's syndrome: randomised controlled trial

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ABSTRACT

Objectives To assess whether supplementation with antioxidants, folic acid, or both improves the psychomotor and language development of children with Down's syndrome.

Design Randomised controlled trial with two by two factorial design.

Setting Children living in the Midlands, Greater London, and the south west of England.

Participants 156 infants aged under 7 months with trisomy 21.

Intervention Daily oral supplementation with antioxidants (selenium 10 µg, zinc 5 mg, vitamin A 0.9 mg, vitamin E 100 mg, and vitamin C 50 mg), folic acid (0.1 mg), antioxidants and folic acid combined, or placebo.

Main outcome measures Griffiths developmental quotient and an adapted MacArthur communicative development inventory 18 months after starting supplementation; biochemical markers in blood and urine at age 12 months.

Results Children randomised to antioxidant supplements attained similar developmental outcomes to those without antioxidants (mean Griffiths developmental quotient 57.3 v 56.1; adjusted mean difference 1.2 points, 95% confidence interval -2.2 to 4.6). Comparison of children randomised to folic acid supplements or no folic acid also showed no significant differences in Griffiths developmental quotient (mean 57.6 v 55.9; adjusted mean difference 1.7, -1.7 to 5.1). No between group differences were seen in the mean numbers of words said or signed: for antioxidants versus none the ratio of means was 0.85 (95% confidence interval 0.6 to 1.2), and for folic acid versus none it was 1.24 (0.87 to 1.77). No significant differences were found between any of the groups in the biochemical outcomes measured. Adjustment for potential confounders did not appreciably change the results.

Conclusions This study provides no evidence to support the use of antioxidant or folic acid supplements in children with Down's syndrome.

Trial registration Clinical trials NCT00378456.

INTRODUCTION

Adults with Down's syndrome seem to age prematurely; many show Alzheimer's-like changes in their

brains in their 30s and 40s.¹ Neuronal changes are evident in infants with Down's syndrome. Postmortem studies have reported neuronal depletion and structural abnormalities of the brain during late gestation and early postnatal life.² Why these changes occur is not fully understood, but involvement of the increased activity of two enzymes, copper/zinc superoxide dismutase and cystathionine β-synthase, has been suggested. Increased activity of superoxide dismutase in children with Down's syndrome is thought to cause oxidative damage to neuronal cells by increasing concentrations of hydrogen peroxide.³

Evidence for a functional folate deficiency in Down's syndrome is based on analytical studies in plasma and in vitro studies. Cystathionine β-synthase catalyses the condensation of homocysteine with serine to form cystathionine. Increased concentrations of this enzyme in Down's syndrome leads to a functional folate deficiency.⁴ In vitro studies have shown that adding selected nutrients to a cultured lymphoblastoid cell line with trisomy 21 causes a shift in one-carbon metabolism to a more normal profile.⁴

Clinical evidence that supplementation in children and young adults with folate, antioxidants, or both might ameliorate the effects of Down's syndrome has been evaluated in a systematic review of four controlled trials.⁵⁻¹² All the trials have significant methodological weaknesses, and no meta-analysis was considered feasible. None of the trials reported any significant effect of antioxidants on cognitive function. Despite these findings, use of vitamin and mineral supplements is widespread in children with Down's syndrome in Europe and the United States. We aimed to determine the benefits of supplementation with antioxidants or folic acid for psychomotor development and the effect on certain biochemical markers of oxidative stress.

METHODS

Between May 2002 and February 2004 in Greater London and the West Midlands and, from January 2003, in Nottingham and the south west of England, we enrolled infants aged under 7 months with Down's

syndrome. We randomised infants to receive a daily oral dose of antioxidants (selenium 10 µg, zinc 5 mg, vitamin A 0.9 mg, vitamin E 100 mg, and vitamin C 50 mg), folic acid (0.1 mg), a combination of the same doses of antioxidants and folic acid, or a placebo. Researchers and parents were blind to allocation. We monitored compliance with supplements from parental reports at each visit or telephone call and by taking blood samples at age 1 year to measure plasma vitamin E concentrations.

