

The Role of Zinc in Down's Syndrome



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Note: A research project is needed to investigate zinc deficiency. The average adult daily intake of zinc from the diet is 10 mg. Evidence suggests this dietary intake will always be inadequate for patients who are at risk, like those who have Down's syndrome.

Zinc deficiency can result in many health problems and we believe it may be the root cause for the universal brain injury we see in Down's syndrome from a very early age. Medical research is needed to find the best treatment therapy. 25 mg per day of zinc may be needed to prevent zinc deficiency in young people and adults with Down's syndrome. Even higher doses (up to 60 mg per day) has been suggested for adults with DS. Babies and young children would get a much smaller dose rate based on body weight.

We cannot offer medical advice to parents, you must discuss these issues with your doctor or paediatrician.

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Introduction

Down's Syndrome: A Brief History

John Langdon Down, whilst working at Earlswood Asylum for Idiots at Redhill in Surrey between 1859 and 1869, noticed that certain children had the same appearance and commented in his paper, Observations on an ethnic classification of idiots (1866) that "their resemblance to each other was such that, when placed side by side, it is difficult to believe that they are not the children of the same parents." He described their physical features in a way which would be immediately recognisable to anyone involved with Down's syndrome people today. This must have been an important first step in recognising that certain children were mentally retarded for the same reason, and that reason had a familial, that is genetic, basis. Down believed that these children were a 'throw-back' to a more 'primitive' race, specifically Mongolians, hence the term 'mongolism'. It was not until 1965 that moves were made to rename the condition, when representatives of the Mongolian People's Republic approached the World Health Organisation and requested of the Director General that the term be abandoned, and the condition thenceforth be known as Down's syndrome.

What is Down's Syndrome?

The genetic cause of Down's syndrome was finally discovered in 1959 by a French man, J. Lejeune. Down's syndrome is the most common recognised chromosomal disorder found in humans, and falls into a category of chromosomal disruptions known as trisomies. This means that instead of there being two copies of a particular chromosome, there are three. When it is chromosome 21 of which there is an extra copy the condition is Trisomy 21, or Down's syndrome. Chromosome 21 is the smallest human chromosome. This may explain why individuals with Trisomy 21 are the only trisomy individuals who survive beyond a few months. In fact most trisomies will cause fetuses to spontaneously abort. Trisomy 21 is

found in three different chromosomal set-ups, known as karyotypes:

- Standard Trisomy 21 appears to be the result of a fault of meiosis or mitosis in either a parental gamete or in the zygote, resulting in every single cell being trisomic. It appears that 90-98% of Down's syndrome individuals are Standard Trisomic 21
- If mutation occurs after the first cell division of the zygote two cell lines will form, those with the normal 46 chromosomes and those with the extra chromosome 21. Depending on when and where the mutation occurs, the trisomic cells can vary from very few to nearly 100%. This is known as Mosaic Trisomy 21 and is found in 2-5% of Down's cases.
- In 3-5% of cases the individual does not have an additional chromosome because the long arm of the extra chromosome 21 has attached itself to another chromosome, Translocation Trisomy 21

It has been suggested that the extra chromosome results in a proportional increase in the expression of the genes represented on chromosome 21, resulting in an imbalance between the products determined by chromosome 21 and products determined by the genes on other chromosomes. This imbalance could be what produces the characteristic Down's syndrome phenotype, but there is not yet evidence ruling out the existence of mutations on the other genes.

Restrictions on applying research findings

Despite the recognisable physical features which characterise Down's syndrome individuals — broad, flat face, protruding tongue, small nose and mouth, distinctive epicanthal folds, and slanted eyes — there is considerably more variability in the presentation of features in Down's syndrome than in the same features in the normal population. This means that the degree of mental retardation and of growth delay (for instance) will vary quite widely from individual to individual; it also means that research findings, especially from small samples, and particularly from individuals sharing a similar environment, should be applied to larger populations with great care.

Nutritional Intervention

When, in 1940, Dr Henry Turkel was approached by the father of a Down's Syndrome child, and asked to treat his child, very little was known about the causes of Down's Syndrome (DS). However, Dr Turkel did know that "all living cells require proper nutrients in optimal quantities for the specific cell to develop normally" (1974). His experience treating diabetes, allergies and arteriosclerosis, using what would now be known as an orthomolecular approach, was the foundation of his methodology. With the aim of "removal of the harmful expression of the genes by removing the accumulating metabolites" he created a series of medicines known as the U series. These were designed to have a "simultaneous and synergistic" (1963) effect. However, though he claimed considerable success in diminishing inborn structural, functional and chemical abnormalities and produced photographs and X-rays of his subjects as evidence, Dr Turkel never performed a controlled trial of his treatment. Since then there have been other claims of success treating DS subjects with nutritional therapies but none that have been sustained (Sylvester, 1984). For instance, Harrell et al (1981) found that nutritional supplements were beneficial for DS children, affecting IQ, height, vision, appearance, and general achievement. However, attempts to replicate these

results have failed so far (Weathers, 1984; Bennett et al, 1983; Smith, 1984), leading to disagreements on the letters pages of The Lancet (Sept. 1983) and The Journal of Paediatrics (March 1985).

Zinc and its links with Down's Syndrome

This paper considers a single nutrient, zinc, and its place in supporting people with Down's syndrome. The importance of zinc is suggested by the many disease states found in DS that have also been observed in subjects with zinc deficiency. These include diabetes mellitus, dwarfism, hypogonadism, atherosclerosis, vitamin A deficiency night blindness, cirrhosis of the liver, myeloid leukaemia (Milunsky, 1970), and hyperthyroidism and hypothyroidism (Napolitano et al, 1990). Fabris et al (1993) cite the importance of zinc in the homeostatic networks found to be altered in DS, namely nervous, neuroendocrine and immune, and their interrelationship, plus a reduced turnover of this mineral, leading to the hypothesis that zinc deficiency could be implicated in at least some of the DS phenotype.

"Zinc forms part of the composition of at least 160 different enzymes. Indeed, zinc is the most widely used mineral in enzymes" (Graham and Odent, 1986).

It is vital for protein, essential fatty acid and carbohydrate metabolism, and for DNA synthesis, and can be used to detoxify lead and mercury (ibid.). The body only has a small pool of biologically available zinc, and a rapid turnover, meaning that deficiency signs appear very quickly (Passwater and Cranton, 1983).

Are Down's Syndrome Subjects Zinc Deficient?

Measuring zinc status

Logic would suggest that the first step towards determining whether zinc is important in DS would be to establish whether DS people are zinc deficient. However, this simple fact, at the time of writing, has yet to be agreed on. Graham and Odent (1986) suggest six methods for measuring zinc status:

- **Plasma concentration;** this is not an accurate reflection of zinc status as results can vary according to the time of day, how recently the subject ate and stress levels, amongst other variables.
- **Hair analysis;** a controversial measure, this is clinically significant but complicated by other factors. Both elevated and depressed zinc levels can be present in deficiency states (Passwater and Cranton 1983).
- **Urine test;** this do not show how much zinc is in the body but how much is being lost. The results are no indication of zinc status but may provide useful extra information.
- **Leucocyte concentration;** this is a useful reflection of zinc status in tissues generally. The measurement does not require much blood and is not technically difficult. Brigino et al (1996) state that assays of zinc concentration in all blood cells have proved to be more accurate and sensitive than plasma assays for indicating functional zinc status.
- **Sweat test;** this is the best indicator of zinc status, but is not widely used and is expensive to administer.
- **Taste test;** this gives a reliable indication of zinc status within broad categories but is

not refined in the data it provides.

