Randomised trial of supplementation with antioxidants and folinic acid for children with Down syndrome.

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Abstract

Objectives –To assess whether supplementation with antioxidants and/or folinic acid improve the psychomotor and language development of children with Down syndrome.

Design – Randomised controlled trial with a 2x2 factorial design.

Setting- Children living in the Midlands, Greater London and South West of England UK.

Participants- 156 infants with Trisomy 21 under 7 months of age.

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

Main outcome measures- 18 months after starting supplementation children were assessed using the Griffiths Developmental Quotient (GQ) and an adapted Mac Arthur Communicative Development Inventory. Biochemical markers in blood and urine were also measured at 12 months of age.

Results Children randomised to antioxidant supplements attained similar developmental outcomes to those without antioxidants: Mean GQ on antioxidants 57.3, mean GQ without antioxidants 56.11 (Adjusted mean difference1.21 points, 95% confidence interval -2.21 to 4.63). Comparison of children randomised to folinic acid supplements or no folinic acid also showed no significant differences in GQ: Mean GQ on Folic acid 57.56, mean GQ without folic acid 55.85 (Adjusted mean difference 1.7, 95% confidence interval -1.73 to 5.14).. There were also no between group differences seen in the mean numbers of words said or signed: For anitoxidants vs none the ratio of means was 0.85 (95% confidence interval 0.6 to 1.2) and for folinic acid vs none the raito of means was

1.24 (95% confidence interval 0.87 to 1,77). There were no significant differences in the biochemical outcomes measured between any of the groups. Adjusting for potential confounders did not appreciably change results.

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

Trial Registration - ***Registered on Clinicaltrials.gov: NCT00378456***

What is already known on the subject

- Developmental delay in children with Down syndrome may occur as a result of neuronal damage due either to increased oxidative stress or abnormal folate metabolism, or both.

- In vitro, cultured neuronal cells from fetuses with Down syndrome undergo apoptotic death more rapidly than those from unaffected fetuses, but this is reversed by addition of antioxidants.

- There is no high quality in vivo evidence whether giving antioxidants or folinic acid affects neurodevelopment in infants with Down syndrome.

What this paper adds

- Daily supplementation with antioxidants and/or folinic acid does not alter the psychomotor or language development in children with Down syndrome.

Introduction

Trisomy 21 (Down syndrome) is the commonest genetic cause of learning disability in the UK with a birth prevalence of 1 per 1000 live births.[1] Adults with Down syndrome appear to age prematurely, with many showing Alzheimers-like changes in their brains in their 30s and 40s.[2] Neuronal changes are evident in infants with Down syndrome. Post mortem studies have reported neuronal depletion and structural abnormalities of the brain during late gestation and early post-natal life.[3] Why these changes occur is not fully understood but it has been proposed that the increased activity of two enzymes, copper/zinc superoxide dismutase (SOD-1) and cystathionine β-synthase, both coded for on chromosome 21, may be involved.

Increased activity of SOD-1 in children with Down syndrome[4] is thought to cause oxidative damage to neuronal cells by increasing levels of hydrogen peroxide. Evidence that oxidative stress may be involved in the premature neuronal degeneration comes from several sources. Firstly, the cerebral cortex from fetuses with Down syndrome was found to have increased activity of SOD-1 without a compensatory increase in glutathione peroxidase activity (GSH-Px).[5] Secondly, cortical neurons from fetuses with Down syndrome have an increased concentration of intracellular oxygen derived free radicals and increased lipid peroxidation compared to controls.[6] Thirdly, fetal neurons in Down syndrome have increased apoptotic degeneration which appears to be prevented by the addition of antioxidants.[7] Finally, studies have reported increased products of lipid peroxidation in blood and urine of people with Down syndrome compared with controls.[8-11]

