

Nutritional supplementation in Down syndrome: theoretical considerations and current status

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Down syndrome (DS) is the most common inherited cause of learning disability¹, and affected individuals are more prone to infections, leukaemia, congenital heart disease and other anomalies, thyroid dysfunction, early senescence and Alzheimer's disease². These complications create a heavy burden for carers of individuals with DS and the social and health services. Consequently, any intervention that ameliorates some of these complications will have a significant impact on the quality of life of individuals with DS and their carers. However, before investing resources in interventions, it is important to ascertain their scientific validity.

The Internet and the lay press make many claims that certain expensive nutritional supplements improve the outcome in DS. These claims have left some health professionals confused and parents of children with DS vulnerable to pressures to spend large amounts of money on nutritional supplements whose benefits have not been proved. This review was undertaken firstly, to determine whether there is a theoretical basis to expect that nutritional supplements may improve the pathology of DS, and secondly, to critically examine published trials of nutritional supplements in DS to determine whether the existing evidence supports claims that nutritional supplements improve the outcome in DS.

Theoretical basis for supplementation

OXIDATIVE STRESS IN DS

Oxidative stress is defined as an imbalance between production of oxygen-derived free radicals and their removal by antioxidants³. The activity of superoxide dismutase (SOD) – a key enzyme in the metabolism of oxygen-derived free radicals – is increased in DS (see below). This increase in SOD

activity may alter the normal steady state equilibrium of reactive oxygen species leading to oxidative injury in DS^{4,5}.

SOD catalyses the dismutation of superoxide radicals to hydrogen peroxide which is further metabolised to water by glutathione peroxidase (GSH-Px) or catalase³. In DS, the SOD:GSH-Px ratio is increased and this imbalance may lead to the accumulation of unmetabolised hydrogen peroxide which may then react with transition metals like iron (Fenton reaction)³ to form the hydroxyl radical⁶ (Fig. 1). The latter is the most reactive oxygen radical known and can, for example, readily initiate lipid peroxidation resulting in damage to cell membranes³. Because the brain is rich in highly polyunsaturated fatty acids which are particularly susceptible to lipid peroxidation, it is potentially vulnerable to this type of damage⁷. Therefore, it is hypothesised that an overexpression of SOD causes free radical mediated damage and that this may contribute to the learning disability and early onset of Alzheimer's disease which are typical of DS. The following, which will be considered in turn, support the above hypothesis: (1) there is an increase in SOD activity, (2) there is evidence of increased lipid peroxidation, (3) there is a compensatory increase in the activities of GSH-Px and the hexose monophosphate shunt (HMPS).

Increased superoxide dismutase (SOD) activity

The presence of an extra chromosome 21 in DS results in overexpression of genes residing on that chromosome. The resulting 'gene dose effect' is thought to account for most of the pathophysiology of DS¹. One of these genes codes for the enzyme SOD⁶. As expected from the gene dose effect, many studies have found an increase in SOD activity of 50% or

more in various tissues of individuals with DS⁸⁻¹⁶.

Similarly, many animal studies have shown 50% or more overexpression of SOD in transgenic mouse models of DS^{5,17-20}.

Increased lipid peroxidation

A number of animal studies have shown that an increase in SOD is associated with increased rates of lipid peroxidation in the brain^{5,17,21-23}.

A 36% increase in lipid peroxidation was also demonstrated in the cerebral cortex of fetuses with DS who were incubated *in vitro* with iron and ascorbate compared with infants without DS¹². In addition, Busciglio and Yankner²⁴ showed that cultured cortical neurons from fetuses with DS had approximately four times more intracellular free radicals and an increased level of lipid peroxidation compared with neurons from individuals without DS. The neurons of fetuses with DS were also more likely to undergo apoptotic degeneration which was prevented by the addition of antioxidants.

Significantly higher levels of the products of lipid peroxidation have also been reported in the blood or urine of individuals with DS than in individuals without DS^{13,25-27}.