This author has not been able to locate research measuring the zinc status of DS using urine, sweat, or taste tests. It is understandable that the latter has not been used given that the majority of DS people exhibit some degree of mental retardation and the taste test requires following complex instructions, complex vocabulary, and analysis on the part of the subject. This author believes that measurement of the zinc levels in urine and in sweat would yield useful new information to help resolve the question of DS people's zinc status.

Plasma levels

By far the most common method used for measuring zinc status is that of measuring plasma concentration, or serum concentration (serum is plasma minus the clotting proteins, and this paper does not concern itself with the difference as it was not found to be relevant). As an equal proportion of serum assays to plasma assays fall into the hypozincaemic findings group as into the normal values group, indicating that neither method is more likely to produce one result than the other, they shall be considered together. The majority of research shows that DS subjects have low zinc status (Milunsky, 1970; Halstead and Smith, 1970; Björkstén et al, 1980; Fabris et al, 1984; Annerén and Gebre-Medhin, 1987; Bruhl et al, 1987; Kanavin et al, 1988; Purice et al, 1988; Stabile et al, 1991; Licastro et al, 1992, 1993, 1994; Kadrabová, 1996). A notable minority of papers find no signs of deficiency (Matin, 1981; Nève et al, 1983, 1984; Noble and Warren, 1988; Laires et al, 1994). Results of both types have been found using both hospitalised subjects and those living at home. Research comparing DS patients with other mentally retarded patients showed normal values (Matin et al, 1981) and low values (Kanavin, 1988), as did research comparing DS patients with normal subjects. Controls were generally age-matched, though apparently not always matched by sex. Every paper analysed for zinc by atomic absorption spectrophotometry, but the method of preparation varied between researchers and was not always fully explained. It is possible that a method of preparing the plasma/serum for atomic absorption spectrophotometry may differently affect plasma from DS subjects than normal plasma samples but this consideration falls outside the scope of this paper. The question of whether or not DS people have low concentrations of plasma zinc remains to be answered conclusively, but it appears likely at present.

Sustrová and Strbák (1994) found that the average serum levels of zinc in DS subjects increased with age: from 66% of control levels for those aged 1 to 6 years to 88% of control levels when aged 15 to 35 years. This is a very interesting finding which prompts many questions about why and how this would be true. Is the survival rate higher amongst DS children with higher zinc levels, leading to a higher average as the population ages? Possibilities based on an improvement in zinc absorption or transport within the body seem unlikely because of the characteristic early senescence of DS people.

Erythrocytes values

A few assays have been made of zinc concentration in the erythrocytes of DS. Most found the values to be significantly higher than in the controls (Milunsky et al, 1970; Nève et al, 1983, 1984; Purice et al, 1988), but Annerén et al found the values to be lower than the controls (1985). The suggestion by the latter author was that previous investigations had used a coagulant that contaminated the samples with zinc. This author does not believe that such contamination would result in the relative values of the DS samples and the control samples

swapping places. Rather, each value would measure extremely high but would still retain the same relationship to the others. This author believes it more likely that DS erythrocytes have high levels of zinc — a fact also suggested by the high levels of SOD-1, a zinc-containing enzyme, found in DS erythrocytes. The role of SOD-1 in DS will be discussed later in this paper.

Muscle concentration

The most unusual piece of research was the measurement of zinc concentration in tongue muscles (Yarom et al, 1987), a rather leftfield piece of research which compared the results of samples obtained from partial glossectomies of DS patients with control samples taken from current autopsies. The finding was that the zinc levels were the same in both groups. However the average age of the 15 DS subjects was 10 years and the average age of the 8 controls was 37 years and 9 months. The control group contained a newborn baby and a 1 year old, bringing down the average considerably; with the two youngest removed the average age rises to 50 years. Given that zinc status decreases with age (Passwater and Cranton, 1983) this is not a very useful comparison, a fact compounded by the cause of death in 2 cases being cancer, in 3 cases being heart disease, and in 1 case being sepsis. These are all states associated with zinc deficiency (Passwater and Cranton, 1983). Considering these two criticisms of the control group this author feels that the research paper shows no more than that the DS children investigated had the same zinc levels as sick, old people.

Hair mineral analysis

Two pieces of research were found which used hair mineral analysis. Barlow et al (1981) compared the concentrations of zinc found in samples taken from DS patients with three sets of controls: other mentally retarded patients in the same hospital, hospital staff, and students in the locality. This broad range of controls is interesting. Barlow found that the concentration of zinc in DS subjects was not significantly different from that in the normal controls, but was significantly higher than that in the control patients. As both the DS patients and the control patients ate the same diet, the question that presents itself is whether the lower values found in these controls is representative of the dietary supply, and the DS subjects had high values because of retarded growth concentrating the zinc levels in their hair; or the DS patients' values reflected dietary intake and the control patients' values were low because of low zinc status. As zinc status is linked with mental health, this comparison needs further clarification before it is useful. The comparison with normal controls is more immediately accessible and does show a normal zinc status.

The second piece of research opens up the picture in an intriguing manner. Björkstén et al (1980) found values for hair mineral analysis and serum for both DS subjects and controls. The finding was that hair values were the same as the controls and the serum values were lower the normal. Can DS people have normal hair levels of zinc while their serum zinc levels are low? Why? Or is this data a reflection of the unsuitability of one or other of these methods for assessing zinc status? Unfortunately there is no information given about the controls, apart from them being healthy, which decreases the usefulness of the data. Björkstén et al also found elevated concentrations of zinc in DS blood clots, but this is a poorly validated index of zinc nutriture (Clinical Nutrition, 1980).

Zinc status in Down's syndrome

It is this author's opinion that taken as a whole the research to date indicates that it is likely that DS individuals have a low zinc status. Several papers reported normalisation of low zinc levels following zinc supplementation (Björkstén et al, 1980; Franceschi et al, 1988; Lockitch et al, 1989; Napolitano et al, 1990; Stabile et al, 1991; Licastro et al, 1992, 1993, 1994; Brigino et al, 1996; Trubiani et al, 1996; Bucci et al, 1999). This indicates a prevalence of zinc deficiency. If zinc status were already at an optimum level it is unlikely that homeostatic mechanisms would allow supplementation to raise zinc levels.

Assessing concentrations of zinc in body tissues is only one way of gathering evidence about the zinc status of DS people. Björkstén et al argue that "The presence of true zinc deficiency in Down Syndrome was supported by the clinical effects of therapy since the response to therapy with zinc is probably one of the most reliable indices for making a diagnosis of zinc deficiency in man" (1980). An example is Franceschi et al's work, which found a significant improvement in certain immune parameters in DS subjects even if their serum zinc levels were normal. In order to assess the effect of zinc supplementation it is necessary to define what responses are being measured, and these would be selected from recognised clinical conditions more often observed in DS people than in the normal population. Those conditions that have also been linked with zinc deficiency are the obvious place to start.

