Trace Elements in Children and Adolescents

Ferdinand Haschke and Christoph Male

Department of Pediatrics, University of Vienna, Austria

Essential trace elements must be present in the organism at a certain concentration in order to maintain life and growth. Thus, every essential trace element has its special range of tissue concentrations that allow adequate maintenance of physiologic and biochemical functions. However, essential trace elements can also become toxic if they accumulate in the body, e.g., copper in patients with Wilson’s disease.

Most studies on trace element requirements have been done in infants and adults, but information in children and adolescents is limited. Biochemical indices of deficiency or toxicity are established for iron but not for other essential trace elements. Trace elements represent essential micronutrients that influence the expression of specific genes, and recent findings on the regulation of gene expression by trace elements will allow a more precise estimate of the requirements of iron, zinc, and copper in the near future.

REQUIREMENTS FOR CHILDREN AND ADOLESCENTS

The physiologic requirement for a trace element should be the basis for calculating a "reference intake." The idealized definition of a physiologic requirement is the amount and chemical form of a trace element which is needed to maintain normal health and development without disturbance of the metabolism of any other nutrient. We need sensitive and reliable methods for assessing the status of trace elements, and a better understanding of the influence of nutrient and nonnutrient components of diets on requirements. Methods which allow us to calculate the physiologic requirements of most essential trace elements for children and adolescents have not been established so far.

"Reference intakes" for children and adolescents (Table 1), published by different expert panels (1-3), are intakes which, on the basis of the best available data, are thought to meet the needs of nearly all (95th centile or mean + 2 SD) healthy children and adolescents in a population. Reference intakes for trace elements have been extrapolated from values for young adults or interpolated between the reference
intakes of infants and adults. The United Kingdom (2) and European Union (EU) panels (3) have moved away from the concept of “recommended intakes,” used so widely today to imply the “amount that should be consumed,” to the concept of “reference values”; this reduces the chance of misunderstanding. It must be emphasized that reference values from different panels are not therefore recommended intakes from the point of view of nutritional evaluation and advice for individuals.

Reference values for iron intake of children and adolescents proposed by the Scientific Committee for Food (3, table 1) are mainly based on estimates of iron requirements for expansion of red cell and muscle mass during growth, studies on iron status of adults, iron absorption (15%), factors influencing iron absorption, and bioavailability of dietary iron. Reference values for zinc are based on interpolating values for basal losses between those of adults and infants, plus increments for growth, assuming 30% absorption. In the case of copper, reference values are based on the tissue copper content in infants and estimates of endogenous losses and absorption rates (50%) in adults. Selenium reference intakes are calculated from adult values on the basis of body weight, with 0.2 ng/g weight gain added for growth requirements. Iodine reference values are based on age-specific energy requirements. No values for manganese, chromium, and other trace minerals have so far been established.

Functions such as immune responses and antioxidant status can be affected by inadequate or excessive amounts of a trace element and may be more sensitive than specific status indices. The approaches to trace element status assessment that may be both sensitive and specific include tests of metalloenzyme function and tracer studies using stable isotopes of minerals. These have been reviewed recently (4). The influence of trace elements on expression of specific genes will be reviewed in the last section of this chapter.

### TRACE ELEMENT DEFICIENCY: RESPONSE OF THE BODY

Golden (5) developed the concept that nutrients can be classified according to the type of response that arises from a deficiency (Table 2).
TABLE 2. Classification of minerals and trace elements according to type of body response during depletion status

<table>
<thead>
<tr>
<th>Type I</th>
<th>Type II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial normal growth</td>
<td>Primary growth failure</td>
</tr>
<tr>
<td>Specific signs</td>
<td>No specific signs</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Iodine</th>
<th>Sodium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron</td>
<td>Potassium</td>
</tr>
<tr>
<td>Copper</td>
<td>Phosphorus</td>
</tr>
<tr>
<td>Calcium</td>
<td>Sulfur</td>
</tr>
<tr>
<td>Selenium</td>
<td>Magnesium</td>
</tr>
<tr>
<td>Manganese</td>
<td>Zinc</td>
</tr>
</tbody>
</table>

From Golden MHN (5).