Compensatory increase in the activity of glutathione peroxidase and the hexose monophosphate shunt

In addition to increased SOD activity, many studies have reported increased activity of GSH-Px in various tissues of individuals with DS^{6,7,11,13,16,28-33}, as well as in animal models¹⁸. Because Wijnen and colleagues³⁴ have localised the gene for GSH-Px on chromosome 3, its increase in individuals with DS is not a gene dose effect. Therefore, it is likely to be a physiological/protective response to cope with the excess hydrogen peroxide produced by the hyperactive SOD system⁶. However, the 50% increase in activity of SOD is much higher than the percentage increase usually reported in activity of GSH-Px^{6,7,16,32,33}. De Haan and colleagues³⁵ reported a two-fold elevation in the SOD:GSH-Px ratio measured in a number of organs of aborted fetuses with DS compared with

control fetuses. These authors also showed that cells with a high SOD:GSH-Px ratio are more likely to undergo apoptosis when challenged with hydrogen peroxide, which supports the findings of Busciglio and Yankner²⁴ cited earlier.

Further evidence of increased oxidative stress in DS was provided by Sinet and coworkers⁷ who demonstrated a 15% increase in HMPS activity in the red blood cells of individuals with DS compared with individuals without DS. Because HMPS is involved in the metabolic pathway that sustains the activity of GSH-Px, its increased activity in individuals with DS is further evidence for the presence of oxidative stress⁷. An increase in HMPS activity has also been reported in mice transgenic for SOD²³.

OXIDATIVE STRESS AND OTHER FEATURES OF DS

Immune dysfunction

It has been hypothesised that abnormal metabolism of reactive oxygen species may also contribute to the defective immunity and increased susceptibility to infections typically seen in individuals with DS. The formation of oxygen radicals is one of the key mechanisms by which phagocytic leukocytes kill pathogens³⁶. The serious consequence of failure in this system is clearly seen in the dramatic increase in severe infections of patients with chronic granulomatous disease³⁷ whose leukocytes cannot form the superoxide radical because of a deficiency of nicotinamide adenine dinucleotide phosphate oxidase³⁸.

There are at least two possible mechanisms by which an increase in SOD activity can reduce immunity in DS. Firstly, a hyperactive SOD system is likely to result in a decrease in the concentration of superoxide radicals, which may in turn cause a reduction in the microbicidal activity of leukocytes^{23,36}. Secondly, an increase in SOD activity may lead to an excess of hydrogen peroxide which may damage immune cells and impair normal signal transduction processes involved in phagocyte activation²³.

In support of these hypotheses, it has been shown that neutrophils from individuals with DS produce less superoxide radicals than individuals without DS^{36,39}. Similarly, Mirochnitchenko and Inouye²³ found that a two-fold overproduction of SOD by intraperitoneal macrophages from transgenic mice, resulted in an inhibition of extracellular release of superoxide radicals, increased intracellular production of hydrogen peroxide, and a reduction in microbicidal and fungicidal activity.

Peled-Kamar and colleagues⁵ have shown that the activity of SOD in the thymus of transgenic mice is increased by two- to five-fold, and that the thymus is more susceptible to lipopolysaccharide-induced apoptotic cell death. The increased SOD activity was also associated with an increased production of hydrogen peroxide and lipid peroxidation. When cultured under stressed conditions (e.g. addition of tumour necrosis factor), the bone marrow cells from the transgenic mice produced two to three times fewer granulocyte and macrophage colonies than control mice. It was suggested that these defects resulted from increased oxidative damage⁵ although the authors did not investigate whether the addition of antioxidants corrected the immune defects.