Importance of the Thyroid

Most investigators have found hypothyroidism in the DS population, with estimates of prevalence as high as 50% (Pueschel, 1990). This means that hypothyroidism is sometimes interpreted as being part of the 'Down's Syndrome Gestalt', and that thyroid function should be one system that is regularly monitored and treated as appropriate (ibid). Indeed much of the controversy surrounding the failure of researchers to replicate the success of Harrell et al (1981) in treating DS children with nutritional supplements hinged on the fact that the original work included thyroid hormone treatment, indicated to be necessary by the Barnes method. Investigators announced thyroid medication did not influence Harrell's final results (Smith et al, 1984), assessed thyroid function by T4 and TSH values and by thyroid palpitation (Bennett et al, 1983) — much less efficient and reliable methods than the Barnes method (Barnes and Galton, 1976) — or did not mention thyroid at all (Weathers, 1983; Bidder et al, 1989). These deviations from the original investigation are believed to nullify the usefulness of the repeat experiments (Rimland, 1983a and b; Davis, 1985), showing the importance of thyroid function when considering DS people. Pueschel also states that failure to recognise thyroid dysfunction early enough can lead to further disturbances to the central nervous system.

It should be noted that, although hypothyroidism is by far the most common thyroid dysfunction in DS and the one with which most researchers choose to work, a higher incidence than hyperthyroidism has also been reported (Pozzan et al, 1990). As certain parameters of thyroid function were universally found to be abnormal it is this author's opinion that both hypo- and hyperthyroidism in DS could very well have the same root cause.

Function of the thyroid and thyroid hormones

The thyroid hormones regulate oxygen use and basal growth rate, cellular metabolism, and growth and development. There are two thyroid hormones under consideration in relation to DS (the third, calcitonin, is unrelated), T3 and T4. T4, also known as thyroxine, is

manufactured in the thyroid from a glycoprotein, thyroglobulin, and is the inactive form. T3, triiodothyronine, is the active form and is made from T4. Reverse T3 (rT3) is also made from T4 but is inactive. This may be a mechanism for disposing of excessive amounts of T4. Thyroid hormones are hydrophobic molecules which usually travel in the blood bound to a protein - specialised alpha globulin or albumin - and are inactive until released. A tiny fraction of the total T3 and T4 is free T3 and T4 (FT3 and FT4), and it is the concentration of free thyroid hormones that determines the effectiveness of these hormones. As they are lipophilic, the thyroid hormones are able to diffuse across plasma membranes and bind to receptors within the cell. This binding increases a receptor's affinity for specific DNA sequences, controlling the rate of transcription of the appropriate gene. Secretion of T3 and T4 is stimulated by thyroid stimulating hormone (TSH, also known as thyrotropin), the secretion of which is stimulated in turn by thyrotropin releasing hormone (TRH). The release of TRH depends on blood levels of TSH, T3, glucose, and on the body's metabolic rate.

Thyroid dysfunction in Down's Syndrome

Research into thyroid function and zinc therapy in DS is still in its infancy and a clear picture has yet to form. The patterns that have emerged so far are that DS subjects exhibit:

- Elevated levels of TSH (Napolitano et al, 1990; Pozzan et al, 1990; Licastro et al, 1992, 1993; Sustrová and Strbák, 1994; Bucci et al, 1999)
- Reduced levels of rT3 (Lejeune, 1990; Licastro et al, 1992, 1993a)
- Normal levels of total T3 and total T4 (Franceschi et al, 1988; Napolitano et al, 1990; Pozzan et al, 1990; Licastro et al, 1992, 1993a)
- Only a small percentage of dysfunctional thyroids explained by antithyroid autoantibodies (Napolitano et al, 1990; Pozzan et al, 1990; Bucci et al, 1999)

The causes of these unusual ratios of hormones are as yet unknown. However, several authors make similar suggestions as to why TSH would be raised and T3 and T4 levels normal - i.e. why the thyroid has not been stimulated to release excess thyroid hormones. It is possible that there is some kind of dysfunction of communication between the hormone and its receptor. Napolitano et al mention some kind of resistance syndrome, Pozzan et al posit the possibility of a less active TSH, and Sustrová and Strbák also put forward the idea, amongst others, of a hormone resistance. Licastro et al (1992) have a different approach, suggesting that there may be an increased rate of T4 degradation in the periphery, and that the body needs to secrete increased amounts of T4 to maintain a homeostasis.

Licastro et al (1992) put forward two possible reasons for the lowered amounts of rT3 found in DS subjects: a decrease in formation T4 due to most of the T5 transforming into T3; or an increased conversion of rT3 into T2 (one of the precursors of T3 and T4). The latter is interesting as Lejeune states that excess SOD1 (discussed later) experimentally increases the transformation of rT3 into (inactive form) T2.

Sustrová's and Strbák's paper adds an interesting dimension to this picture as they separated their subjects by age into 3 groups: DS1 = 1-6 years, DS 2 = 6-15 years, and DS3 = 15-35 years. They found that all three groups had high TSH levels, high thyroxine binding globulin (TBH) levels, and low FT3 and FT4 levels. However, the T3 and T4 results told a more complicated story. DS1 had high levels of T3 and T4, DS 2 had high T3 and low T4, and DS 3 had T4 and T3 which were in lower concentration than the controls. Were the other researchers finding 'normal' levels of thyroid hormones because they were combining the

high levels of the youngest subjects with the low measurements of the oldest? This could be true of Napolitano et al and Bucci et al whose DS subjects spanned the Sustrová's and Strbák's groups, though Bucci did find a significant inverse correlation between age and both T3 and FT3 levels. Licastro et al (1993a) fail to give the ages of the "children" in their 1993 paper - they could be as old as 17 or 18- but in their 1992 paper their DS subjects are 6-15 years old. Though this is the same age as the DS 2 group, Licastro et al (1993a) found normal T3 and T4 levels, thus contradicting Sustrová and Strbák. However, the idea that thyroid hormone production declines with age in DS people is worth further investigation and if true would beg many questions. Does TSH effectiveness decline? Does the ability of the thyroid to manufacture its hormones decline?

How could thyroid dysfunction be associated with the pathology of Down's syndrome?

The thyroid hormones, along with insulin and human growth hormone, are responsible for accelerating body growth. It has been found that low rT3 levels could impair growth hormone stimulation (Lejeune), and that all DS children with elevated TSH exhibit more severe growth delay (Bucci et al). Increased weight gain often becomes apparent in many individuals with DS (Pueschel), a common symptom of hypothyroidism as the thyroid controls basal metabolic weight. Indeed Pueschel states that one cause of weight gain in DS is a decreased intracellular metabolic rate. Thyroid dysfunction interferes with the hypothalamic-pituitary-thyroid axis which modulates thymic activity, thus affecting immunity (Napolitano et al) and DS subjects are characterised by an unbalanced immune control including poor performance by the thymus (Serra and Neri, 1990). Both thyroid hormone deficiency (Barnes and Galton) and DS (Lejeune) are associated with mental retardation. Lejeune discusses the role of the thyroid in directing tubulin organisation, and points out that only three conditions exhibit neurofibrillary tangles: Alzheimer's disease, hypothyroidism, and DS. It is interesting that many of the physical characteristics of cretinism (extreme thyroid deficiency during foetal or early life) correspond with those of DS such as enlarged tongue, open mouth, broad face and flat nose.

What role does zinc play?

Altered zinc levels have been observed in both DS subjects and in hypothyroid patients when compared to controls (Napolitano et al; Bucci et al), though the roles zinc plays are only beginning to be teased out.

Thyroid hormone receptors require zinc ions (Licastro et al, 1992; Sustrová and Strbák) which facilitate folding into their active shape (Bucci et al). Zinc is also required for binding thyroid hormone receptors to the target DNAs, called thyroid response elements (Licastro, 1992; Bucci et al). A zinc deficiency may require more of a hormone to be secreted in order that enough is taken up. Sustrová and Strbák suggest that if the pituitary receptors were affected, normal thyroid hormone concentrations would not inhibit TSH secretion. This author wonders whether TSH receptors are rendered less active by zinc deficiency, meaning more TSH must be secreted to maintain a normal level of T3 and T4.