Developmental outcomes—A trained assessor measured the primary outcome, age adjusted general quotient on the Griffiths mental developmental scales, 18 months after enrolment. The Griffiths scales combine observations on how the child interacts with test equipment, together with developmental questions to parents. Parents used a diary to record prospectively the date their child achieved major motor milestones such as sitting without support and walking. We assessed language development at the 18 month home visit.

Biochemical outcomes—We determined whether supplementation had any detectable effect on the antioxidant enzymes copper/zinc superoxide dismutase and glutathione peroxidase in red blood cells. We measured urinary isoprostane concentrations as a marker of lipid peroxidation.

Analyses—We based all analyses on the intention to treat principle. In the primary analyses, we compared children who received antioxidants with those who did not and those who received folic acid with those who did not. We used regression analyses to estimate the differences between groups for each intervention, adjusted for the effect of the other intervention, area of residence, and baseline stratification variables.¹³ We adjusted for age at assessment. In secondary analyses, we further adjusted for variation in age, maternal ethnicity, social class, and neonatal problems. We tested for an interaction between the interventions.

RESULTS

Enrolment, follow-up, and compliance

In all, 215 families were referred to the research team, of whom 59 either did not meet the inclusion criteria or declined to participate; 156 infants (mean corrected age 4.2 months) were randomly assigned to one of four

groups. Baseline characteristics were similar in the four groups. Of the 17 (11%) children lost to follow-up, three died, three developed leukaemia, and four moved abroad. The mean age at completion of the trial was 22.9 months (range 18.6-35.9 months). We assessed 139 children for the primary outcome of Griffiths developmental quotient after 18 months.

More of the children taking antioxidants than taking folic acid alone or placebo stopped taking supplements (15/74, 20% *v* 2/65, 3%; relative risk 6.5, 95% confidence interval 1.5 to 27). Only children taking antioxidants stopped supplements because of vomiting or distress (10/74 *v* 0/65; *P*=0.002). No other notable adverse events were reported. For the children who continued on supplements, reported compliance was good; 78% (94/122) of parents reported missing fewer than 10% (<54/547 days) of daily doses, and only 6/122 (4%) missed more than 20% of doses (>104/547 days). We measured mean plasma vitamin E per millimole of cholesterol in 95 children, and this was almost twice as high in those taking antioxidants as in those taking placebo or folic acid alone (10.76 *v* 5.92 µmol/mmol cholesterol; *P*<0.0001).

Effects on development

Table 1 shows the unadjusted mean Griffiths developmental quotients by group. Table 2 shows results for clinical and biochemical outcomes, adjusted for variables used to stratify randomisation. We found no significant differences between groups randomised and not randomised to antioxidants or between those randomised and not randomised to folic acid on Griffiths developmental quotient or measures of language (table 2).

Supplementation had no effect on the recorded age at attainment of motor milestones. Comparing infants allocated to antioxidants with those who were not, the hazard ratio for age of sitting without support was 1.10 (95% confidence interval 0.77 to 1.56) and that for standing was 1.25 (0.88 to 1.78). The results for children on folic acid compared with those not on folic acid were 1.25 (0.88 to 1.78) for sitting and 1.14 (0.76 to 1.71) for standing. None of these results changed appreciably after adjustment for area of residence, maternal ethnicity, birth weight, and social class.

Table 1 | Unadjusted means* of Griffiths developmental quotient for all combinations of groups in factorial design

Antioxidants	Folic acid		Total
	Yes	No	
Yes:	(Group A; n=36)	(Group B; n=37)	(Group A+B; n=73)
Mean GQ	58.7 (9.3)	57.4 (9.8)	58.0 (1.1)
No:	(Group C; n=32)	(Group D; n=33)	(Group C+D; n=65)
Mean GQ	57.8 (11.9)	56.1 (9.8)	56.9 (1.3)
Total:	(Group A+C; n=68)	(Group B+D; n=70)	(Group A+B+C+D; n=138)
Mean GQ	58.3 (1.3)	56.8 (1.2)	57.5 (0.9)

GQ=general quotient; group A=antioxidants and folic acid; group B=antioxidants only; group C=folic acid only; group D=placebo.
*Standard deviations in parentheses for individual group entries; standard errors shown for entries for totals.

