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## **70th Anniversary Conference on ‘Vitamins in early development and healthy aging: impact on infectious and chronic disease’**

### **Symposium 1: Vitamins and cognitive development and performance Vitamins and neural and cognitive developmental outcomes in children**

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The role of vitamin status in the development of the brain and the subsequent functioning of the brain was considered. There are data with a range of vitamins, from animal studies and human studies in developing countries, suggesting that a clinical deficiency during the critical period when the brain is developing causes permanent damage. To date there is, however, with the exception of cases of clinical deficiency such as those that might be associated with a vegan diet, little evidence that variations in the diet of those living in industrialised countries have a lasting developmental influence. Similarly, later in life clinical deficiencies of various vitamins disrupt cognition although there is to date limited evidence that variations in the intake of single vitamins in industrialised societies influence functioning. It may well be, however, unreasonable to expect that vitamins examined in isolation will be associated with differences in cognitive functioning. The output of the brain reflects millions of metabolic processes, each potentially susceptible to any of a range of vitamins. A diet poor in one respect is likely to be poor in other respects as well. As such, the preliminary reports in double-blind placebo-controlled trials that aspects of cognition and behaviour respond to supplementation with multi-micronutrients may indicate the way forward.

#### **Brain development: Cognition: Intelligence: Multi-vitamins: Vitamins**

Vitamins as essential nutrients inevitably play a role in the initial development of the brain, and as co-enzymes influence metabolism throughout life. In fact as the brain is the most metabolically active organ in the body<sup>(1)</sup> it will be potentially among the first to reflect an inadequate diet. However, although in principle an inadequate supply of vitamins can influence both the development of the brain and its subsequent functioning the important question is how often, if at all, is the diet deficient to the extent that a problem develops? Although problems are inevitable in developing countries when the diet is grossly inadequate, does the provision of vitamins in industrialised countries give grounds for concern?

When considering cognitive development sensitive and critical stages of development are often distinguished; periods when environmental conditions, such as diet, have a long-lasting influence. A critical period has a relatively

precise beginning and end during which the body is susceptible to a particular environment condition, whereas a sensitive period is of longer duration. It is known that a dietary deficiency at a critical stage of development can result in permanent changes in brain structure and therefore cognitive functioning. Good examples include protein and energy malnutrition or a deficiency of Fe or iodine<sup>(2)</sup>, where a dietary insufficiency while the brain is developing adversely influences cognitive functioning for the rest of life. The possibility of similar roles for vitamins will be considered. Do deficiencies of the mother's diet, or the infant's diet in the first months of life, cause permanent structural damage with consequences for cognitive functioning?

There is increasing interest in metabolic programming, more specifically whether the level of vitamin intake during pregnancy can have long-term implications. An

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imbalance between folate and vitamin B<sub>12</sub> can increase the incidence of later insulin resistance<sup>(3)</sup>. Male rats whose mothers during pregnancy were fed a diet containing ten times the recommended level of a range of vitamins were more likely to develop aspects of the metabolic syndrome in adulthood, possibly reflecting changes in the ability of the brain to control food intake<sup>(4)</sup>. A study in India related nutritional status during pregnancy to outcomes measures at 6 years of age. Mothers with low levels of circulating vitamin B<sub>12</sub> while pregnant had offspring with a high prevalence of insulin resistance<sup>(5)</sup>. To the extent that such an inadequate vitamin status in later life predisposes to the poorer glucose tolerance associated with the metabolic syndrome, poorer memory could potentially result. Particularly in older adults, but also in younger individuals<sup>(6)</sup>, there is an association between poorer glucose tolerance and a poor memory. Such examples suggest one way in which the effect of diet on cognition may potentially take place after a considerable delay. The role of folate status in the incidence of neural tube defects<sup>(7)</sup> is well established but during pregnancy deficiencies of folate, riboflavin, vitamin B<sub>6</sub>, vitamin B<sub>12</sub> are associated with increased levels of homocysteine, a risk factor for low birth weight and pre-term delivery<sup>(8)</sup>. Low birth weight is associated with lower intelligence in later life<sup>(9)</sup>. A double-blind trial gave vitamin C and E supplements to pregnant women with pre-eclampsia and found that they decreased the incidence of the disorder<sup>(10)</sup>. It has, however, been emphasised that we need to consider the time when dietary intake is manipulated. Although conception, gestation and lactation represent a continuum, a nutritional insult will have different outcomes depending on when it occurs<sup>(8,11)</sup>.

As with the rest of the body the brain is potentially susceptible to problems resulting from dietary inadequacies at key stages of development. In fact, it can be argued that the brain is likely to be particularly susceptible to dietary status as in the peri-natal period it grows particularly rapidly, more quickly than the rest of the body. At birth although the infant is about 6% of the adult body weight, the brain is already 25% of its final weight. By 2 years of age the brain will be about 77% of its final weight while the body is about 20% of the adult level<sup>(12)</sup>. Such a rapid rate of growth inevitably places demands on the diet to supply the molecules required for brain development. During pregnancy the mother must be well nourished, and nutrition during the first 2 years of life is crucial. However, although the brain initially grows rapidly its development continues into the teenage years raising the possibility that the nature of diet at later ages may be influential. As judged by head circumference, cortical thickness, degree of neuronal branching and the electroencephalograph profile, periods of brain growth have been described at 7, 12 and 15 years of age<sup>(13)</sup>. At such times it is possible, using randomly controlled trials, to examine the influence of supplementation with either single or multiple vitamins, although the question has been almost totally ignored.

Although nutritional status at these early stages may potentially have implications for the development of the brain's basic structure it may also have implications for general metabolism throughout life. The adult brain is about 2% of the weight of the body, yet it accounts for

about 20% of BMR. In addition, the brain tissue of children, below 10 years of age, uses glucose at twice the rate of the same amount of adult brain. Given the considerable metabolic activity of the brain<sup>(1)</sup> its moment to functioning will depend in part on an adequate supply of micro-nutrients acting as co-enzymes. The question arises as to whether minor differences in vitamin status influence the brain and hence mood, cognition and behaviour?