Zinc is required by thyroid hormone deiodinase, which modulates the deiodination activity vital for the homeostasis of the thyroid hormones. Thyroid hormone deiodinase converts T4 to T3, and removes the iodine ions from excess T1 and T2 (thyroid hormone precursors) to be

reused in the synthesis of more T3 and T4. Perhaps a zinc deficiency would affect the rate of conversion of T4 to rT3 via the deiodinising enzyme?

It is impossible to ignore the connection between the function of the thyroid and of the thymus, especially when discussing the importance of zinc. There is a close correlation between zinc, the thymic hormone and the pituitary-thyroid axis. Thymulin - the thymic hormone - is associated with an improvement in thyroid function (Bucci et al) and each thymulin molecule contains a zinc ion (thymic function is discussed in more detail further on).

Zinc may affect the action of the binding proteins that carry thyroid hormones, and this could interfere with the pituitary-thyroid axis (Napolitano et al).

Zinc deficiency appears to affect the utilisation of thyroid hormones in the peripheral tissues (Licastro et al, 1992).

What effect does zinc supplementation have on thyroid function?

Every piece of research this author found which measured the effects of zinc supplementation on DS thyroid function found significant changes. However these changes were different in almost every case, and sometimes contradictory. Each piece of research supplemented the subjects by os, that is 1mg of supplement per kg body weight per day. Though most stated the use of zinc sulphate, some, such as Licastro (1992), did not make clear whether the measurement was of elemental zinc or a zinc compound. This would affect the amount of elemental zinc the children were receiving. This could be one of the reasons behind variations in results. 1mg per kg per body weight is a high dose when compared with the governmental Estimated Average Requirement for the normal population. Using growth charts for children with DS (Cronk et al, 1988) to estimate bodyweight a 1-3 year old would be given 12.5mg while the EAR is 3.8mg per day, and a 15 year old would be supplemented with 55mg instead of the EAR 7.3mg.

In both their 1992 and 1993 papers, Licastro et al found that TSH levels returned to normal with zinc supplementation. Bucci et al note that it was the hypozincaemic DS subjects which exhibited high TSH and that they experienced a significant decrease after supplementation. Conversely, Napolitano et al found that TSH remained the same after 6 months supplementation and Sustrová and Strbák found that after a year of alternating three months with supplementation with three months off, ending on three months off, the TSH levels were found to rise. The only papers to measure rT3, both by Licastro et al, found a rise to normal levels after supplementation. These papers also found T3 and T4 remained the same while Napolitano et al found a rise in T3, and Sustrová and Strbák found a drop in T4. Napolitano et al found a drop in FT3 after zinc treatment, and Bucci et al found that FT4 levels reduced significantly, also reducing the FT4/FT3 ratio.

Clearly zinc deficiency is implicated in DS thyroid dysfunction, but to come closer to understanding its actions, the benefits of supplementation and the best kind of regime, it would be appropriate to:

- Subdivide the subjects into age groups (as with Sustrová and Strbák)
- Use hypozincaemic and normozincaemic DS people as subjects and controls (as with Bucci et al)

- If cycling treatment programme, take final measurements after a cycle being supplemented (such as Bucci et al who found that previously normalised TSH levels rose again after a period off zinc) rather than after a cycle without treatment (such as Sustrová and Strbák, who reported higher levels of TSH after supplementation)
- Measure all the hormones mentioned, i.e; TSH, T3, T4, rT3, FT3, FT4, perhaps even T1 and T2 to give a clearer picture of thyroid hormone manufacture and degradation

This author believes that more research is necessary before any conclusions can be drawn beyond that of zinc having an important and beneficial part to play. As it is important for the thyroid to be functioning optimally for growth and development from as early an age as possible research which observes the effects of zinc supplementation from birth could prove to be very useful.

Immunodeficiency - The Thymus

“Patients with Down’s syndrome suffer from frequent infections and have an increased mortality in infectious diseases compared to a normal population. Several laboratory studies have demonstrated abnormalities of cell-mediated and humoral immune capacity and of phagocyte function.” (Björkstén et al, 1980). As zinc is vital for the functioning of the immune system (Meek, 1996) it makes sense to consider the role of zinc when questioning why the DS immune system is weak, and how it can be supported. It has been found that young animals and humans, when receiving insufficient zinc, exhibit:

- **Rapid thymic atrophy**
- **Decreased production of thymic hormones**
- **Impaired lymphocyte proliferation after phyto mitogen stimulation**
- **a) decreased number of, and dysfunction of, T-lymphocytes b) abnormal T-helper and/or suppressor cell function**
- **Deficiency of natural killer cells**
- **Significantly reduced antibody and cell-mediated responses**
- **Delayed hypersensitivity reaction**
- **Decreased spleen and lymph nodes**
- **Generalised defective development of lymph tissue**

(Franceschi et al, 1988; Stabile et al, 1991; Brigino et al, 1996)

Therefore the first step is to investigate whether DS people also exhibit any of these dysfunctions. In fact DS individuals have many complex immune disturbances (Opitz and Gilbert-Barnes, 1990) into which research is only beginning to make inroads.

- It is in the thymus that immature T-cells differentiate and mature and thymic hormones are produced which perform such functions as inducing the expression of T-cell markers and modulating T-cell function. In healthy individuals, much of the thymic tissue is replaced by fat and connective tissue after puberty, and by maturity the thymus has atrophied — though it is still continuing to function. However in DS subjects there is rapid and premature thymic atrophy (Fabris et al, 1993), suggesting a reason for at least some of the characteristic immune weakness.

- Measurement of thymulin — a thymic hormone which induces expression of several T-cell markers and modulates T-cell function — has shown significantly lowered levels in DS (Franceschi et al, 1988; Licastro et al, 1994; Brigino et al 1996). Franceschi et al differentiated between DS normozincaemic children and hypozincaemic children, and found that both groups had low thymulin levels.
- DS is undoubtedly concurrent with a significantly reduced ability of lymphocytes when stimulated with mitogens (Björkstén et al, 1980; Franceschi et al, 1988; Lockitch, 1989; Stabile et al, 1991; Fabris et al, 1993; Licastro et al, 1994)
- A number of papers report a reduction in T-lymphocyte numbers and in distribution of subsets, though they are sometimes contradictory in how the distribution varies from normal (Noble and Warren, 1982; Franceschi et al, 1988; Fabris et al, 1993; Licastro et al, 1994; Brigino et al, 1996). In 1990, Serra and Neri reported “reduced expansion of T-cell precursors in the thymus, which results in incomplete T-cell subsets, a lowered response to antigens, and consequently a profound impairment of T-cell-mediated immunity”. Lockitch et al (1989) and Stabile et al (1991) found normal numbers of total lymphocytes and of total T-cells. Lockitch et al report finding lower T-lymphocyte, and lower T helper cell and T suppressor cell counts in earlier research. Interestingly Stabile used the most closely age-matched controls and subjects; unfortunately Lockitch does not give mean age or standard deviation for their subjects or controls. Lockitch did not investigate subset distribution, but Stabile found only elevated CD8+ (precursor of cytotoxic T-cells) levels and a raised ratio of CD8+ to CD4+ (precursor of T-helper cells).
- Fabris et al (1993) reports raised levels of natural killer (NK) cells and lowered NK cell activity. Stabile also found elevated levels of NK cells. Most other papers investigating DS and the role of zinc in immune potency do not mention NK cells, but Noble and Warren observed normal NK cell activity and Lockitch et al found normal NK cell numbers.
- No research was found directly investigating the activity of DS antibodies in relation to zinc status. Franceschi et al report low levels of B-cells, and both Stabile and Lockitch found normal levels.
- Only Licastro et al and Björkstén et al measured delayed hypersensitivity reactions, a class of allergic reaction where either CD4+ or CD8+ T cells secrete cytokines that activate allergen-eating macrophages. Both used harmless skin tests, and both found that their DS subjects had a diminished response.
- And 9. There is no work, at the time of writing, which investigates the development and functioning of lymph and spleen tissue in DS subjects in relation to zinc.