Evidence for a functional folate deficiency in Down syndrome is based on analytical studies in plasma and in vitro studies. The enzyme cystathionine β-synthase catalyses the condensation of homocysteine with serine to form cystathionine. Increased levels of this enzyme in Down syndrome leads to significantly reduced plasma concentrations of homocysteine, methionine, S-adenosylhomocysteine and S-adenosylmethionine and thereby to a "folate trap" and a functional folate deficiency.[12] In vitro studies have shown that adding selected nutrients (methionine, folinic acid, methyl B_{12}, thymidine and dimethylglycine) to a cultured lymphoblastoid cell line with trisomy 21 causes a shift in one-carbon metabolism to a more normal profile.[13]

Clinical evidence that supplementation with folate[14] and/or antioxidants[15] might ameliorate the effects of Down syndrome has been evaluated in a systematic review.[16] Four randomised controlled trials of various forms of antioxidant vitamin and mineral supplementation were analysed in children and adults with Down syndrome, although none included folate or folinic acid supplements.[17] None of the trials reported any significant effect of antioxidants on cognitive function but all were small and of poor quality. Despite these findings, use of vitamin and mineral supplements is widespread in children with Down syndrome in Europe and the USA due to the marketing of commercial preparations claiming substantial benefits for children with Down syndrome. This study aimed to address uncertainty about the benefits of supplementation with antioxidants or folinic acid for psychomotor development and determine the effect on certain biochemical markers of oxidative stress.

Participants and 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 less than 7 months old with Down syndrome. Children with chromosome mosaicism or translocation, severe cardiac defects or other serious long term illness, and from non-English speaking families were excluded. The study was publicised through clinicians and parent groups and interested families were visited at home. We used a 4 arm factorial design to randomise infants to receive a daily oral dose of a) antioxidants (selenium 10μg, zinc 5mg, vitamin A 0.9mg, vitamin E 100mg and vitamin C 50mg,); b) folinic acid (0.1mg,); c) a combination of the same doses of antioxidants and folinic acid; or d) a placebo. Randomisation was stratified by sex and presence of congenital heart disease according to a random sequence generated by a Minim computer programme by the pharmacists who retained the allocation lists. Supplements were prepared and packaged by Quintiles (Edinburgh UK), stored in pharmacy and mailed direct to the parents as numbered identical sachets of powder that could be mixed with food or drink. Parents were shown how to mix and administer the supplements at the enrolment visit and the dosage was increased by 30% after the child's first birthday. Researchers were not aware of treatment allocation until completion of the analyses. Parents were blind to allocation until after the final outcome assessment but could thereafter request information directly from the pharmacy.

To detect a clinically important difference of 6 points on the Griffiths Mental Developmental Quotient (equivalent to 0.5 of a standard deviation) with 85% power we

required 68 patients in each combined treatment group (antioxidants vs. no antioxidants and folinic acid vs no folinic acid). We planned to recruit 200 infants allowing for a 33% loss to follow-up.

The primary outcome, age-adjusted General Quotient on the Griffiths Mental Developmental Scales (birth to two years, 1996 revised version), was measured by one of four trained assessors 18 months after enrolment. The Griffiths Scales combine observations on how the child interacts with test equipment together with developmental questions to parents. The number of "successes" the child achieves is converted to a developmental age equivalent. Scores are also produced on five sub sections (locomotor, personal-social, hearing and language, co-ordination and performance) and age-adjusted sub-quotients calculated.

Parents used a diary to prospectively record the date their child achieved major motor milestones such as sitting without support and walking. Missing records were completed based on parental recall at visits 9 and 18 months after enrolment. Treatment differences in recorded age of attainment of milestones were estimated using Cox regression.

We assessed language development using a modified version of the MacArthur Communicative Development Inventory, a five section postal questionnaire.[18] As this was designed for the USA we replaced the standard word list (section B) with one used for UK children[19] and administered it to the parents at the 18 month home visit. We

scored signed as well as spoken words and calculated the total number of gestures, phrases understood and words said, signed and said or signed. Results were age adjusted.

We monitored compliance with supplements in two ways. Firstly, at each visit or telephone call, we asked parents how many doses had been missed in the past 9 months. Secondly, we collected blood samples at approximately one year of age to measure plasma vitamin E concentrations. Venous samples were collected into lithium heparin tubes, separated within 3-4 hours of collection and stored at -80°C prior to analysis. Plasma vitamin E (α-tocopherol) was measured using high performance liquid chromatography with fluorimetric detection by a modification[20] of the method of Buttriss and Diplock.[21] Vitamin E concentrations were expressed per plasma cholesterol concentrations measured enzymatically [22] on a COBAS Fara analyser using a kit supplied by ABX Diagnostics, Montpelier, France.