Table 2 | Developmental, speech, and biochemical outcomes for children randomised to antioxidants versus no antioxidants or to folic acid versus no folic acid. Values are mean (SD) unless stated otherwise

Outcomes	Antioxidants v no antioxidants			Folic acid v no folic acid		
	Group A+B	Group C+D	Mean difference or ratio of means (95% CI)	Group A+C	Group B+D	Mean difference or ratio of means (95% CI)
Griffiths mental developmental scales*						
No	73	65	–	68	70	–
Total GQ	58.1 (9.5)	56.9 (10.8)	1.2 (–2.2 to 4.6)	58.4 (10.5)	56.7 (9.7)	1.7 (–1.7 to 5.1)
Griffiths subscales*:						
Locomotor	54.1 (12.0)	51.1 (12.5)	3.0 (–1.1 to 7.0)	53.1 (11.6)	52.3 (12.9)	0.9 (–3.2 to 5.0)
Personal-social	62.6 (12.9)	61.5 (13.2)	1.1 (–3.2 to 5.4)	62.7 (12.5)	61.5 (13.5)	1.2 (–3.2 to 5.6)
Hearing and language	56.5 (10.3)	56.8 (14.3)	–0.3 (–4.5 to 3.9)	56.9 (11.6)	56.3 (12.9)	0.6 (–3.6 to 4.9)
Eye and hand	61.9 (11.6)	60.9 (12.3)	1.0 (–3.0 to 5.1)	62.6 (12.9)	60.2 (10.7)	2.3 (–1.8 to 6.4)
Performance	60.8 (15.7)	59.3 (15.5)	1.5 (–3.8 to 6.8)	61.8 (16.5)	58.5 (14.6)	3.3 (–2.1 to 8.6)
Receptive language‡						
No	73	65	–	69	69	–
Total gesture score	31.3 (11.9)	32.4 (11.4)	–1.1 (–5.1 to 2.9)	32.0 (11.2)	31.7 (12.1)	0.3 (–3.8 to 4.4)
Phrases understood	15.7 (6.6)	16.5 (7.4)	–0.8 (–3.2 to 1.6)	16.3 (6.8)	15.8 (7.2)	0.5 (–2.0 to 2.9)
Expressive language‡						
No	73	65	–	69	69	–
Mean No of words child says§	3.2 (3.6)	4.9 (6.3)	0.82 (0.60 to 1.12)	4.7 (6.2)	3.4 (3.7)	1.05 (0.76 to 1.45)
Mean No of words child signs§	6.0 (7.8)	6.1 (7.6)	0.93 (0.66 to 1.31)	7.2 (8.5)	4.9 (6.5)	1.33 (0.93 to 1.89)
Mean No of words child says or signs§	8.2 (8.3)	9.7 (9.6)	0.86 (0.61 to 1.21)	10.4 (9.9)	7.3 (7.7)	1.24 (0.87 to 1.77)
Blood analysis¶						
No	52	47	–	50	49	–
Superoxide dismutase (SOD-1) (U/mg Hb)	4.0 (1.1)	3.8 (1.1)	0.2 (–0.2 to 0.6)	3.9 (1.2)	3.9 (0.9)	0.1 (–0.4 to 0.5)
Glutathione peroxidase (GSH-Px) (U/mg Hb)	66.3 (34.8)	65.3 (37.7)	4.2 (–9.3 to 17.7)	71.7 (41.4)	60.2 (29.4)	7.6 (–6.0 to 21.3)
Ratio of SOD-1 to GSH-Px§††	0.077 (0.06)	0.077 (0.05)	0.99 (0.79 to 1.24)	0.077 (0.06)	0.077 (0.04)	1.01 (0.81 to 1.27)
Urine analysis¶						
No	26	26	–	23	29	–
Isoprostanes§ (pmol/mmol creatinine)	2264 (2202)	2306 (2399)	1.10 (0.61 to 2.00)	2243 (2143)	2318 (2419)	0.92 (0.52 to 1.61)