The significance of the thymus

“The majority of immune alterations observed in DS subjects seem to depend on defective thymic function.” (Fabris et al, 1993). This would include low levels of thymulin; a reduction in and/or disruption to the subset division of, T-lymphocytes; a reduction in B-lymphocytes (the proliferation of which is controlled by the T helper cells) and diminished delayed hypersensitivity — all part of what is known as the adaptive immune system. Napolitano et al (1990) claim that low zinc levels are responsible for the early atrophy of the thymus. One obvious link between the thymus and low zinc status is that zinc is required to transport vitamin A from the liver, and vitamin A is necessary for the growth hormone which maintains the thymus (Meek, 1996).

The importance of functional assays

Before the effects of zinc supplementation on these parameters is considered, a ground-breaking piece of research by Fabris et al in 1984 must be considered. Having realised that plasma concentrations of thymic factors are not necessarily a reliable index of the functional activity of the gland, the investigators measured the levels of active thymulin and the levels of thymulin inhibitory activity in young DS subjects and in healthy controls. They then added zinc sulphate in vitro and assayed again. The finding was that the DS individuals and the normal subjects over 50 years old had an inverse correlation between plasma active thymulin and thymulin-inhibitory activity. In normal people up to 20 years old thymulin levels were highest of all those measured and thymulin-inhibitory activity was not detected in healthy subjects until they reached 30. Conversely thymulin-inhibitory activity was high even in the youngest DS children. In both the elderly people and DS subjects plasma zinc was below the normal range for healthy adults. Once zinc sulphate was added to the plasma samples from both these groups the active thymulin levels become the same as those found in healthy young adults and the thymulin-inhibitory activity completely disappeared. These inhibitory factors have not yet been identified, though some experiments suggest an anti-thymulin antibody is involved. However, as Fabris et al point out, this is highly unlikely in their work, as there is a strict inverse correlation between the thymulin and the thymulin inhibitory activity, which is reversed by the addition of zinc sulphate. Their interpretation is that the inhibitory substance is in fact biologically inactive thymulin, which is still able to bind to thymulin receptor sites, and that the thymulin is activated by zinc. This is a very neat assumption, which accords with other research. Interestingly, though the DS active/inactive thymulin ratio was completely corrected by the addition of zinc sulphate, in the normal subjects this was only partial, suggesting that in physiological ageing other factors interfere with thymulin turnover. The overall picture is that DS people's thymuses do produce sufficient thymulin, that insufficient zinc is available to activate it, and, importantly, simply measuring levels of thymulin in the plasma was not telling the whole story. This paper plainly shows that measuring levels and counting numbers is not the same as assaying what is biologically available. In deed Lockitch et al acknowledge in their paper that "lymphocyte number and subset distribution are relatively static indexes of immune system capability and that functional assays such as in vitro antibody or interleukin production may be more sensitive indicators for future studies."

Does zinc improve the functioning of the immune system in Down's syndrome?

Franceschi et al found that zinc significantly raised active thymulin and lowered inactive thymulin, echoing Fabris's findings, but Brigino found no increase in thymulin levels despite normalised cellular zinc levels. These findings can not really be compared as Brigino used only 5 subjects, all of whom presented with recurrent infections such as pneumonias and chronic otitis media. It is quite possible that such conditions, which did improve with supplementation, were utilising the extra available zinc. Franceschi et al used 18 subjects and Fabris et al used 72, all off whom were basically healthy.

Zinc supplementation appears to increase lymphocyte proliferation (Stabile et al, Licastro et al, Brigino et al), which may be because zinc is essential for cell division. This is because DNA polymerase is the enzyme central to DNA replication, and cannot function without

zinc.

The aforementioned three papers also all found a reduced incidence of recurrent infections. Lockitch et al however, found no improvement in the frequency of infections. It is this author's opinion that the methods of assessing frequency of infectious episodes prior to and after zinc supplementation were far too inexact to be useful. For example Licastro et al asked parents to remember the number and type of infections their child had had the previous year and Stabile et al give no indication how they collected the information. The parents in Lockitch's investigation were instructed in detail how to fill in an infection log, which lends more credence to this research, but still relied on parental judgement on what would be considered normal. Possibly more objective means of assessing day-to-day infectious status, such as a daily temperature chart, should be investigated.

Other papers found improvements such as increased T-lymphocytes (Franceschi et al), improved skin responsiveness (Björkstén), or improved utilisation of interleukin 2 (IL-2) (Licastro et al), but until these tests are repeated it is not possible for this author to confidently comment on their validity. Lockitch et al found no improvement in any of the parameters they measured, and indeed found that lymphocyte proliferation decreased even further. The researchers themselves suggest that "although low doses raise serum zinc values, a much higher intake is needed to correct cellular deficiencies," but Licastro et al propose the possibility that "the zinc supplementation was given for a longer period (6 months in the [Lockitch] study). Prolonged zinc administration (6 months versus [Licastro's] 4 months) might suppress immune functions. An excessive zinc intake has indeed been shown to impair... lymphocyte [proliferation] in humans, polymorphonuclear migration response to the chemotactic factors and granulocyte phagocytosis of opsonized bacteria."

Dosage Levels

Thus it is still unclear what would be the basic zinc dosage to work from (moving up or down until an optimum dose is found). Most researchers based dosage on body weight, whether by os or a proportion of their own devising, sometimes referring to elemental weight of the supplement and sometimes referring to the compound weight. Others stuck with a set dose for all their subjects. Björkstén used 600mg (135mg elemental zinc) for all, meaning that the youngest, who at 8 years old would have been receiving about 24mg if dosed by os, were receiving a huge amount compared to those in Franceschi et al's and Licastro et al's papers. Lockitch's dosages did not rise correspondingly as weight rose, which may partly explain her failure to repeat other papers' successes. Perhaps only one or two of her subjects were receiving sufficient zinc to make a difference? The other paper to have little success with zinc supplementation was that of Stabile et al, and it should be noted that these investigators only gave zinc to the DS children with low serum zinc status. This author considers normal serum zinc to be a poor indicator to whether the DS immune system needs more zinc; perhaps if all the children in Stabile's research had been supplemented there would have been a higher proportion of children benefiting?

The risk of serious infectious disease is very real for the DS child, and, in this author's opinion, the research to date shows that zinc has a role to play in supporting the adaptive immune system and reducing infectious episodes. Zinc activates thymulin, and may increase lymphocyte proliferation, restore delayed hypersensitivity, and normalise other immune parameters. As too little zinc will not have the ideal effect and too much can suppress immunity, finding an optimum dosing regime is of immediate importance. This author also

wonders whether long-term zinc supplementation, started early, would slow thymus atrophy and if this would increase the T-lymphocyte pool. Perhaps other lymph tissues would also benefit from long term supplementation? Before such research can ethically take place it is vital that the optimum dosage for DS infants is set. The other aspect of this research that needs to be improved is the method of assessing infectious episodes, finding more objective methods that can be used daily by parents.