Biochemical outcomes

We determined whether supplementation had any detectable effect on the antioxidant enzymes: copper/zinc superoxide dismutase (SOD-1) and glutathione peroxidase (GSH-Px) in red blood cells. We used a COBAS Fara analyser and kits supplied by Randox Laboratories Ltd (County Antrim, UK). SOD-1 was measured using the RANSOD kit, which is based on the original method of McCord and Fridovich.[23] GSH-Px was measured using the RANSEL kit which is based on the method of Paglia and Valentine.[24] Both SOD 1 and GSH-Px activities were expressed per haemoglobin concentrations measured as cyanmethaemoglobin.

Urinary isoprostane (8isoPGF$_{2\alpha}$) concentrations were measured as a marker of lipid peroxidation. 8isoPGF$_{2\alpha}$ was extracted by a specific affinity sorbent supplied by Cayman Chemicals Company (Ann Arbor, US) and estimated by gas chromatography-mass spectrometry by a modification of the method of Bessard et al [25] using deuterated 8isoPGF$_{2\alpha}$ as an internal standard. The mass spectrometer was operated in the selected ion monitoring mode with ions at *m/z* 481 and 485 (deuterated compound used for quantification). Concentrations of 8isoPGF$_{2\alpha}$ were expressed per urinary creatinine which was measured using a COBAS Fara analyser and a kit supplied by ABX Diagnostics, Montpelier, France, based on the Jaffe reaction.[26]

Analyses

All analyses were based on intention to treat. In the primary analyses, we compared children who received antioxidants with those who did not and those who received folinic acid with those who did not. For continuous variables we used regression analyses to estimate the differences between groups for each intervention and their confidence intervals, adjusted for the effect of the other intervention, area of residence and baseline stratification variables.[27] For dichotomous variables we used logistic regression analyses to produce similarly adjusted estimates of the odds ratios and confidence limits. Where these measures were not age standardised we adjusted for age at assessment. The number of words said, signed or said or signed were log-transformed to ensure approximate normality of the residuals. Effect sizes were then expressed as ratios of means adjusted for the effect of the other intervention, area of residence and the baseline stratification variables.[28] In secondary analyses, we further adjusted for variation in age,

maternal ethnicity, social class and neonatal problems. We also tested for an interaction between the interventions.

The study was approved by The London Multi-Research Ethics Committee and written informed consent was obtained from the parents or legal guardians.

Results

Patient enrolment

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 4.15 months) were randomly assigned to one of four groups (Figure1). The planned sample size of 200 infants was abandoned due to slow recruitment and funding restrictions. Baseline characteristics were similar in the four groups (Table 1).

Of the 17 (11%) children lost to follow-up, 3 died, 3 developed leukaemia and 4 moved abroad. The mean age at trial completion was 22.9 months (range 18.6-35.9). 139 children were assessed for the primary outcome of Griffiths Developmental Quotient after 18 months. Follow up for other outcomes is shown in Figure 1.

More of the children on antioxidants stopped taking supplements (15/74, 20%) than those on folinic acid or placebo (2/65, 3 %); RR 6.5 (1.5-27). Only children on antioxidants stopped supplements because of vomiting or distress (10/74 vs 0/65, p=0.002). No other significant adverse events were reported. For the children who continued on supplements, reported compliance was good; 78% (94/122) of parents reported missing less than 10% (<54/547days) of daily doses and only 6/122 (4%) missed more than 20% of doses (>104/547days). Mean plasma vitamin E per cholesterol concentrations were measured in 95 children and were almost twice as high in those on antioxidants compared with those on placebo or folinic acid (10.76 vs 5.92 µmol/mmol cholesterol, p=<0.0001).

At the end of the trial we asked parents to guess which of the four supplements their child had been taking during the trial. Only 44/138 (32%) parents felt able to guess and of these only 11/44 (25%) guessed correctly which is consistent with chance.