GQ=general quotient; group A=antioxidants and folic acid; group B=antioxidants only; group C=folic acid only; group D=placebo; Hb=haemoglobin.
*Mean (SD) and confidence interval adjusted for area of residence, sex, and congenital heart disease.
†Mean (SD) and confidence interval adjusted for area of residence, age, sex, and congenital heart disease.
‡Unadjusted mean (SD); confidence interval adjusted for area of residence, age, sex, and congenital heart disease.
§Mean difference calculated on log scale and back transformed to give ratio of means on original scale.
¶Unadjusted mean (SD); confidence interval adjusted for area of residence, sex, and congenital heart disease.
**After exclusion of outlier with GSH-Px of 312.
††After exclusion of outlier with SOD:GSH-Px ratio of 6.4.

Enzyme activities and oxidative stress

We obtained blood at 1 year of age from 107 children and measured enzyme activities on 99 samples. We obtained urine from 106 children and estimated isoprostane concentrations in 52. We found no significant effect of antioxidant or folic acid supplementation on superoxide dismutase or glutathione peroxidase activities or on the superoxide dismutase to glutathione peroxidase ratio or urinary isoprostane concentrations (table 2).

DISCUSSION

We found no evidence that either the antioxidants or the folic acid supplements used in this trial had any effect on psychomotor development or language acquisition in children with Down's syndrome.

Activities of the antioxidant enzymes and urinary isoprostane concentrations (a marker of lipid peroxidation) were similar in all groups, indicating that supplementation did not affect oxidative stress.

These findings are supported by a systematic review that included four randomised controlled trials of high dose vitamin supplements compared with placebo.⁷ Concerns that the design of previous studies could have been biased in favour of no effect, owing to small sample size, short duration of supplementation, and late age of starting supplements, were considered in our study. Our sample size was sufficient to detect a clinically small effect in the main developmental outcome, and loss to follow-up was only 11%. Infants were started on supplements at a mean age of 4 months and continued for 18 months.

WHAT IS ALREADY KNOWN ON THIS TOPIC

Developmental delay in children with Down's syndrome may result from neuronal damage due to increased oxidative stress, abnormal folate metabolism, or both

Cultured neuronal cells from fetuses with Down's syndrome undergo apoptotic death more rapidly than those from unaffected fetuses, but this is reversed by addition of antioxidants

No high quality in vivo evidence exists to show if giving antioxidants or folic acid affects neurodevelopment in infants with Down's syndrome

WHAT THIS STUDY ADDS

Daily supplementation with antioxidants, folic acid, or both did not alter psychomotor or language development in children with Down's syndrome

Reported compliance was good and confirmed by increased plasma vitamin E concentrations in those children on supplementation. Concealment of allocation was good, and blinding proved to be effective as only 8% of parents correctly guessed which supplement their child was taking.

One limitation of our study was the relatively low dose of supplements compared with commercially available preparations,¹⁴ which may have been inadequate to affect biochemical pathways. The doses used in the study were 100% of the recommended daily allowance for vitamin E, zinc, and selenium and 200% of the recommended daily allowance for vitamin C and folic acid.¹⁵ We were reluctant to use higher doses, as data on the safety of high doses for young children are lacking.

The mechanisms responsible for the neuronal changes in Down's syndrome are likely to be complex. The variable phenotype of Down's syndrome could result from an interaction involving any of the genes or gene products coded on chromosome 21.¹⁶ An aneuploid mouse strain carrying human chromosome 21 has recently been developed, and this might provide further insights into the complex mechanisms involved in Down's syndrome.¹⁷

The only short term side effect we found was a significant increase in vomiting in infants taking antioxidants. However, the side effects of higher dose commercially available preparations used over a long period are unknown.

Conclusion

Our study provides no evidence to support the use of antioxidants or folic acid in young children with Down's syndrome. Parents who choose to give supplements to their children need to weigh their hope of unproved benefits against potential adverse effects from high dose, prolonged supplementation.

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