Malignancy

There is now evidence linking increased oxidative stress with increased DNA damage in DS^{27,40}. Jovanovic and coworkers²⁷

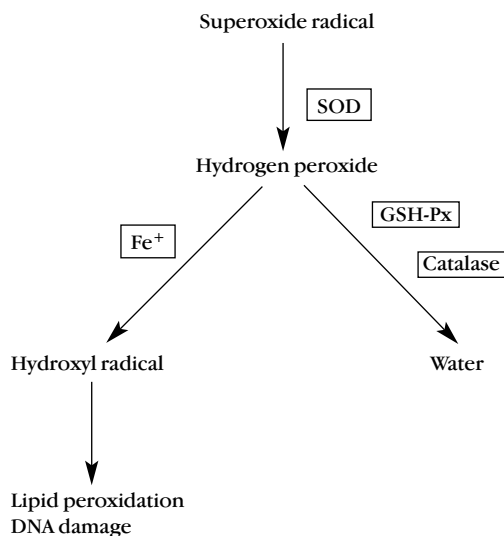


Figure 1: Hypothesised pathway for increase in SOD leading to increased oxidative stress in DS.

compared the levels of 8-hydroxy-2-deoxyguanosine (a biomarker of oxidative damage to DNA) in 166 matched pairs of individuals with DS and their siblings, and found a significantly increased concentration of 8-hydroxy-2-deoxyguanosine in the urine of individuals with DS. Pincheira and colleagues⁴⁰ found an increase in chromosomal damage in lymphocytes of individuals with DS compared with individuals without DS, which could be reduced by more than 50% by the addition of vitamin E to the cell culture. As vitamin E is a powerful antioxidant, it was hypothesised that the increased chromosomal damage in DS resulted from increased oxidative stress. These studies, therefore, not only provide further direct evidence for increased oxidative stress in DS but also suggest a possible explanation for the increased malignant potential associated with the syndrome.

Mental development

There is some evidence of an association between mental development in DS and oxidative stress. Sinet and colleagues⁷ found a highly significant positive correlation between GSH-Px activity and IQ in 22 individuals with DS and concluded that GSH-Px may play an important role in preserving the cerebral status of individuals with DS. As GSH-Px is an endogenous antioxidant, it is possible that supplementing individuals with DS with exogenous antioxidants may offer similar protection to their cerebral status. This is supported by the protective effect of antioxidants on DS neurons in culture, referred to earlier²⁴. A randomised controlled trial of vitamin E in Alzheimer's disease⁴¹ found significant beneficial effects. Because individuals with DS almost invariably develop Alzheimer's disease, this trial suggests a role for oxidative damage in the pathology of both conditions.

Premature ageing

De Haan and colleagues³⁵ have carried out several studies suggesting that increased oxidative stress could be implicated in ageing. They found (1) a significant increase in the SOD:GSH-Px ratio ($p < 0.005$) and susceptibility to lipid peroxidation ($p < 0.005$) in normal mouse brain during ageing, (2) that cultured murine cells which have been transfected to have an increased SOD:GSH-Px ratio showed the characteristic features of senescence, (3) that normal mouse cells exposed to hydrogen peroxide in culture also showed features of senescence, and (4) that cells derived from children with DS showed features of senescence which were not seen in cells from age-matched control children.

CONCLUSIONS AND SOME IMPLICATIONS OF INCREASED OXIDATIVE STRESS IN DS

In summary, the evidence for increased oxidative stress in DS is reasonably strong and includes: gene dose overexpression of SOD, increased lipid peroxidation in human individuals with DS and transgenic mice models, compensatory increases in GSH-Px and HMPS activities, increased products of oxidative DNA damage, increased chromosomal damage reduced by 50% *in vitro* by the addition of vitamin E, and most importantly there are increased intracellular free radicals and enhanced apoptosis in neurons of fetuses with DS which can be prevented by addition of antioxidants. Therefore, there is good evidence that increased oxidative stress may play a role in the complications of DS. This means that an excess of oxygen-derived free radicals could result in

an extra demand for antioxidant nutrients like vitamins C and E, β -carotene, and selenium (cofactor for GSH-Px). Thus even normal serum concentrations of these nutrients could be functionally deficient in the face of excess demand. This opens the possibility that antioxidant nutrient supplementation might help to ameliorate the pathology of DS. We would, therefore, hypothesise that supplementation with increased amounts of antioxidant nutrients could benefit individuals with DS. The following section reviews trials of nutritional supplementation and a selection of other non-nutritional/pharmacological interventions which have been carried out in individuals with DS.