Effects on development

We found no evidence for clinically or statistically significant effects of antioxidants or folinic acid on any of the outcomes measured. The unadjusted mean Griffiths Developmental Quotients by group are shown in Table 2. Results for clinical and biochemical outcomes, adjusted for variables used to stratify randomisation, are shown in Table 3. No significant differences were found between groups randomised to antioxidants or not, or those randomised to folinic acid or not on Griffiths Developmental Quotient or measures of language (Table 3).

Supplementation also had no effect on the recorded age at attainment of motor milestones. Comparing infants allocated to antioxidants with those who were not, the hazard ratios for age of sitting without support was 1.10 (95% CI 0.77, 1.56) and for standing was 1.25 (95% CI 0.88, 1.78). The same results for children on folinic acid compared with not were 1.25 (95% CI 0.88, 1.78) for sitting and 1.14 (95% CI 0.76, 1.71) for standing.

None of these results changed appreciably after adjusting for area of residence, maternal ethnicity, birth weight and social class.

Enzyme activities and oxidative stress

We obtained blood at 1 year of age from 107 children and enzyme activities were measured on 99 samples. Urine was obtained from 106 children and isoprostane concentrations were estimated in 52. We found no significant effect of antioxidant or folinic acid supplementation on SOD 1 or GSH-Px activities, or on the SOD/GSH-Px ratio and urinary isoprostane concentrations. (Table 3)

Discussion

We found no evidence that either antioxidants and/or folinic acid supplements had any effect on psychomotor development or language acquisition in children with Down syndrome. Activities of the antioxidant enzymes (red cell SOD-1, red cell GSH-Px) and urinary isoprostane concentrations (a marker of lipid peroxidation) were similar in all groups, suggesting 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.[16] Concerns that the design of previous studies could have biased in favour of no effect, due to small sample size, short duration of supplementation (3-8months), and late age of starting supplements, were addressed in the present study. Our sample size was sufficient to detect a clinically small effect in the main developmental outcome (6 GQ points) 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. Reported compliance was good and confirmed by increased plasma vitamin E concentrations in those children on supplementation. Allocation concealment 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 (Nutrivene-D & Euro TNI).[29] Doses used in the study were based on known safety levels but may have been inadequate to affect

biochemical pathways. Our results do not exclude the possibility that subtle effects of supplementation on development might be detectable given longer term supplementation and follow up.

The mechanisms responsible for the neuronal changes in Down syndrome are likely to be complex. SOD-1 and cystathionine β-synthase are just two of many gene products coded for on chromosome 21. The variable phenotype of Down syndrome could result from an interaction involving any of the genes and/or gene products coded on chromosome.[30] Recently an aneuploid mouse strain carrying human chromosome 21 has been developed and this might provide further insights into the complex mechanisms involved in Down syndrome.[31]

As Down syndrome has a profound effect on the lives of children and their families, it is likely that parents will have a low threshold for trying interventions. Commercially available nutritional supplements cost between £15 and £30 a month and, as they are food supplements, are not required to be produced to the same high standards as prescription drugs. The only short term side effect we have shown was a significant increase in vomiting in those taking antioxidants but the side effects of higher dose preparations, used in children over a long period, are unknown. The widely held belief that vitamins are harmless has been challenged by a recent systematic review and meta-analysis of controlled trials suggesting that antioxidant supplementation may be associated with an increased risk of mortality across a range of conditions.[32, 33]

In summary, our study provides no evidence to support the use of antioxidants or folinic acid in young children with Down syndrome. Parents who choose to give supplements to their child need to weigh their hope of unproven benefits against potential adverse effects from high dose, prolonged supplementation.