Type I Deficiency

Low intakes of a trace element in the diet result in a reduced tissue concentration and an identifiable defect in type I deficiency. In the case of iron deficiency, low serum ferritin indicates reduced tissue stores, and anemia can easily be detected. "Cutoff points" as well as specificity and sensitivity of laboratory tests are well defined and the prevalence of the deficiency and the steps in which the symptoms arise are known.

Type II Deficiency

The response to a type II deficiency is primary cessation of growth, without a reduction in tissue concentration, and it is not normally associated with specific signs and symptoms. Clinical symptoms may appear only during profound and prolonged deficiency, long after growth has ceased. Growth failure is the main symptom of energy, protein, zinc, potassium, phosphorus, and magnesium deficiency. The response to a deficiency—"stunting" is the same for each of the nutrients. Growth failure cannot be used to differentiate one deficiency from another. Energy can be stored in the body, but for zinc and the other nutrients, there are no body stores and the body needs a continuous dietary supply. In the case of zinc deficiency, concentrations in body tissues such as plasma or hair concentrations can be normal, and identifiable clinical symptoms such as dermatitis, impaired wound healing, and alopecia may be found only during prolonged and profound deficiency.

IRON DEFICIENCY DURING CHILDHOOD: STILL A PUBLIC HEALTH PROBLEM?

During late infancy, socioeconomic factors, the prevalence and duration of breastfeeding, the age at which whole cow’s milk is introduced into the diet, and the extent
and duration of the feeding of iron-fortified formulas are important variables to explain the prevalence of iron deficiency in a population. Studies in the USA from 1979 and 1980 indicated that 30% of infants seen in a Public Health Service clinic had anemia (6), as compared with 8% who had anemia in a private practice (7). Studies in a group of 9- to 23-month-old U.S. middle class children indicated a decline in the prevalence of anemia from 7.5% in the 1969–1973 to 2.8% in 1982–1986 (8).

The prevalence of iron deficiency depends on the criteria used to establish the diagnosis. Anemia in young children can be a result of iron deficiency, but common infections also play an important role. Inclusion of more sophisticated and expensive laboratory methods during pediatric population screening for iron deficiency provides detailed information on the state of iron nutrition. Low ferritin values indicate depletion of body iron stores. An upregulation of transferrin receptor is found when red cells perceive a need for additional iron. Unlike serum ferritin, which only identifies iron deficiency, the serum transferrin receptor measures its severity (9). Another important feature of the serum transferrin receptor is that, unlike many other iron measurements, the level remains normal in patients with the anemia of infection (10) and therefore assists in identifying iron deficiency in pediatric populations where infections are common.

Recent detailed epidemiologic data from different European regions indicate that iron deficiency is still a public health problem in early childhood. Hemoglobin, mean corpuscular volume (MCV), transferrin saturation, serum ferritin, and serum transferrin receptor values of 514 healthy 1-year-old children from 11 European cities were measured as part of the Euro-growth study (11). Percentages of European children with values below or above the respective cutoff values are indicated in Table 3. Preliminary results from the Euro-growth study indicate that the prevalence of anemia at 1 year of age was 9.8%. However, only 46% of the children with anemia (Fig. 1) had low ferritin or raised transferrin receptor values or both. Anemia related to infections and other causes therefore seems to be common in Europe (12), so it is unlikely that a majority of European children with anemia of unknown origin will benefit from a therapeutic trial with oral iron, which has been proposed in the USA (13,14). Depletion of iron stores with normal hemoglobin values was found in 19.6% and high cellular need for iron without any further signs of iron deficiency was present in 3.7% (Fig. 1).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cut-off value</th>
<th>% below/above cut-off</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin</td>
<td>&lt;11 g/dl</td>
<td>9.8</td>
</tr>
<tr>
<td>MCV</td>
<td>&lt;70 fl</td>
<td>8.8</td>
</tr>
<tr>
<td>Serum ferritin</td>
<td>&lt;12 µg/l</td>
<td>23.7</td>
</tr>
<tr>
<td>Transferrin receptor</td>
<td>&gt;4.2 µg/ml</td>
<td>9.4</td>
</tr>
<tr>
<td>Transferrin saturation</td>
<td>&lt;10%</td>
<td>22.3</td>
</tr>
</tbody>
</table>