Thus, the diet is responsible both for the building blocks from which the brain is constructed and the energy required for it to function. However, all such analyses demonstrate that the diet has the potential to influence intellectual functioning: the important question is how often, if at all, is the diet in industrialised countries deficient to the extent that a problem develops? Initially a series of individual vitamins will be briefly examined and then whether multivitamin administration is influential will be considered.

### Vitamin A

Vitamin A plays a critical role in visual perception and a deficiency is the leading cause of childhood blindness in developing countries. It has been estimated that 127 million preschool-aged children and seven million pregnant women are vitamin A deficient<sup>(14)</sup>. A deficiency increases the risk of many problems, including diarrhoea, respiratory disease, measles and can result in death. Vitamin A also has a role in cell differentiation, the immune system and reproduction. Deficiency is particularly important during periods of rapid growth, both during pregnancy and in early childhood. Globally, 4.4 million preschool children have xerophthalmia (dry eye) that is caused specifically by a lack of vitamin A. Six million mothers suffer night-blindness during pregnancy. There is clear evidence that severe vitamin A deficiency can impair the visual system<sup>(15)</sup>. Vitamin A in combination with specific proteins (opsin) forms rhodopsin or visual purple. It is found in both types of photo-receptors in the retina, although in greater quantities in rods rather than cones. Under conditions of mild to moderate vitamin A deficiency dark adaptation is compromised and night-blindness results. With more severe and prolonged deficiency the eye becomes dry; small foamy spots form over the conjunctival surface, so-called Bitot's spots; ulcers may develop and total blindness result.

Vitamin A derivatives, retinoids, are involved in a complex signalling pathway that regulates gene expression. As the fetus develops it must not be exposed to too much or too little vitamin A, as either can have teratogenic consequences<sup>(16)</sup>. Pregnant women in industrialised countries should ensure that any vitamin supplements do not result in too large an intake.

The role of vitamin A in the brain is poorly understood. The retinoids control the differentiation of neurones and a role has been suggested in memory, sleep, depression, Parkinson disease and Alzheimer disease<sup>(17)</sup>. For example, studies in the rat have found retinoid receptors in the hippocampus, an area of the brain important for memory, and that vitamin A deficiency disrupts memory<sup>(18)</sup>. We await evidence of similar effects in human subjects, although

they are most likely to be observed in those in developing countries with a clinical deficiency. Given the typical intakes in industrialised countries it is less likely that variations in vitamin A intake within the normal range will have functional implications.

Vitamin A deficiency is also associated with an increased risk of morbidity, mortality, wasting and stunting in children. Reductions of child mortality of 19–54% following vitamin A treatment have been reported<sup>(14)</sup>. A meta-analysis of trials of vitamin A supplementation found a decrease of 23% in mortality<sup>(19)</sup>. Although in those with clinical signs of deficiency growth is delayed, when a review considered seven double-blind trials of vitamin A supplementation they found little or no evidence that it increased the rate of growth<sup>(20)</sup>. However, the evidence was not sufficient to comment on the benefit to children with clinical symptoms of vitamin A deficiency. Indirect benefits may result. In Sri Lanka, Mahawithanage *et al.*<sup>(21)</sup> examined the influence of vitamin A supplementation on the absenteeism of school children. In a randomised controlled trial over a period of 13 months, vitamin A status improved school attendance but not the anthropometric status of these children.

An examination of vitamin A status in the US National Health and Nutrition Examination Surveys I to III found some but not many individuals with low values<sup>(22)</sup>, but values not so low that there was a high likelihood of functional impairment. These low values were more common in blacks than whites and those below the poverty line.

As supplementation increases the levels of serum retinol in those with baseline values less than 1.05 µmol/l, this value has been taken as an indication of potential sub-optimal status. In the USA 16.7–33.9% of children aged 4–8 years and 3.6–14.2% of children aged 9–13 years, depending on sex and racial/ethnic group, had serum values below this level<sup>(23)</sup>.

A Cochrane review<sup>(24)</sup> considered forty-three trials, involving in total 215 633 children; it concluded that the supplementation of children aged 6 months to 5 years with vitamin A can reduce death and diseases. In total, it reduced the risk of death by 24%. They recommended the universal supplementation in areas at risk of vitamin A deficiency although further trials are needed to compare different doses and means of delivery.

### Thiamine

There is evidence from animal studies that the developing brain is influenced by a reduced thiamine intake. The activity of three thiamine-dependent enzymes was measured in the brain tissue of the offspring of mothers fed a thiamine-deficient diet<sup>(25)</sup>. In all cases enzyme activity was lower, although replenishment of the vitamin restored enzymic activity to normal. It was noted that as thiamine-dependent enzymes play an important role in establishing adult patterns of brain energy metabolism and in myelin synthesis, there could be serious metabolic consequences if a deficiency occurs at a critical period of brain development. Similarly, animals born to thiamine-deficient mothers displayed lipogenesis<sup>(26)</sup>.

An Israeli study examined the impact of thiamine deficiency during early infancy on aspects of language at 5–7 years of age<sup>(27)</sup>. The children had been fed during their first year a milk deficient in thiamine, resulting in a high rate of problems with syntactic and lexical retrieval. Ninety-seven percent of thiamine-deprived children had language impairment compared with 9% of controls. It was suggested that thiamine deficiency in infancy causes long-lasting language impairment. Similarly, in a study of the offspring of Korean mothers suffering with thiamine deficiency, visual alertness was better in infants whose mothers had received thiamine supplements<sup>(28)</sup>.

It is accepted that the thiamine requirements of the mother are increased during pregnancy and lactation, and that in the third trimester of pregnancy the vitamin is preferentially taken up by the fetus<sup>(29)</sup>. It seems clear that during pregnancy a clinical deficiency of thiamine has long-term consequences for cognitive functioning although it should be remembered that the evidence of problems to date has come from examples of clinical rather than sub-clinical deficiency.