Immunodeficiency - Leukocyte Function

It has been found that the chemotaxis, phagocytosis, and other anti-foreign microorganism actions of leukocytes are reduced (Fabris et al, 1993). Licastro et al (1993b, 1994) investigated the ability of DS neutrophils to produce chemically active molecules, such as the superoxide anion, when stimulated. They observed that chemical activity was low before supplementation but after zinc therapy neutrophil reaction to a stimulus was normalised. The authors point out that protein kinase C is believed to play an important role in the activation of human neutrophils, including superoxide generation, and that the activity of protein kinase C is regulated by zinc ions. Possibly the actual fault is an impaired activation of protein kinase C because of a poor supply of zinc ion. Rates of infection amongst the subjects were found to decrease with supplementation, but the same problems with this data apply as previously discussed, especially the compilation of a record of each child's infection history for the year preceding the experiment from what the parents recalled.

Neutrophil chemotaxis was also found to be reduced in DS patients by about 30%, but normalised after zinc therapy (Björkstén et al, 1980). Björkstén et al relate zinc enhancement of neutrophil activity to possible membrane phenomena and also mention intracellular activity, as leukocyte locomotion is very complex. Licastro et al (1993) also followed up their subjects a year after ceasing zinc therapy and found that neutrophil activity had dropped again, supporting their assumption that zinc had improved their functioning. Unfortunately Björkstén did not follow up his subjects so a similar comparison is not available.

That zinc supplementation may improve neutrophil deadliness is very important in attempting to understand how to reduce the incidence of infection in DS people. Further investigation to expand understanding in this area could prove very fruitful, including investigation into the role of protein kinase C and its relationship with zinc.

Maturation of blood cells

Differentiation is the process by which subsets of a family of cells are formed from parent, or 'stem' cells by the acquisition of specific functions. It is of particular importance in haemopoiesis, the process by which blood cells are formed, and the consequent development of T-lymphocytes. Differentiation is a gradual process with cells passing through various stages before achieving maturity, and is stimulated by various growth factors. Apoptosis is a mechanism of programmed cell death, which occurs as a response to an external factor or the withdrawal of a growth factor. It is an important part of cell differentiation for both the immune system and haemopoiesis as it culls excess cells to maintain a suitable subset

balance. The enzymes concerned in breaking apart DNA fragmentation during apoptosis are endonucleases.

Inefficient cell maturation

DS individuals have been found to have an unusual presence of immature myeloid cells (found in the earliest stages of haemopoiesis) in peripheral blood circulation associated with low levels of zinc plasma (Trubiani et al, 1996a, 1996b). Both pieces of research found that six months of zinc therapy induced the disappearance of the immature myeloid cells, but the authors offer different possible explanations:

- That zinc is required for the process of programmed cell suicide: “ The results here show that zinc therapy in Down’s patients induces cell death of undifferentiated and ineffective myeloid cells, recovering a mechanism related to cellular differentiation of the haemopoietic system” (1996a)
- That zinc is required for normal cell differentiation: “Since leukocytes contain high levels of zinc and this level varies with cell maturity, being lowest in the most immature cells, we suggest that low plasma zinc levels in DS subjects could be responsible for a reduced rate of myeloid differentiation resulting in accumulation and in escape from the bone marrow of immature cells reaching the peripheral blood” (1996b)
- These reasons are not mutually exclusive and most likely zinc supplementation is supporting both processes (Trubiani et al, 1996a).

Inappropriate apoptosis

A paper was published the following year, by essentially the same researchers (Antonucci et al, 1997), which evaluated the presence of apoptosis in the peripheral blood cells in DS subjects before and after six months of zinc supplementation. It was found that there were signs of programmed cell death before the zinc treatment, which decreased in all the patients following supplementation. The authors state that endonucleases are inhibited by normal plasma zinc concentrations and suggest that in DS individuals low levels of plasma zinc activate the endonucleases. Zinc therapy would therefore inhibit the apoptotic process leading to a decrease in the number of apoptotic cells.

Meanings of these results

It would appear that programmed cell suicide is happening at an inappropriately high rate in mature DS peripheral blood cells. This author believes that when combined with the weakened cell maturation process discussed above these findings represent a serious decrease in the numbers of healthy, efficient blood cells in circulation, and the apparent success in treating this deficiency with zinc has important implications for supporting DS people’s health. Murphy et al (1995) found that DS spleens are markedly missing T cells “suggesting the inefficient release of mature T cells from the DS thymus to the DS spleen”. Could this lack of mature T cells be related to a poor rate of stem cell maturation? Or to a rapid destruction of healthy, mature cells by a high rate of apoptosis? Or both? How does this confusion of the cell differentiation process relate to the 10 to 20 times higher risk DS people have of developing myeloid leukaemia (Milunsky et al, 1970)? The question must also be asked as to why these scientists found that zinc deficiency appears to reduce programmed cell

death in immature cells and promote it in mature cells. It is unfortunate that at present one group of investigators has done all the research and it is hoped that in the future other scientists will pick up the baton.

DNA Repair

Premature ageing is a universal problem for DS people, including a far higher risk of developing Alzheimer's disease (Opitz and Gilbert-Barness, 1990). This observation suggests a fault in the integrity of the DNA repair system. Chiricolo et al (1993) investigated whether zinc supplementation affected the maintenance of DNA integrity by damaging lymphocyte DNA with radiation *in vitro*, before and after four months of zinc supplementation, and observing the rates of repair. The finding was that before supplementation the DNA damage (which was normal) was repaired extraordinarily rapidly when compared with the cells from normal children. The authors suggest that persistent oxidative stress may mean that DNA repair enzymes are activated in higher numbers in DS, a possible explanation for the increased rate of repair. It may even be that zinc deficiency contributes to this oxidative stress. They also suggest that the high speed of repair is likely to mean more mistakes are made and that "it could contribute to neurodegeneration and precocious ageing which are both hall-marks of the system." (Chiricolo et al). After the period of zinc therapy the damage received by the DS lymphocytes was the same as before, but the rate of DNA repair was significantly reduced, back down to a normal, and presumably more accurate, speed.

Thus the paper shows that zinc does not appear to have a protective effect against DNA damage - at least not radiation damage - but rather modulates the speed of repair and so probably its accuracy. The authors speculate that this is because four months of zinc therapy was enough time to reduce the oxidative stress, but this author believes it is also possible that zinc is a requirement for one or more enzymes which regulate DNA repair. Possibly this could be investigated *in vitro*, without zinc supplementation *in vivo*, to observe the effect of immediate availability of zinc ions rather than a slow build up of effects associated with a gradual rise of zinc status to normal. Though this is only one paper it appears to be the first that demonstrates a nutritional intervention apparently affecting DNA repair, and so is a ground-breaking paper.

Growth Delay

"Growth retardation is a cardinal characteristic of Down syndrome" (Annerén et al, 1990). It is not known what the underlying mechanism is responsible for the retardation of growth. DS children have normal levels of human growth hormone (hGH) and low levels of somatomedin C — also called insulin-like growth factor (IGF-1). IGF-1 is regulated by hGH postnatally, and it appears that in DS there is a delayed, possibly incomplete, transfer from foetal IGF-1 to the hGH regulated IGF-1 (Annerén et al, 1990). Growth is mainly restricted between the ages of 6 months (when hGH starts to regulate growth) and 3 years. Some, but not all, researchers have found that growth after this age is near to normal, just starting from a smaller stature (Annerén et al, 1993). Napolitano et al (1990) found that the level of IGF-1 rose after zinc supplementation, especially in children over 7. Zinc levels diminish with age in DS people, as with normal people, though this happens earlier in DS, so it is conceivable that the results were more pronounced with older children as they had a greater need for extra zinc.