Nutritional supplementation trials in DS

We found many published trials of supplementation with nutrients and pharmacological agents in individuals with DS, including zinc, selenium, megavitamin/mineral preparations, vitamin A, vitamin B6 and its precursors, 'targeted nutritional intervention (TNI) supplements', vasopressin, and 'U series' (see below). The results have been varied and will now be briefly reviewed. Unfortunately, only a few of the studies were randomised trials and although, as indicated above, there is a theoretical rationale for antioxidant supplementation, none of the trials was specifically designed to evaluate antioxidant therapy.

ZINC SUPPLEMENTATION

Zinc is part of the cytosolic copper-zinc-SOD enzyme⁶. Zinc supplementation trials have been justified mainly because of reports of relatively low serum zinc in DS. Of 16 studies which have compared serum levels of zinc in individuals with DS and individuals without DS, 13 showed significantly reduced zinc concentrations in individuals with DS⁴²⁻⁵⁴, while three found no significant difference⁵⁵⁻⁵⁷.

We found only one randomised controlled trial of zinc in DS⁵⁸. These investigators assigned 64 individuals with DS aged 1 to 19 years to receive 25 to 50 mg of zinc/day (depending on age) or placebo for 6 months with a crossover for another 6 months. Outcome criteria consisted of laboratory measures of immune competence and an infection log which included respiratory symptoms such as coughing. They found no significant changes in lymphocyte function, complement levels, or number of infections. However, the trend was in favour of the zinc-treated group ($p = 0.07$) for days coughed and they had significantly ($p = 0.03$) fewer episodes of cough. Also, among children less than 10 years old, the zinc-treated group had significantly fewer cough days ($p = 0.01$) than placebo controls.

There have been seven uncontrolled zinc trials with pre- and posttreatment measurements with a total of 168 individuals with DS aged 2 to 22 years^{43,45,47,49-51,53}. All the studies consistently reported mainly laboratory evidence for beneficial effects of zinc supplementation on the immune function of individuals with DS. However, as these studies had no placebo treated controls or blind assessment of outcome, they are difficult to interpret.

We found two *in vitro* studies with zinc in DS. Fabris and colleagues⁴⁴ reported that adding zinc to the serum of individuals with DS increased their serum thymic factor (FTS) to levels normally seen in individuals without DS and also reduced the concentration of FTS inhibitory factor. Chiricolo and coworkers⁵⁹ showed that individuals with DS who were

supplemented for 4 months with 1 mg of zinc/kg/day had an increase in the *in vitro* incorporation of thymidine into their phytohaemagglutinin (PHA) stimulated lymphocytes similar to individuals without DS. In addition, following gamma radiation induced damage to DS cells, zinc supplementation reduced the abnormally high DNA repair rate (which predisposes to mutations and increases malignant potential) in DS cells to normal levels⁵⁹. However, as these studies were conducted *in vitro* their *in vivo* significance remains uncertain.

In summary, although there are encouraging results from uncontrolled studies and *in vitro* experiments suggesting that zinc supplementation may enhance immunity and reduce malignant potential in individuals with DS, there is no rigorous or consistent evidence from clinical trials to show that this is the case.