Thiamine may also have an influence at later stages. An early, yet well-designed study by Harrell<sup>(30)</sup> produced a surprising and informative, although largely forgotten, finding. She monitored for a year 120 children who lived together at an orphanage, a 200-acre farm, where they all ate the same food cooked in the same kitchen. On two occasions the food on offer was weighed and the thiamine intake was assessed by Columbia University. The intake was calculated as 0.9 and 1.0 mg/d for these two periods; that is, it achieved the levels recommended for the children being studied. Two matched groups were created and randomly, and under a double-blind procedure, received either 2 mg thiamine or a placebo each day for a year. At the end of the year those who consumed thiamine were more intelligent, had better visual acuity, faster reaction times, better memory and were taller. This is an interesting finding in that this range of improvements occurred in children who received a supplement that, as judged by the conventional measures of nutritional need, was not required. Although too much attention should not be given to a single isolated study, there are similar reports that individuals, who as judged by activation of the erythrocyte transketolase enzyme were well nourished, benefitted from supplementation. For example, in a double-blind trial thiamine supplementation was found to improve mood, although as judged by the usual physiological index of bodily status the 283 subjects were well nourished prior to supplementation<sup>(31,32)</sup>.

### Vitamin B<sub>6</sub>

One function of vitamin B<sub>6</sub> is as a coenzyme associated with the decarboxylase enzymes that are essential for the production of various amino-acid neurotransmitters including serotonin and noradrenaline. A study of rats that were prenatally vitamin B<sub>6</sub> deficient, found structural impairment of the hippocampus an area of the brain important for memory<sup>(33)</sup>. Similarly, vitamin B<sub>6</sub> deficiency during gestation and lactation has been found to change the

functioning of *N*-methyl-D-aspartate receptors, important in glutamatergic neurotransmission, learning and memory<sup>(34)</sup>.

Such findings have attracted attention as, although a severe deficiency of vitamin B<sub>6</sub> is rare, a mild deficiency is relatively common. For example, in the USA the Second National Health and Nutrition Examination Survey found that 71% of males and 90% of females consumed less than the RDA. In particular, there has been a concern about deficiency during pregnancy with associated implications for brain development.

Low vitamin B<sub>6</sub> status has been associated with the slower growth of breast-fed infants<sup>(35)</sup>. In Egyptian infants, the level of vitamin B<sub>6</sub> in the mothers' milk predicted how often the child cried. Mothers with low levels of vitamin B<sub>6</sub> were less responsive to their child's distress. The authors concluded that low vitamin B<sub>6</sub> status influenced both the behaviour of the child and the mother<sup>(36)</sup>. Given the role played by vitamin B<sub>6</sub> in the development of the nervous system a Cochrane review considered the influence of its supplementation during pregnancy as, especially in open trials, it had been reported to increase birth weight and basic functioning at birth. They found five trials involving in total 1646 women<sup>(37)</sup> but 'not enough evidence to detect clinical benefits of vitamin B<sub>6</sub> supplementation in pregnancy and/or labour other than one trial suggesting protection against dental decay'. There is a need for trials that specifically consider neurological development.

There has been a particular interest in the relation between vitamin B<sub>6</sub> intake and autism. Rimland<sup>(38)</sup> obtained case reports from parents who reported or did not report an improvement in children with autism after vitamin supplementation. He found that vitamin B<sub>6</sub> was a common factor when an improvement was reported. Early intervention studies from France gave some support, although most studies failed to randomly allocate subjects to treatment. Typically, using as much as 1 g vitamin B<sub>6</sub> and 0.5 g Mg daily resulted in positive reports<sup>(39)</sup>. There was said to be a minority, about a third to a half of children with autism, who responded. For example LeLord *et al.*<sup>(40)</sup> found that 15 out of 44 children showed a noticeable improvement. More recently, the area has been critically reviewed<sup>(41)</sup>. Most early studies were rejected on methodological grounds leaving only three studies that were of satisfactory design. They concluded that due to the small number of studies, small sample sizes and the poor methodological quality, the use of vitamin B<sub>6</sub>-Mg as a treatment for autism could not be recommended. Even if a positive response can be demonstrated it is unclear as to whether this is a nutritional problem, a pharmacological response or a problem of absorption and metabolism.

### Vitamin B<sub>12</sub>

Vitamin B<sub>12</sub> has a significant role in the formation of erythrocytes, the synthesis of DNA and plays a role in the metabolism of the fatty acids needed to produce myelin, the sheath around the axon of the neurone. Vitamin B<sub>12</sub> neuropathy results in neuronal degeneration and thus irreversible brain damage. As vitamin B<sub>12</sub> only occurs in animal products a diet lacking such items can offer

inadequate amounts, although eggs, milk and cheese can offer sources for the vegetarian. Vegans, who choose to eat no animal products, are particularly at risk, although vegan foods may be fortified. A review concluded that the potential nutritional problems associated with vegetarianism include a shortage of protein, Fe, Zn, Ca, vitamin D, riboflavin, vitamin B<sub>12</sub>, vitamin A, *n*-3 fatty acids and iodine. There is, however, no suggestion that nutritional requirements cannot be met by a vegetarian diet although some foods may be fortified<sup>(42)</sup>. During pregnancy the fetus absorbs vitamin B<sub>12</sub> through the placenta. Among pregnant women with a vitamin B<sub>12</sub> deficiency the levels transported to the fetus decline<sup>(43)</sup>.

As a vitamin B<sub>12</sub> deficiency results in brain damage, its provision is essential for pregnant and breast-feeding women as well as infants. The consequence of vitamin B<sub>12</sub> deficiency in infancy is a clinical syndrome of irritability, anorexia and retarded development of the brain<sup>(44)</sup>. A clear example was a 14-month-old boy who was found by brain imaging to have severe frontal and fronto-parietal atrophy<sup>(45)</sup>. As a consequence of the vegan diet of the mother the child suffered with a severe vitamin B<sub>12</sub> deficiency. However, after vitamin B<sub>12</sub> supplementation for 6 weeks the pattern of brain waves normalised and brain imaging found that the gross structural abnormalities had disappeared. However, at 2 years of age cognitive and language development remained seriously retarded so it appeared that vitamin B<sub>12</sub> deficiency in infancy has lasting consequences.