Other members of the V&M trial team

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Contributors:

CA, SGM, RM and DPRM were applicants on the original grant to the Down's Syndrome Association and Fondation Jérôme Lejeune. TM gave statistical advice in the design phase. CA, SGM, SL, RG and JE wrote the protocol and applied for MREC approval. HK, JE, RP and VE set up the database. JE was trial co-ordinator and together with RM and SL supervised research assistants SR, MR, SD, RP and VE who visited families, collected and imported data. AS gave advice on developmental assessment and other aspects of trial conduct. Griffiths assessments were done by JE, SD, RP and SL. HG and DM carried out the biochemical analyses. HK supervised data cleaning by RP and VE and with JE, SL and RG did the preliminary analysis with advice from AS. WH did the final adjusted analysis. JE, RG, DM and SL drafted the paper which was commented on by the other authors.

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Competing interest statement

All authors declare that the answer to the questions on your competing interest form (http://bmj.com/cgi/content/full/317/7154/291/DC1) are all No and therefore have nothing to declare.

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Figure1: Trial Profile

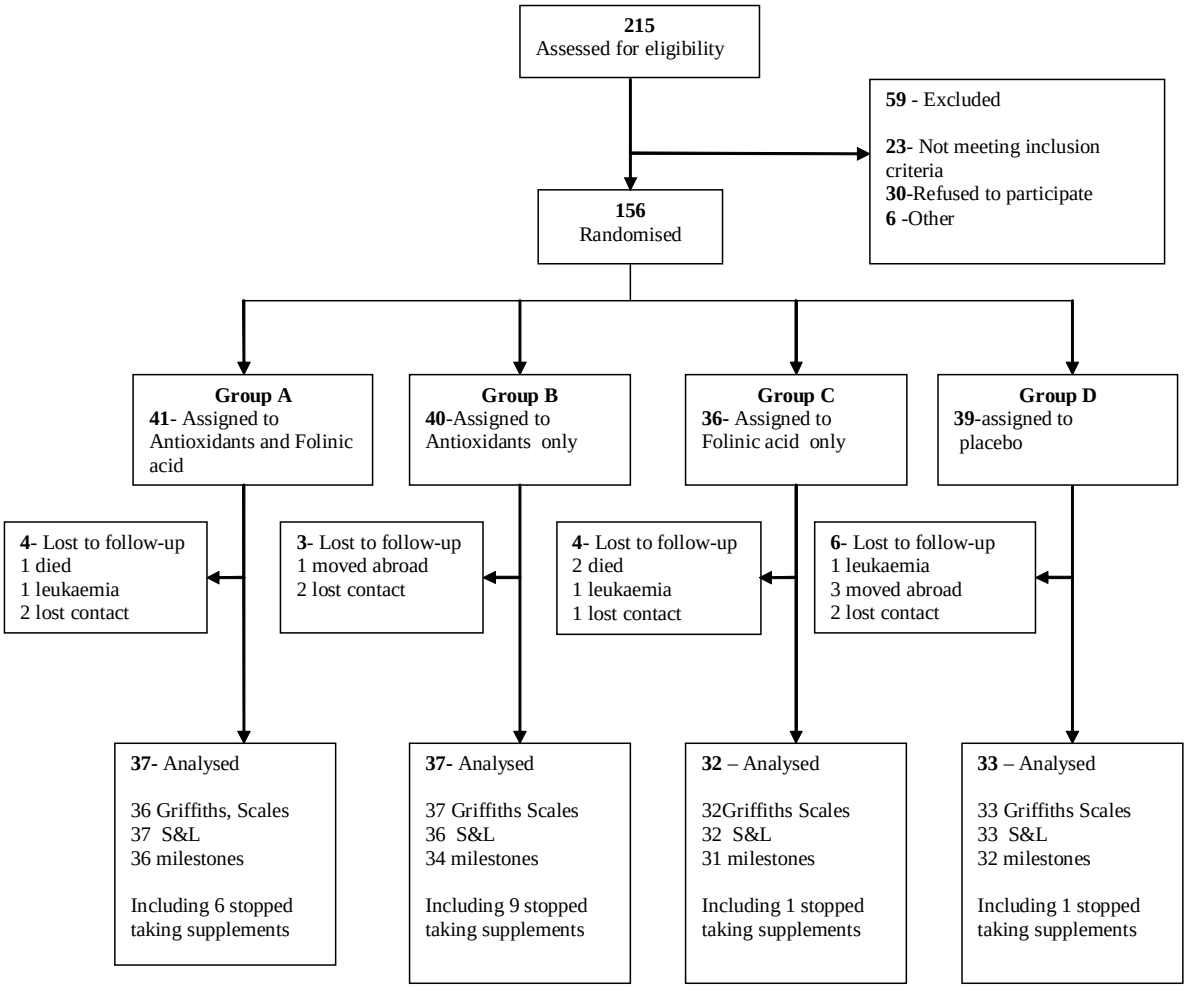

Table1: Characteristics of trial participants at baseline

	Group A **Antioxidants and folinic acid (number= 41)**	*Group B* **Antioxidants only (number= 40)**	*Group C* **Folinic acid only (number= 36)**	*Group D* **Placebo (number= 39)**	**A+B+C+D Total (number= 156)**
Infant Characteristics					
Mean age – months (range)	4.1 (0.4, 6.3)	4.3 (1.2, 6.2)	4.6 (1.5, 6.8)	3.7 (1.3, 6.9)	4.15 (0.4, 6.9)
Mean age of assessment (range)					
Mean birth Weight (range)	2.8 (0.9, 4.1)	2.8 (1.4, 4.3)	2.8 (1.1, 3.8)	2.8 (1.3, 4,5)	2.8 (0.9, 4.5)