The effect of zinc supplementation

Strangely, despite the well documented link between zinc deficiency and growth delay (Passwater and Cranton, 1983), this author found only one paper investigating the effects of zinc supplementation on growth in DS children. Napolitano et al studied 22 DS children whose growth velocity was calculated for the six months before the period of supplementation and during the six months of therapy. They were supplemented by os, and their rate of growth compared with special growth charts for DS children (Cronk et al, 1988). It was found that 15 of the subjects moved into a higher centile in their growth charts and, interestingly, that the children older than 7 years showed a greater differential in growth velocities before and during supplementation than the children aged 4-7 years. This particularly noteworthy when compared to the finding that suppressed growth occurs earlier. Unfortunately, no children under 3.8 years were included. The results for children under 4 years, and over 10 years (girls) or 12 years (boys) were considered separately to avoid interference by first childhood and pubertal growth spurts.

This author considers that there could be huge potential for zinc therapy to minimise growth retardation. Zinc is essential for DNA and RNA polymerase and thus for cell proliferation; it is necessary for protein metabolism; and as it is essential for the functioning of hormone receptors (such as protein kinase C) zinc is possibly required for hGH and/or any of the hGH regulated growth factors to function efficiently. The results of Napolitano et al's work are encouraging, but need to be repeated with measurements of the foetal variant form of IGF-1, and assays into numbers and activity of hGH binding sites in DS would be useful. Further research using younger children would be extremely valuable in illuminating the role of zinc in hGH functioning, transfer to hGH regulated IGF-1, and growth delay. Napolitano et al suggest the possibility that thymus hormones may have a role in regulating the secretion of hGH in DS children. Considering the importance of the thymus and thymic factors already discussed, this link raises many more possibilities.

Gene over-expression as a drain on zinc

Extra SOD-1 gene

The best known gene mapped to chromosome 21 is that for copper-zinc superoxide dismutase (SOD-1), and it is estimated that 99% of DS people have three copies of this gene (de Haan et al, 1997). Most researchers found an elevated level of zinc in the erythrocytes (Milunsky et al, 1970; Nève et al, 1983, 1984; Purice et al, 1988) and as there is believed to be a 50% increase in SOD-1 activity in DS subjects (Jezirowska et al, 1988), it seems "highly probable that the increase of red cell... zinc levels in trisomy 21 is partly attributable to the increased SOD-1 activity" (Nève et al, 1983). Kadrobova et al point out that "adaptations for permanent oxidative stress and changed biochemical functions in DS may lead to the increased requirements of an organism for zinc" (1996). This means that it is highly likely that DS people have an increased need for zinc as they use a larger percentage of that available for manufacturing SOD-1. It also alludes to the effects of increased SOD-1 activity.

The function of SOD-1

The task of SOD-1 is to remove the superoxide radical, but it performs only half of the process of clearing up by transforming the free radical into a much less volatile free radical called hydrogen peroxide. It is glutathione peroxidase (and possibly catalase) which finishes the process by dismantling hydrogen peroxide, otherwise prone to forming the hydroxy radical, the most destructive of the free radicals. While SOD-1 activity is elevated because of gene dosage, glutathione peroxidase and catalase are only present in the normal quantities, quite possibly leading to an accumulation of hydrogen peroxide which could result in considerable oxidative damage.

Zinc as an antioxidant

As well as its role in the formation of SOD-1, zinc has an antioxidant effect in its own right, and stabilises cell membranes (Kadrobova et al, 1996). It is possible that the permanent oxidative stress of excess production of hydroxy radicals uses up a higher proportion of zinc than is used for antioxidant activity in normal people. If oxidative stress were to be a drain on zinc resources it could be expected that exercise would increase this load, as exercise increases oxygen metabolism thus increasing the production of free radicals. Two pieces of research look at the effects of exercise on the plasma and erythrocyte zinc levels. The first, by Laires et al (1994) found that exercise did not change these measurements, but the period of exercise was only twenty minutes rowing. As the paper reports that DS people find such a length of time of concentrated exercise difficult, it is quite possible that many subjects either did not complete twenty minutes, or did so in smaller sections. The second paper, by Monteiro et al (1997), looks instead at the effects of aerobic exercise over the period of time. The subjects' regime was increased gradually until they were performing 25 minutes of aerobic activity 3 times a week. After 16 weeks their plasma zinc and erythrocyte zinc levels were compared with their starting values, and the plasma zinc was found to be significantly lower. The authors suggest either an activated expression of antioxidant mechanisms or elevated sweat loss as possible reasons. This author considers sweat loss less likely because sweat, as well as having a cooling action, is a mechanism for excreting excess ions. There is no evolutionary advantage to excreting a trace element that is already in short supply. There is of course a possibility that DS people have a faulty sweat mechanism but this author found no mention of this in relation to zinc status in any papers. Thus this author considers this research supportive of the possibility that excess SOD-1 is placing a drain on zinc supplies.

Not all DS subjects express excess SOD-1

It should be added that some researchers have found that it is possible for the clinical features of DS to exist without elevated levels of SOD-1 (Jeziorowska et al, 1988; De La Torre et al, 1996) — a reminder that “among numerous abnormalities reported in DS no finding except for the extra chromosomal material is constant” (Jeziorowska et al). However it is this author's opinion that excess SOD-1 is likely to be a factor in the majority of DS people.

Over-expression of other proteins

Another known gene on chromosome 21 is that for the protein subunit S100 β . An increase of this could also increase the requirements for zinc (Lejeune, 1990). According to Lejeune it is

also possible that there is excessive adenosine formation, mainly produced by a zinc-requiring enzyme, which could be another drain on the available zinc. Trubiani et al (1996) also mention other zinc binding proteins which appear to be over-expressed in DS subjects: polymerase and various kinases which are necessary for cellular metabolism and differentiation.

The possibility of malabsorption

A common explanation for nutritive deficiencies in subjects with a normal diet is that of malabsorption, and several authors consider it a possible reason for low serum zinc in DS (Bruhl et al, 1987; Kanavin et al, 1988; Licastro et al, 1993). Sylvester (1984) considers malabsorption to be a significant reason behind low levels of nutrients. He states that the shortages of some trace metals to which DS people are prone are lifelong, and points out that their absorption from the intestines, measured using the xylose absorption test, has been found to be reduced. Abalan et al (1990) measured the absorption in 4 DS patients by microscopically examining their stools for meat fibres after a measured diet. The meat was minced to negate the effect of insufficient chewing due to poor dentition – DS children are prone to abnormalities of tooth formation, and to periodontal disease (Pueschel, 1990). The finding was a high meat fibre count, strongly supporting the malabsorption hypothesis. As Abalan et al point out, this test does not indicate what the cause of the malabsorption could be, suggesting as possibilities pancreatic insufficiency, reduced intestinal absorptive capacity, or other causes. This author would add hypochlorhydria, and food allergies as possible causes. In the wake of reports of a high incidence of coeliac disease in DS, Strong (1993) performed a study which found all of his ten subjects displayed raised IgE and IgG to one or more of 13 common food allergens. Though only a preliminary study this indicates a possible role of allergies in DS malabsorption. Both of these studies are small-scale, uncontrolled investigations; larger, controlled investigations would provide valuable data regarding DS absorptive status.