Longer-term implications of early vitamin B<sub>12</sub> deficiency have been described<sup>(46)</sup>. The cognitive functioning of children aged 10–16 years was considered, who had consumed a vegan diet up to an average age of 6 years, but subsequently ate a vegetarian or omnivorous diet. Vitamin B<sub>12</sub> deficiency before the age of 6 years resulted in poor fluid intelligence in adolescence with a significant correlation between the degree of vitamin deficiency and the measure of intelligence. In Mexico, a prospective study related the vitamin B<sub>12</sub> status of the mother to the child's development at 12 months. Mothers who consumed more or less than 2 µg vitamin B<sub>12</sub>/d were distinguished. Although there was no associated difference in psychomotor ability the offspring of mothers who had low levels of vitamin B<sub>12</sub> performed cognitive tasks more poorly at 12 months<sup>(47)</sup>.

Similarly, a study in India examined children at 9 years of age whose mothers while pregnant had a lower, rather than higher, vitamin B<sub>12</sub> status. In fact, the mothers' vitamin B<sub>12</sub> status while pregnant predicted the levels in the children when aged 6 years. At 9 years of age, tests of frontal lobe functioning (perceptual tracking and sequencing tasks) and memory were better in the offspring of mothers who had higher levels of vitamin B<sub>12</sub>. The association remained after controlling for a range of possibly confounding variables<sup>(48)</sup>. In contrast, another study in India that related the level of vitamin B<sub>12</sub> during pregnancy to cognition at 9 years of age found no consistent association<sup>(49)</sup>.

In summary, although there is increasing evidence that a clinical deficiency of vitamin B<sub>12</sub> during the period of brain development causes permanent brain damage,

a review concluded that the mechanisms underlying the action of vitamin B<sub>12</sub> are unclear and further work is needed<sup>(43)</sup>. An increased risk of vitamin B<sub>12</sub> is associated with bypass surgery, a vegan diet and having Crohn's or celiac disease.

### Choline

Although choline can be produced by the body, in the USA it is classified as an essential nutrient as dietary sources may not satisfy all bodily needs. A critical role when the fetus is in the womb has been proposed; Zeisel<sup>(50)</sup> concluded that 'it influences stem cell proliferation and apoptosis, thereby altering brain and spinal cord structure and function and influencing risk for neural tube defects and lifelong memory function'. The modulation of stem cells and apoptosis alters the structure and functioning of the brain. In the newborn, the level of plasma choline is higher than adult, with levels falling throughout the first year of life. In later life, it acts as a precursor for phospholipids, the neurotransmitter acetylcholine and the methyl donor betaine.

There is a report that the memory of rodents was enhanced permanently when towards the end of gestation they were supplemented with choline. Similarly, a deficiency of choline at this stage of brain development resulted in problems of memory in later life<sup>(51)</sup>. A review of thirty-four animal studies concluded that 'choline supplementation during development results in improved performance of offspring in cognitive or behavioural tests'<sup>(52)</sup>. Similarly, it is suggested that developmentally the provision of choline influenced the hippocampus with its importance for memory<sup>(53)</sup>.

We await similar evidence of a role for choline in human neural development. Given the evidence from animal studies that prenatal choline supplementation results in improved memory when adult, and that a deficiency during pregnancy has a long-term negative influence on cognitive functioning, in human subjects the level of choline in blood taken from the umbilical cord was related to intelligence assessed at 5 years of age<sup>(54)</sup>. In a sample of 404 children, the levels of free and total choline were measured in the mother's blood at 16–18, 24–26, 30–32 and 36–38 weeks of gestation and finally in cord blood. These values were related to measures of intelligence, visuo-spatial processing and memory assessed at 5 years of age but no significant association was found. Similarly, a review of studies that had supplemented infant formulae with choline was unable to find evidence that it improved the cognitive development of human infants who were already receiving a standard infant formula<sup>(55)</sup>.

### Folate

A review noted that it has long been acknowledged that folate status during pregnancy is related to various outcomes; for example, an adequate status is known to decrease the incidence of pregnancy-induced megaloblastic anaemia<sup>(56)</sup>. Folate plays a key role in brain development, reflecting a role in nucleotide synthesis, DNA integrity and

transcription<sup>(57)</sup>. It is received wisdom that folate deficiency in the initial stages of pregnancy is a risk factor for neural tube defects<sup>(7)</sup>. Since 1998, both in Canada and USA, there has been a policy of folic acid fortification, although after the introduction of this policy the taking of folic acid supplements has not further decreased the incidence of neural tube defects, suggesting that the diet is now adequate in this respect<sup>(58)</sup>.

Although animal studies and the consideration of neural tube defects raise the possibility that folate status may influence human brain development, the topic has been the subject of very limited study. This is the more surprising as it is known that pregnancy increases the demand for folate to the extent that on occasions it is associated with overt folate deficiency. However, blood folate levels are elevated in the newborn such that there must be placental folate transport against the concentration gradient<sup>(56)</sup>.

Although there is evidence that in animals a folate deficiency has detrimental consequences<sup>(59,60)</sup> the topic has been little considered in human subjects. There is a report that infants born to a mother with a severe folate deficiency during pregnancy showed delayed development<sup>(61)</sup> and that folic acid supplementation during pregnancy reduced the incidence of immaturity<sup>(62)</sup>.

Tamura *et al.*<sup>(63)</sup> associated the folate status of mothers during pregnancy and the cognitive development of their offspring. Blood levels of folate and homocysteine were assessed at 19, 26 and 37 weeks of gestation and at 5 years of age a range of cognitive tests were performed. When the offspring of mothers with poor or adequate levels of folate were compared there were no differences in the scores of the two groups. It was suggested that the discrepancy between these results and a previous study<sup>(61)</sup> may have reflected the classification of low-folate status relying on biochemical measures, rather than clinical signs such as megaloblastic anaemia. As both these studies took place before the fortification of grain products it will prove impossible to further consider the phenomenon in countries where this has occurred.

A study in India examined the association between folate and vitamin B<sub>12</sub> status during pregnancy and the cognitive functioning of the offspring when aged 9–10 years. The cognitive scores increased by 0.1–0.2 of a standard deviation for every standard deviation increase in maternal folate concentrations. These associations with learning, memory and attention occurred independently of a range of relevant demographic variables. There were, however, no consistent associations with the levels of maternal vitamin B<sub>12</sub> and homocysteine. An interesting suggestion was that, in the context of neurodevelopment, the level that defines folate deficiency should be set lower than at present<sup>(49)</sup>.