SELENIUM SUPPLEMENTATION

Selenium is a component of GSH-Px which is part of the body's endogenous antioxidant system⁶. In a study by Anneren and coworkers⁶⁰, 10 µg of selenium/kg/day was administered to 48 individuals with DS aged 1 to 16 years for 6 months, and concentrations of immunoglobulin G2 and G4 increased by up to 33% and 75% respectively. The participants also reported fewer infections during the study. However, as this study was uncontrolled and almost half of the sample was lost during follow-up, the result is impossible to interpret. In another study, Antila and coworkers⁶¹ gave 15 to 25 µg of selenium/kg/day to seven individuals with DS aged 1 to 54 years for a period of 0.3 to 1.5 years and reported a 25% increase in the activity of GSH-Px and a 24% reduction in the SOD:GSH-Px ratio compared with 10 unsupplemented individuals with DS.

MEGAVITAMIN/MINERAL SUPPLEMENTATION

In 1981, Harrell and colleagues⁶² randomised 22 children aged 5 to 15 years with learning disability (five of whom had DS) to receive either a megavitamin/mineral preparation or placebo for 4 months initially. After the first phase, all the participants received the megavitamin/mineral supplement for another 4 months. The supplement consisted of 11 vitamins and eight minerals in high doses and included two antioxidants: vitamin C, 1500 mg; and vitamin E, 600 IU, daily. The investigators reported dramatic improvements in IQ, growth, physical appearance, language, educational attainment, and general health of the treated participants. This study had significant problems in that the loss of participants reduced the already small sample from 22 to 16 and only four of these had DS. However, the findings stimulated several more trials of megavitamin/mineral supplementation.

Six randomised controlled trials⁶³⁻⁶⁸ attempted to replicate the findings of Harrell and coworkers⁶² using similar vitamin/mineral supplements. These studies consisted of a total of 161 individuals with DS aged between 6 months and 40 years and none of the studies showed any improvement in IQ, physical appearance, or general health.

VITAMIN A SUPPLEMENTATION

We found only one small trial of vitamin A (retinol) in DS. Palmer⁶⁹ paired 23 individuals with DS aged 3 to 15 years with their own siblings and randomly assigned each pair to receive either 1000 IU/kg/day of vitamin A or placebo for 6 months. At baseline, the participants with DS in both groups experi-

enced significantly more frequent infections than their siblings ($p < 0.01$). However, during follow-up, the difference in frequency of infections between the vitamin A treated participants with DS and their siblings gradually reduced, becoming insignificant ($p > 0.05$) by the fifth month of the study. In contrast, the difference remained significant ($p < 0.01$) between the untreated participants with DS and their siblings throughout the study. However, the analyses were difficult to interpret because the treated and control groups were not statistically compared and the frequency of infections of individual children were summed. Also, it was not clear if the assessment of infections was performed 'blind'. This trial was conducted on the basis of reports of poor absorption and reduced serum vitamin A concentration in DS^{25,56,69}. However, this rationale is weak as impaired absorption of vitamin A was not reported in a larger study⁷⁰ and others have not found reduced serum vitamin A concentrations^{14,70-73}.

VITAMIN B6 / 5-HYDROXYTRYPTAMINE (5-HTP) SUPPLEMENTATION

Individuals with DS have been treated with vitamin B6 or 5-HTP in order to increase their serotonin level⁷⁴, which is frequently reported to be reduced⁷⁵⁻⁷⁷. Despite two uncontrolled studies^{76,78} which reported improvements in the muscle tone of 23 babies and children with DS treated with 5-HTP, two randomised controlled trials^{74,79} failed to find any significant clinical improvements in a total of 108 babies with DS treated with vitamin B6 or 5-HTP for 3 years.

TARGETED NUTRITIONAL INTERVENTION (TNI) SUPPLEMENTATION

Supplementation with TNI is probably the most popular nutritional therapy currently advocated for individuals with DS judging by its extensive coverage on the Internet and in lay publications. Its proponents claim to have identified the biochemical abnormalities in DS and have formulated a supplement to 'target' these abnormalities. A typical TNI supplement contains about 56 nutrients including vitamins, minerals, enzymes, amino acids, electrolytes etc. Unfortunately, we found no published trial on the safety or efficacy of this supplement. In addition, we found that a typical TNI preparation contains 1000 mg of vitamin C which may be unsafe in children, given that a daily intake of 500 mg of vitamin C has been shown to have pro-oxidant effects in adults⁸⁰.