A cautious note is struck by Licastro et al who point out that some elements, such as copper and magnesium, are found in the normal range in DS, an argument against malabsorption as an explanation for low zinc levels.

Transportation

Zinc ions need to be bound to a carrier to travel in the bloodstream. Another possible explanation for decreased plasma zinc status is “a perturbation of the plasma zinc transport due to a defect in its transport protein (Purice et al, 1988). Usually, approximately 80% is associated with albumin, 15-19% is associated with alpha-2-macroglobulin and much smaller amounts (less than 1%) are associated with amino acids or metalloenzymes. It has been suggested that alpha-2-macroglobulin is a transport protein for zinc and that, as DS adults have been found to have abnormal levels of this protein, this may explain the low levels of zinc found in their plasma (Halstead and Smith, 1970; Purice et al, 1988). The problem with this suggestion is that the research that found these low levels of alpha-2-globulins was performed in the 1950's and 1960's, using institutionalised patients. Liver disease amongst such patients occurred frequently and this could well have affected the formation of alpha-2-globulins (Milunsky et al, 1970). Research into the relationship between zinc status and transport proteins is surprisingly patchy, especially since the 1970's, considering how important it could be. Milunsky et al found that plasma zinc was low in DS children, all living at home, and that their alpha-1 and alpha-2-globulin levels were normal. Annerén and

Gebre-Medhin (1987) assayed plasma zinc, alpha-2-macroglobulin, albumin, and other serum proteins in home-residing DS children, using their siblings and other healthy children as controls. It was found that the DS children had significantly higher alpha-2-macroglobulin concentrations than their siblings, but similar albumin concentrations. The authors could find no correlation between the amounts of circulating zinc and alpha-2-macroglobulin, a state they believe points to a 'true' zinc deficiency. The suggestion is that there is a high concentration of alpha-2-macroglobulin because of subclinical infections. However the activity of the alpha-2-macroglobulins has not been measured. Could the alpha-2-globulin be failing to transport zinc satisfactorily? Could the presence of a fault in the protein being produced mean that a negative feedback loop is not being completed and the liver continues to make more? There were found to be normal concentrations of albumin, but it is possible that albumin is not working efficiently. It is also possible that carriers are transporting the wrong ions; the concentrations of heavy metals is beyond the remit of this paper, but it is known that cadmium, aluminium, and copper are zinc antagonists and can replace zinc ions. This author believes that problems with transport proteins could play a role in DS low zinc status, and the way forward is to measure the activity of all the proteins known to be involved.

Food choices

One possible reason behind low zinc status in DS subjects which has been not just completely, but deliberately, over looked is that of food choices and eating habits. Most papers do not mention this aspect at all, but those that do dismiss it in one sentence. Two typical examples are:

- Bruhl et al, working with institutionalised subjects: "A diet prepared to Recommended Dietary Allowance standards was offered to all" (1987)
- Annerén et al, working with children living at home: "No systematic investigation of the dietary intake was performed, but an interview did not reveal any obvious deviations in dietary habits, pica phenomena or food preferences" (1985)

As Pipes (1992) points out, just because food is offered to institutionalised patients it does not mean it is consumed. Patients can choose what to eat, probably unobserved, when there are other retarded patients who also require assistance. And asking parents whether they regard their children's diet as normal is not very illuminating. Mention has already been made of the poor dentition from which most DS people suffer, and it would not be unreasonable to presume that food choices would be amongst those foods easy to chew. DS children develop more slowly than normal children, and spend longer in developmental stages (Cunningham, 1988), meaning that weaning could be a problematic, drawn out process for parents. Children are drawn to sweet foods, and as taste develops will often reject other foods the first time they are tried before accepting them on reintroduction (Bennett, 1988). If meal times are hard work for a parent it is easy to see that they could begin to rely on the foods first accepted instead of continually trying to reintroduce other foods, especially once the DS child is of an age when a normal child would be feeding itself. Once food habits are formed it is very unlikely they will change to much degree (*ibid.*).

It is worth noting that almost all foods known to be good sources of zinc require thorough chewing, including meat (though the antagonistic effect of iron may negate meat's usefulness), wholegrains, canned fish (with accompanying problematic small bones), nuts and seeds, and shellfish. Only eggs can be prepared in a way that is easy to masticate. "Clinical experiences of the author have indicated that low blood levels of zinc... in

preschool children with DS have resulted from inadequate dietary intake” (Pipes, 1992). Amongst older children and young adults with some responsibility for food choice and preparation, mental retardation could mean it is difficult for them to understand the concept of a balanced diet. “Individual observations indicate that many convenience and already-prepared foods are used. An abundance of sweet foods of high calorific density are consumed and little support is given in helping the individuals in selecting an adequate diet” (ibid.). A diet high in sugar will affect zinc status not just due to sugary foods taking the place of zinc-rich foods in the diet, but because sugar increases the excretion of zinc, as the zinc-containing hormone insulin is required to rebalance blood sugar. There is the possibility that DS affects the taste buds, which would also have an effect on food choices. In all the literature this author read, only Pipes even mentioned food choices and eating habits in DS, and she found them to often be disturbed or inadequate. It is vital that this area is investigated, and in this author’s opinion should be a priority, when considering reasons for zinc deficiency in DS.

Conclusion

The aim of this paper was to determine whether DS people suffer zinc deficiency, what symptoms they exhibit which could be the result of zinc deficiency, whether zinc supplementation improves any of these symptoms, and what factors could cause zinc deficiency in DS. The conclusions have been drawn from a critical review of all the research papers available on the subject of DS and zinc.

It appears that the majority of DS individuals are zinc deficient, and that they exhibit symptoms classically associated with zinc deficiency:

- **Thyroid dysfunction**
- **Immunodeficiency**
- **Retarded growth**
- **Faulty DNA repair**

Supplementation will raise DS zinc status to normal. Correction of zinc deficiency seems to:

- **Improve thyroid function, though it is not clear yet what parameters are being affected**
- **Raise active thymulin levels and concomitantly lower inactive thymulin**
- **Possibly increase lymphocyte proliferation**
- **Possibly restore delayed hypersensitivity**
- **Improve neutrophil function**
- **Possibly normalise lymphocyte subset distribution**
- **Possibly minimise growth retardation**
- **Regulate DNA repair**
- **Regulate myeloid cell differentiation and apoptosis**

There several possible reasons behind DS zinc deficiency, which are most likely to be working in some combination:

- **Over-expression of genes**
- **Malabsorption**
- **Dysfunction of transport proteins**

- **Food choices**

It is difficult to recommend an optimum strategy for zinc supplementation as researchers use different regimes. However dosing by os is the most common method and therefore known to be safe, and as effective as any other method. As it is possible for too much zinc to suppress the immune system it would be wise to regularly test an immune parameter, such as lymphocyte proliferation, or to alternate periods of supplementation and periods without. It is possible that zinc therapy could benefit DS babies from birth but at present it is not possible to recommend this as there is no evidence even as to its safety.

It may be that the typical DS diet is low in zinc-rich foods, and it is vital to find ways of preparing such foods so as to appeal to a DS person and to educate individuals to incorporate such food in their diet.

It could also be sagacious to investigate such potential sources of malabsorption as food allergies, hypochlorhydria, and pancreatic insufficiency.

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