In summary, the limited data prevent the drawing of conclusions. The inconsistencies may reflect the extent of folate deficiency, the nature of the assessment of cognitive functioning and the age at which assessments were made. There remain, however, important questions. A study of the folate status of adolescents living in Europe suggested that pockets of deficiency might exist: as there is a wide variation in intake, a deficiency was likely in some sections of the population<sup>(64)</sup>.

## Vitamin D

Vitamin D receptors are found throughout both the fetal and adult brain, in fact they occur in more than fifty tissues<sup>(65)</sup>. In the human adult brain, the expression of many genes has been shown to be influenced by calcitriol<sup>(66)</sup>.

There is increasing concern that vitamin D insufficiency is common in industrialised countries<sup>(67)</sup>, a reflection of changes in lifestyle that include less exposure to the sun, and obesity that results in sequestration of this fat-soluble vitamin by adipose tissue. Thus, a possible developmental role has been considered and a review concluded that a deficiency in pregnancy 'can result in low birth weight, pre-term labour, pre-term birth, infections, and pre-eclamptic toxemia'<sup>(67)</sup>. However, another review of eighteen studies of vitamin D levels in the first trimester of pregnancy found insufficient evidence to suggest an association between low levels and adverse outcomes<sup>(68)</sup>.

It has been concluded that there is 'ample biological evidence to suggest an important role for vitamin D in brain development' although the direct effects are subtle and 'the current experimental evidence base does not yet fully satisfy causal criteria'. It was, however, recommended that vitamin D supplementation should be given to at-risk groups including nursing infants<sup>(69)</sup>. Kesby *et al.*<sup>(70)</sup> noted that our knowledge of the role in the brain is rudimentary although vitamin D receptors appear early in its development and they increase in number throughout gestation.

In animals, vitamin D-deficient diets have been fed to mothers at particular development stages. Many molecular and structural changes have been reported in the brains of the offspring of mothers fed in this way<sup>(70)</sup>, for example, decreased cortical thickness and larger lateral ventricles<sup>(71)</sup>. In addition, the functioning of the neurotransmitter dopamine is altered by a pre-natal lack of vitamin D<sup>(70)</sup>.

Although a good case can be made for a vitamin D deficiency being likely to influence human brain development there is little evidence of lasting problems in industrialised societies. However, an interesting hypothesis is that there is an association between being born in the winter and an increased risk of developing schizophrenia in later life, particularly in more northerly latitudes. In such situations, there is a lack of exposure to the sun and hence a low vitamin D production. A study has related the giving of vitamin D supplements during the first year of life with the later development of schizophrenia<sup>(72)</sup>. A reduced risk of developing schizophrenia was found in males but not females who consumed the supplements.

## Multi-vitamin supplementation

Although the traditional scientific approach tends to initially consider one physiological aspect in isolation, there are reasons in the present context to question the use of this approach. Although on occasions there may be a deficiency of a single nutrient, these occasions are likely to be less common than more general nutritional deficiencies. An individual problem of absorption or metabolism may result in a particular deficiency or alternatively a poor diet can

cause problems. However, on many occasions a diet inadequate in one respect will also be deficient in others.

In such circumstances, it may prove difficult to establish that a particular deficiency exists, as nutrients do not function in isolation. Particularly when considering the brain, with its considerable complexity, any output will reflect the integration of many mechanisms that each in turn will be the consequence of a long series of metabolic reactions. If, for example, a specific vitamin deficiency inhibits the functioning of a particular enzyme, supplementation may only solve that precise problem. As such, a metabolic bottle neck may be moved to a later stage in a chain of reactions that in turn is inhibited by the lack of another nutrient. As a poor diet will typically result in a sub-optimal consumption of a number of nutrients, it is more likely that a positive response will come from the use of a vitamin and mineral supplement. Alternatively such an approach may be viewed as more cost-effective. If there is no response to a vitamin and mineral intervention it is unlikely that there will be a response to a single nutrient given in isolation. If a response is demonstrated with a multi-nutrient approach then the active ingredients and optimal dose can be subsequently established. If this perspective has merit then on occasions a beneficial response to the supplementation of a single deficient nutrient may not have been demonstrated, when in fact there was a deficiency. Thus, when considering behaviour, a failure to demonstrate a positive response to the supplementation of a single nutrient may not necessarily demonstrate that a deficiency does not exist.

Given this perspective three examples of the use of multi-vitamins will be considered that have used aspects of psychological functioning as dependant variables. The first example is the use of supplementation while the brain is initially growing. It is characteristic of all mammals, and in particular human subjects, that the brain initially grows more rapidly than the rest of the body, particularly during the last 3 months of pregnancy and the first 2 years of life. This rate of growth requires appropriate nutrition and there is an interest in understanding the manner in which variations in nutrition can produce long-lasting changes to the structure of the brain. The basic principle that an insufficient intake of protein, energy, Fe and iodine during this critical stage of brain development can reduce cognitive capacity for life is widely accepted<sup>(2)</sup>. Thus, a clinical deficiency can produce irreversible changes to brain structure. There is, however, less understanding of the influence of sub-clinical deficiencies; is it possible to stimulate intellectual functioning by consuming an optimal diet? Although the details are unclear, there is evidence that small changes in the diet of infants that are relatively well fed can have a long-term influence on the structure of the brain and cognitive functioning. This conclusion relies to a large extent on the outcome of a long-term trial in which premature babies were fed at birth by nasal-tube, with either a standard cow's-milk-based formula or one enriched with micronutrients (water-soluble vitamins, vitamins D, E and F but also Na, Ca, P, Cu and Zn) plus additional protein<sup>(73)</sup>.

Although these different diets were consumed for a median of 4 weeks, at 18 months there was evidence of advanced social and motor development<sup>(73)</sup>. At 8 years

the intelligence of boys, but not girls, was higher if the enriched formula had been consumed in infancy<sup>(74)</sup>. These differences were still apparent at 16 years of age where intelligence scores were still greater in males<sup>(75)</sup>. Interestingly, brain imaging found a larger caudate nucleus in the brains of those who were administered the enriched formula<sup>(75)</sup>. Thus, a difference in nutrition for a few weeks in infancy produced long-term changes in intelligence and even changes in the gross structure of the brain.