First born Number (%)	17 (41)	17 (43)	15 (42)	19 (49)	68 (44)
Sex					
Male Number (%)	23 (56)	24 (60)	21 (58)	21 (54)	89 (57)
NICU					
Admitted Number (%)	14 (34)	24 (60)	16 (44)	15 (38)	69 (44)
Ventilated Number (%)	4 (10)	3 (8)	5 (14)	4 (10)	16 (10)
Congenital heart disease					
Cyanotic heart disease*	7 (17)	5 (13)	2 (6)	4 (10)	18 (12)
Ventriculo- septal defect	4 (10)	6 (15)	7 (19)	3 (8)	20 (13)
Medication for failure	6 (15)	2 (5)	4 (11)	2 (5)	14 (9)
Family Characteristics					
Mean maternal age (age range)	32.8 (19.0, 44.0)	34.7 (19.0,44.0)	34.8 (20.0,46.0)	33.0 (20.0,46.0)	33.8 (19.0, 46.0)
Social class					
1 (Highest)	19 (46)	20 (50)	16 (44)	14 (36)	69 (44)
2	8 (20)	11 (28)	9 (25)	11 (28)	39 (25)
3	10 (24)	4 (10)	11 (31)	13 (33)	38 (24)
4 (Lowest)	4 (10)	5 (13)	0 (0)	1 (3)	10 (6)
Maternal ethnicity					
White	26 (63)	28 (70)	27 (75)	31 (80)	112 (72)
Black	4 (10)	6 (15)	3 (8)	4 (10)	17 (11)
Asian	7 (17)	5 (13)	1 (3)	2 (5)	15 (9)
First Language					
Yes Number (%)	31 (76)	32 (80)	31 (86)	36 (92)	130 (83)
Area resident					
Birmingham	17 (41)	14 (35)	5 (14)	19 (49)	55 (35)
London	18 (44)	23 (58)	23 (64)	17 (43)	81 (52)
South West	6 (15)	3 (7)	8 (22)	3 (8)	20 (13)

* -includes children with atrioventricular septal defect or Fallots tetralogy

Table 2: Unadjusted mean differences in Griffiths Developmental quotient (GQ) for all combinations of groups in the factorial design

		Folinic Acid		Total
		Yes	No	
Antioxidants	Yes Mean GQ	Group A (n=36) 58.70	Group B (n=37) 57.35	Group A+B(n=73) 58.01
	No Mean GQ	Group C (n=32) 57.77	Group D (n=33) 56.11	Group C+D (n=65) 56.94
	Total Mean GQ	Group A+C (n=68) 58.27	Group B+D (n=70) 56.77	Group A+B+C+D (n=138) 57.51

Table 3 – Development, speech and biochemical outcomes for children randomised to antioxidants vs no antioxidants, or to folinic acid vs no folinic acid