MISCELLANEOUS TREATMENT TRIALS IN DS

In addition to the nutritional supplements discussed above, individuals with DS have also been treated with various pharmacological agents and two of these will now be briefly reviewed.

Following reports that vasopressin enhances learning in animals⁸¹, Eisenberg and colleagues⁸² treated nine individuals with DS, aged 10 to 42 years, with vasopressin or placebo for 10 days using a double-blind randomised crossover design. They found no significant improvements in tests of learning or memory.

Bumbalo and coworkers⁸³ conducted a double-blind randomised controlled trial of a preparation called the 'U series of drugs' on 24 children with DS aged 3 months to 11 years and reported no significant treatment effects after 1 year. The 'U series of drugs' was developed by Henry Turkel⁸⁴ and has been a popular therapy for DS in many countries. The supplement contained 48 items which, in addition to vitamins and minerals, included substances such as rutin, naphazoline

hydrochloride, propyl paraben, and pentylene tetrazole. No theoretical rationale was given for most of the items included in this supplement.

Comment on published supplementation trials in DS

Almost all the supplementation studies discussed above had major methodological shortcomings. In most studies, the design was poor and only a few were randomised controlled trials. Many lacked control subjects, had small sample sizes, ran for too short a duration, and targeted older individuals with DS.

None of the studies had a sample size large enough to detect small treatment effects, and thus they were all prone to type II statistical error⁸⁵. We have calculated the minimum sample size required to detect a 6-point (half a standard deviation) difference in IQ to be 170 individuals with DS (i.e. 85 in each of the treatment and control groups), assuming a power of 90% and 5% level of significance.

Most of the studies were of short duration. It may be too optimistic to expect subtle physiological improvements to be translated into detectable physical and mental changes within a short time period. For example, in a clinical trial of vitamin E in Alzheimer's disease an interim analysis performed after 1 year showed no significant treatment effects but significant effects were subsequently observed after 2 years⁴¹.

In most of the trials reviewed, the study participants had a very wide age range and comprised of older children and adults. Scientific pragmatism would suggest that the best outcomes would be among the youngest participants in whom the brain is developing rapidly and before damage has been done by the complications of DS. Wisniewski and colleagues⁸⁶ have shown that pathological changes in the brain of children with DS begin in late pregnancy which suggests that interventions to limit this damage should begin soon after birth. Thus most previous investigators may have studied individuals who have been too old to benefit maximally.

As already noted, some of the studies lacked a sound theoretical basis so that they had no scientific rationale to expect treatment effects and even if observed, there would have been no rational or plausible scientific explanation.

Conclusion

There is an increasingly good body of evidence to suggest that increased oxidative stress may be involved in the pathology of DS. Therefore, it is theoretically possible that using antioxidant nutrients to scavenge oxygen-derived free radicals may ameliorate some of the complications of DS. Despite this possibility there have been no clinical trials which have specifically evaluated the effects of antioxidant nutrient supplementation on the health and development of children with DS. Indeed, we believe that to date there has been no consistent or rigorous proof that any form of nutritional supplementation improves the outcome in DS. There is, therefore, an urgent need for a well conducted clinical trial to evaluate the hypothesis that antioxidant supplementation may improve the outcome in DS.

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Notice

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This book, which was reviewed in the July 1999 issue of *Developmental Medicine & Child Neurology*, is available from The Friends of the Cheyne Centre for Children with Cerebral Palsy, Tadworth Court, Tadworth, Surrey KT20 5RU, priced £5, plus 55p postage.