This study establishes the principle that small changes in diet at critical stages in development, even in those who traditionally would have been thought to be well fed, can be beneficial. Do vitamins play an important part in this finding? The extent to which particular micronutrients are important rather than higher levels of protein is unclear. If the study was being planned today then it is probable that the enriched formula would have contained PUFA but the Lucas study<sup>(73,74)</sup> was planned at a time before the interest in fatty acids had developed and they were not included.

These children were premature and hence the brain was at an earlier stage of development than with full-term babies. Therefore, the extent to which the findings are generalised to those continuing to full-term is unclear. If the age of the child is critical then perhaps we should be considering the nutritional status of the mother late in pregnancy. In this context a study in Indonesia is relevant<sup>(76)</sup>. In a double-blind trial 31 290 pregnant women took either a multi-micronutrient supplement or a combination of Fe and folic acid. In those taking the multi-micronutrients, rather than Fe and folic acid, infant mortality was 18% lower and the incidence of low birth weight occurred on 14% fewer occasions. An Indian birth cohort related the level of maternal plasma folate, vitamin B<sub>12</sub> and homocysteine during pregnancy to the cognitive development of the offspring<sup>(49)</sup>. When memory, reasoning, attention, visuo-spatial and verbal abilities were considered performance tended to be higher as maternal folate levels rose. The association was not, however, observed with maternal vitamin B<sub>12</sub> and homocysteine levels.

At a later developmental stage Indian children, aged 6–15 years from a middle-income background, were given for 14 months a drink fortified with micronutrients<sup>(77)</sup>. The consumption of the supplementation was associated with improved measures of attention but not intelligence, memory or achievement in school. Similarly, when 7–11-year-old Indian children consumed a supplement containing not only seven vitamins but also iodine, Fe and Ca, the performance on tests of memory and attention, but not on intelligence, improved<sup>(78)</sup>.

More recently, there has been an interest in contrasting the influence of vitamins, minerals and fatty acid supplementation. In India for 12 months the 'Champion' study compared the effect of 15 and 100% of the recommended intake of a range of micronutrients, vitamins A, B<sub>2</sub>, B<sub>6</sub>, B<sub>12</sub>, C, folate in addition to Fe, Ca and Zn. These were administered with either 900 mg  $\alpha$ -linolenic acid plus 100 mg DHA or 140 mg  $\alpha$ -linolenic acid<sup>(79)</sup>. In children 7–9 years of age, the higher doses of micronutrients improved memory at 6 but not 12 months. However, the lower dose was more beneficial when fluid reasoning was examined at both 6 and 12 months. The high micronutrient treatment

was associated with greater height after 12 months. There were, however, no significant differences between the high and the low doses of fatty acids or any interaction between the micronutrient and fatty acid treatments.

It is natural to ask to what extent such findings generalise to those living in western industrialised societies, although it is more controversial to suggest a benefit of micronutrient supplementation in those who are presumed to be initially better nourished. In Wales, a double-blind placebo-controlled trial gave children, aged 12–13 years, a placebo or a vitamin and mineral supplement for 8 months<sup>(80)</sup>. Performance on a non-verbal intelligence test increased substantially although a measure of verbal intelligence was not affected by supplementation. When after 10 years the research generated by this initial finding was reviewed, ten out of thirteen studies reported a positive response in at least a sub-group of children. It was, however, apparent that it was a minority of children who responded and it was hypothesised that it was those with an initially poorer diet<sup>(81)</sup>. In addition it was important that when a response had been found it was always with non-verbal measures and never when verbally based tests were used. Such a highly selective response suggested a genuine phenomenon, particularly as such a response is theoretically predicted. There is a distinction between crystallised and fluid intelligence<sup>(82)</sup>. Fluid intelligence is the ability to reason; an ability that is independent of education and experience that is perceived to reflect basic biological functioning. In contrast, crystallised intelligence reflects past experience and involves verbal ability and acquired information. Fluid intelligence can be assessed using non-verbal intelligence tests whereas crystallised intelligence is measured using verbal tests. Logically given that a micronutrient supplement cannot be expected to increase vocabulary or basic knowledge, whereas it could enhance basic biological functioning, the pattern of findings is exactly as would be predicted.

More recently, the Nutrition Enhancement for Mental Optimization study looked at the effects of micronutrient supplementation in well-nourished and marginally nourished school-aged children in Australia and Indonesia<sup>(83)</sup>. For 12 months, children aged 6–10 years received either micronutrients, folate, vitamins A, B<sub>6</sub>, B<sub>12</sub>, and C as well as Fe and Zn; *n*-3 fatty acids; both micronutrients and fatty acids; a placebo. An improvement in verbal learning and memory was observed in those consuming micronutrients but not fatty acids. Although it did not occur with boys, the girls who consumed the micronutrients showed an improvement in verbal learning and memory but not intelligence or attention. Fatty acids never influenced the performance of cognitive tests. The study concluded that 'in well-nourished school-aged children, fortification with multiple micronutrients can result in improvements in verbal learning and memory'.

A systematic review considered trials published from 1970 to 2008 that had administered at least three micronutrients and considered the cognition of healthy children. For children between 5 and 16 years 'the pooled random-effect estimates for intervention were 0.14 SD (95% CI -0.02, 0.29; *P* = 0.083) for fluid intelligence and -0.03 SD (95% CI -0.21, 0.15; *P* = 0.74) for crystallised

intelligence, both of which were based on twelve trials. Four trials yielded an overall effect of 0.30 SD (95% CI 0.01, 0.58;  $P = 0.044$ ) for academic performance<sup>(84)</sup>. Another review of the area concluded that it was at an early stage and needed large-scale trials that considered the composition of the supplement, the dietary styles of the children and the nature of the influence on cognition<sup>(81)</sup>. The relative contribution of various vitamins and minerals has not been considered and the possibility of synergistic interactions has not been addressed. Questions concerning the optimal dose have not been considered? To date, the impression given is that not all children respond, if anything in industrialised societies it is a minority. A working hypothesis is that it is children who are poorly nourished who respond. For example, a study considered seven schools in Belgium where children kept a dietary diary for 15 d<sup>(85)</sup>. The minority, who responded to supplementation, had diets that offered a low intake of a range of micronutrients, tended to come from schools for the less academically able and from less economically privileged homes. It is clearly the case that 'more research is required, however, before public health recommendations can be given'<sup>(84)</sup>.