	Group A+B Antioxidants	**Group C+D** No Antioxidants		**Group A+C** Folinic acid	**Group B+D** No Folinic Acid	
Griffiths Mental Developmental Scales	**Mean (SE)$** (number=73)	**Mean (SE)$** (number=65)	**Mean difference (95% CI)$**	**Mean (SE)$** (number=68)	**Mean (SE)$** (number=70)	**Mean difference (95% CI)$**
Total GQ	57.30	56.11	1.19 (-2.20, 4.59)	57.56	55.85	1.70 (-1.73, 5.14)
Griffiths subscales						
Locomotor	53.10	50.14	2.97 (-1.07, 7.00)	52.06	51.18	0.89 (-3.19, 4.96)
Personal-social	61.31	60.22	1.09 (-3.24, 5.41)	61.37	60.16	1.21 (-3.17, 5.58)
Hearing and Language	56.21	56.50	-0.29 (-4.46, 3.88)	56.67	56.04	0.63 (-3.58, 4.85)
Eye and Hand	61.42	60.38	1.03 (-3.03, 5.10)	62.06	59.74	2.32 (-1.80, 6.43)
Performance	59.90	58.39	1.50 (-3.76, 6.77)	60.77	57.52	3.25 (-2.07, 8.58)
Receptive language	**Mean (SE) #** (n=73)	**Mean (SE) #** (n=65)	**Mean difference (95% CI)#**	**Mean (SE) #** (n=69)	**Mean (SE) #** (n=69)	**Mean difference (95% CI)#**
Total gestures	30.85	31.92	-1.08 (-5.05, 2.90)	31.53	31.24	0.29 (-3.77, 4.35)
Phrases Understood	15.47	16.25	-0.78 (-3.17, 1.61)	16.08	15.63	0.46 (-1.99, 2.90)
Expressive language	**Mean (SD) Δ** (n=73)	**Mean (SD) Δ** (n=65)	**Ratio of means(95% CI)#**	**Mean (SD) Δ** (n=69)	**Mean (SD) Δ** (n=69)	**Ratio of means(95% CI)#**
Mean no. of words child says*	3.2	4.9	0.82 (0.60, 1.12)	4.7	3.4	1.05 (0.76, 1.45)
Mean no. of words child signs*	6.0	6.1	0.93 (0.66, 1.31)	7.2	4.9	1.33 (0.93, 1.89)
Mean no. of words child says or signs*	8.2	9.7	0.86 (0.61, 1.21)	10.4	7.3	1.24 (0.87, 1.77)
Blood Analysis	**Mean (SE) Δ** (number=52**)**	**Mean (SE) Δ** (number=47)	**Mean difference (95% CI)$**	**Mean (SE) Δ** (number=50)	**Mean (SE) Δ** (number=49)	**Mean Difference (95% CI)$**
SOD-1 (U/mg Hb)	3.98	3.82	0.19 (-0.22, 0.61)	3.94	3.88	0.05(-0.38, 0.47)
GSH-Px (U/mg Hb) ^	66.3	65.3	4.2 (-9.3, 17.7)	71.7	60.2	7.6 (-6.0, 21.3)
Ratio SOD-1/GSH-Px *^^	0.065	0.064	0.99 (0.79, 1.24)	0.061	0.067	1.01 (0.81, 1.27)
Urine analysis	**Mean (SE) Δ** (number=26)	**Mean (SE) Δ** (number=26)	**Ratio of means (95% CI)$**	**Mean (SE) Δ** (number=23)	**Mean (SE) Δ** (number=29)	**Ratio of means (95% CI) $**
Isoprostanes * (pmol/mmol creatinine)	1462	1318	1.10 (0.61, 2.00)	1400	1379	0.92 (0.52, 1.61)

$ Adjusted for area of residence, sex and congenital heart disease.
# Adjusted for area of residence, age, sex and congenital heart disease
Δ Unadjusted mean
* Mean difference calculated on log–scale and back-transformed to give ratio of means on original scale
^ After exclusion of outlier with GPx of 312.
^^ After exclusion of outlier with SOD/GPx ratio of 6.4

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