There is a second series of well-controlled trials in which the impact of micronutrient supplementation has been considered. There are suggestions that the consumption of a diet, sub-optimal in micronutrients, may predispose to anti-social behaviour<sup>(86)</sup>.

Juveniles with a diagnosis of 'Aggressive' using Diagnostic and Statistical Manual of Mental Disorders-III criteria, while in prison received a vitamin and mineral supplement or a placebo<sup>(87)</sup>. For 3 months the incidence of violence was monitored and was found to be 28% less in those who had received the micronutrients. Blood samples were used to establish micronutrient status before and after supplementation. The incidence of violence did not alter when vitamin status did not change during the study. In contrast, in those whose vitamin status improved there was a marked decline in the incidence of violence. It was argued that an increase in vitamin levels over the study indicated a poor vitamin status at baseline, which had been improved by supplementation. A similar study was also carried out in school children<sup>(88)</sup> where violent and anti-social behaviour was monitored using the official school disciplinary record. During a 4-month period in a double-blind trial those who received supplements were less likely to have been disciplined than those taking the placebo. It was concluded that there been a decline in 'impulsive misconduct'.

The disciplinary records of young offenders, while incarcerated, were monitored in a well-designed study when they received both micronutrients and fatty acids or alternatively a placebo<sup>(89)</sup>. There was a decrease in the incidence of anti-social behaviour in the active group from 16 to 10.4 incidents per 1000 person days, whereas the placebo group showed a non-significant change in the rate of offending. The greatest reduction was in the incidence of serious incidents including violence. In this study, the extent of the response to vitamins, minerals or fatty acids is unclear. Although some have talked about a role for fatty acids the dose provided is lower than one which in

other situations, such as depression, would be sufficient to offer an active dose.

The Dutch government responded to the British findings<sup>(89)</sup> by running a replication study in which 116 young prisoners for between 1 and 3 months received micronutrient supplements in addition to a higher dose of fatty acids than in the British study, while 105 received placebos<sup>(90)</sup>. The incidence of violence in prison decreased by 47% in those taking the supplements although it increased by 13% in those consuming the placebo.

Thus, there is a series of well-designed studies that have reported that anti-social behaviour was less when micronutrients were administered, either with or without fatty acids. Although there are reports of decreases in anti-social behaviour when micronutrients are administered alone<sup>(87,88)</sup>, a meta-analysis also reported that *n-3* fatty acids when administered alone were also effective<sup>(86)</sup>. Similarly, to what extent, if any, there is a synergistic interaction between micronutrient and fatty acid supplementation is unclear.

In summary, there is an increasing number of double-blind placebo-controlled trials in which multi-vitamins and minerals have had a positive influence on both measures of intelligence and aggression. Although the findings are suggestive, additional evidence will be required before the phenomenon is widely accepted and the active ingredients and doses have been established.

## Discussion

In much of the population there appears to be a ready predisposition to see diet as both the cause of behavioural problems and the means of solving them. More specifically, there is a widespread belief that micronutrient intake is deficient. It is often suggested that deficiency results from the consumption of too many refined foods or is a consequence of farming methods in industrialised countries. A British survey found that 32% of boys up to 10 years of age took supplements, although it fell to 14% in an older group. The comparable figures for girls were 23% when under 7 years, 16% between 7 and 14 years and 22% after this age. The probability of taking supplements was related to social background with the children of manual workers being less likely to consume supplements<sup>(91)</sup>. The high number of parents who purchase micronutrient supplements demonstrates a widespread concern that dietary deficiencies may occur.

Typically, nutritional surveys in industrialised countries find that the diet of children is a cause for only limited concern. For example, the British survey of the diet of 2600 children concluded that 'average intakes of all vitamins except vitamin A were well above reference nutrient intakes'<sup>(91)</sup>. Blood analysis showed a generally good nutritional status for vitamins A, B<sub>12</sub> and E. There was, however, some evidence that some individuals had a poor nutritional status for vitamin C, vitamin D, folic acid, riboflavin and thiamine. Biesalski *et al.*<sup>(92)</sup> considered the risk of micronutrient deficiencies in Europe and concluded that although a balanced diet is generally available the intake of some micronutrients is often marginal including

folic acid, vitamins D and B<sub>12</sub>. A later review similarly noted an insufficient intake of vitamins D and B<sub>12</sub> in some European children<sup>(93)</sup>. The question is whether such marginal intakes are a cause for concern.

Although the present review presents evidence that clinical deficiencies in developing countries adversely impact on the functioning of children, the evidence is unclear as to whether in industrialised countries a marginal intake of a vitamin is a problem. The experience of micronutrient supplementation in other contexts causes many nutritionists to doubt whether it is likely to affect the behaviour of children. For example, there has been a general failure of long-term supplementation with antioxidant micronutrients, in studies involving tens of thousands of participants, to benefit a range of diseases<sup>(94)</sup>. These negative findings have occurred despite the theory that free radicals play a role in the progression of heart disease, cancer and dementia. With this background, it seems improbable that significant changes will result in small-scale, short-term studies of the behaviour of children who are relatively well fed. It is, however, necessary to distinguish those whose diets are seriously deficient from those with more minor inadequacies. There can be no dispute that deficiency diseases can occur, mainly in developing countries, where there are inevitable negative consequences for the brain and the rest of the body. An adverse affect of severe iodine, Fe and vitamin A deficiencies is well described<sup>(2)</sup>. More contentious is the suggestion that sub-clinical deficiencies in industrialised countries can influence behaviour.

The view that deficiencies are unlikely relies greatly on the relationship between assessments of dietary input and the population needs for micronutrients, as summarised as Dietary Reference Values or Recommended Daily Amounts. The preamble to the British Dietary Reference Values<sup>(95)</sup> puts these norms into context. It states that 'some nutrients may have a variety of physiological effects at different levels of intake. Which of these effects should form the parameter of adequacy is therefore to some extent arbitrary'. In no instance were psychological or behavioural indices the measure of adequacy used when deriving these reference values. Yet, Benton<sup>(31)</sup> noted that the first symptoms associated with micronutrient deficiency are often psychological and that in well-controlled double-blind trials micronutrient supplementation has been reported to improve mood, memory and attention. For example, in a double-blind trial thiamine supplementation was found to improve mood, although as judged by the usual physiological index of bodily status, erythrocyte transketolase activation, the population was well nourished prior to supplementation<sup>(96)</sup>. Similarly, Harrell<sup>(30)</sup> reported that improved cognition resulted from thiamine supplements in children whose diet already supplied the recommended level. In children, although the findings require clarification, an improvement in non-verbal intelligence scores in at least some of the children who received micronutrient supplements has been reported in a number of double-blind studies<sup>(81)</sup>.

Why might psychological variables prove more susceptible to micronutrient status than disease states? The brain is the most complex and metabolically active organ in the

body, and thus behaviour reflects the summated outcome of countless millions of metabolic processes. In this way, even minor metabolic inefficiencies could create a cumulative adverse effect. Small changes in micronutrient status, responsible for differences of only a few percent in the activity of a single enzyme, could, when multiplied several million fold, result in a noticeably different output. Thus, *a priori* it might be expected that psychological measures would be susceptible to relatively minor nutritional deficiencies, given the complexity and the intense metabolic activity of the brain. The timescale of responses may also be relevant. A disease state may develop over many years, such that the role of diet can be difficult to demonstrate<sup>(97)</sup>. In comparison, the brain output is rapid, measured in milliseconds, potentially making an association with diet easier to establish.

Although it is beyond the scope of this review to fully consider the role of micronutrients on neurochemistry, an example will illustrate one of many ways in which marginal deficiencies could potentially influence neural function. The decarboxylase enzymes are important in the metabolism of a range of neurotransmitters including serotonin, dopamine and noradrenaline and have as a co-enzyme pyridoxal phosphate, the form in which vitamin B<sub>6</sub> occurs most commonly in the diet. There is evidence of marginal intakes of this vitamin. Using a biochemical measure of pyridoxal phosphate status there was a subgroup of about 10% of British children who were deficient<sup>(91)</sup>. In males, the incidence of deficiency increased with age. In young British adults, 27.7% of males and 36.6% of females were deficient as judged by the same measure<sup>(96)</sup>. Bender<sup>(98)</sup> concluded that although a gross deficiency was rare a marginal inadequacy of vitamin B<sub>6</sub> was relatively common, to the extent that it affected amino-acid metabolism and the functioning of steroid hormones. In rats, varying the dietary intake of vitamin B<sub>6</sub> was found to increase the serotonin content of various areas of the brain. It was suggested that this reflected the role of pyridoxal phosphate in the regulation of the decarboxylation of 5-hydroxytryptophan. A continuum existed from deficiency to a moderate excess of pyridoxal phosphate that was associated with the rate of the synthesis and release of serotonin<sup>(99)</sup>.

However, any attempts to use nutrition to either stimulate the development of cognitive functioning, or slow its decline, need to be kept in context. It should be remembered that although diet influences bodily functioning it is by no means the only important factor. Logically all that diet can do is to modify biological functioning: it can increase potential but the exploitation of that potential, with resulting enhanced cognitive functioning, also requires a stimulating and supportive psychological and social environment. An interaction between nutrition and a stimulating environment can have long-term implications.

Naturally, there is evidence that a child's development is modified by factors other than diet. The developmental implications of providing psychosocial stimulation (structured play) for malnourished children have been studied<sup>(100)</sup>. Six months after discharge from hospital those who had stimulating play were developmentally ahead of a similar malnourished group, although both had received

remedial nutrition. A combination of both an improved diet and stimulations resulted in development similar to a group of children without a history of malnutrition.

A study in Jamaica followed up children who had been stunted in early childhood. Beginning at 9–24 months, for 2 years they received dietary supplements, stimulation (demonstrations of the use of toys) or both<sup>(101)</sup>. Perceptual-motor ability benefitted from stimulation, although supplementation only helped the children of mothers who had better verbal ability. After 2 years, supplementation and stimulation had an additive effect, although this was no longer apparent after 4 years. The sample was further followed up at 11–12 years of age, although no benefit of supplementation was found in terms of growth or cognitive functioning<sup>(102)</sup>. In contrast, children who had received stimulation had significantly higher intelligence scores. At 17–18 years of age the group was again examined and there was no significant effect of nutritional supplementation, although stunted non-stimulated participants had poorer scores than the non-stunted children<sup>(103)</sup>. Again a history of stimulation was associated with higher intelligence scores.

It seems as if the effects of dietary supplementation, if provided by itself, might be short-lived. Clearly, the intention of improving diet is to provide long-term benefits. When implementing such a programme, or alternatively developing a food item with supposed beneficial consequences, the extent to which it is reasonable to expect diet by itself to have an impact should be considered. It is reasonable to suggest that dietary interventions should only be one part of a coordinated approach. After critical early stages in brain development have passed, a change in diet will have a short-term influence on biological potential. Without the opportunity to exploit that potential offered by a stimulating environment, the dietary intervention cannot have a long-term impact. A similar conclusion is associated with epidemiological studies and short-term controlled trials. There is a need to monitor and take into account many aspects of the environment. Diet is likely to be influential under some circumstances but not others. Placing diet in a broader picture will greatly increase the chance of generating significant findings.

In summary, there are data with a range of vitamins, from animal studies and human studies in developing countries, suggesting that a clinical deficiency during the critical period when the brain is developing causes permanent damage. To date there is, however, with the exception of cases of clinical deficiency such as those that might be associated with a vegan diet, little evidence that variations in the diet of those living in industrialised countries have a lasting developmental influence. Similarly, in later life clinical deficiencies of various vitamins disrupt cognitive functioning, although in industrialised societies there is limited evidence that variations in the intake of single vitamins influence functioning. It may well be, however, unreasonable to expect that vitamins examined in isolation will be associated with differences in cognitive functioning. The output of the brain reflects millions of metabolic processes, each potentially susceptible to any of a range of vitamins. A diet poor in one respect is likely to be poor in others. As such, the preliminary reports, in double-blind

placebo-controlled trials, that some aspects of cognition and behaviour respond to supplementation with multi-micronutrients may indicate the way forward.

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