MCV, mean corpuscular volume.
During the second year of life, the factors that predispose to iron deficiency during late infancy should gradually become less pronounced. Less iron is required for growth, and a more bioavailable source of iron is offered with a mixed diet, which is less dominated by milk. The risk of anemia decreases further beyond 2 years of age. Indeed, studies in the USA in the 1970s confirmed the decreasing risk of anemia with age, at least in middle class children. Between 1982 and 1986, the prevalence for the age group between 2.0 and 6.9 years was as low as 2–3% (8). However, recent European studies in preschool children from all socioeconomic groups indicated prevalences of 25% and 22.8% in London (15) and Valencia (16). Groups at high-risk for iron deficiency are the poor, some vegetarians (14,17), children recovering from malnutrition, and children with short stature during treatment with recombinant human growth hormone (18).

The adolescent growth spurt markedly increases the requirement for dietary iron and, in females, menstrual losses have to be replaced. The cumulative effect of high-iron needs and the tendency of female adolescents to eat less than males or to adhere to special diets explain why the prevalence of iron deficiency among females appears to increase throughout adolescence (19,20).

There are no published epidemiologic data on systemic manifestations of iron deficiency anemia in children and adolescents. Alterations in cognitive performance, impaired exercise capacity, functional alterations in the small bowel, koilonychia, beeturia, increased intracranial pressure leading to pseudotumor cerebri, pica, and blue sclerae have been mentioned (21–27).

**ZINC DEFICIENCY AND GROWTH**

We must ask if it is likely that zinc deficiency limits growth in any substantial population group. Because there are no data on the precise requirements for normal
growth of children or on requirements for catch-up growth, the question of dietary adequacy—or inadequacy—cannot be answered at present (28,29).

Prasad et al. (28,30) described zinc deficiency in adolescent Iranian and Egyptian boys with stunted growth and delayed sexual maturation. The effects of zinc deficiency on insulin-like growth factor-1 (IGF-1) (5,31,32) and testicular development (33) have recently been published.

Low zinc content of the diet and consumption of dietary and other substances that lower zinc bioavailability are important factors in the pathogenesis of primary zinc deficiency. Inhibitors of zinc absorption include phytate, dietary fiber, oxalate, products of Maillard browning, casein, iron, calcium, and cadmium (34–36). Phytate, which is present in whole-grain cereals and legumes, is probably the most important dietary inhibitor of zinc absorption. Ferguson et al. (37) confirmed that a high intake of phytic acid relative to zinc was a risk factor for inadequate zinc nutrition and stunted growth in Malavian children.

The existence of a growth-limiting mild zinc deficiency syndrome in otherwise healthy children from industrialized countries (Table 4), which responds to treatment with oral zinc, was postulated in a low-income population of Mexican–American background in Denver, Colorado, USA (38), in southern Ontario, Canada (39,40), and in Japan (32). In the Denver study (38), 2- to 6-year-old children with height for age below the 10th centile of the National Center of Health Statistics (41), low zinc intake, and plasma or hair zinc below the cutoff values, received a daily supplement of 5 mg zinc. When the double-blind, pair-matched study was finished 12 months later, height velocity in the zinc-supplemented children \( p < 0.05 \), in particular of boys \( p < 0.001 \), was significantly greater. A double-blind, pair-matched, 12-month study in Canadian 5- to 7-year-old boys (39,40) with hair zinc below the cutoff value and height below the 15th percentile of the reference (41) found that a daily zinc supplement of 10 mg resulted in a higher mean change in height for age z-score than in the control group. In addition, the study of Gibson et al. (40) clearly showed that zinc supplementation in an unselected cohort of children with short stature is useless,

<table>
<thead>
<tr>
<th>Country</th>
<th>(ref)</th>
<th>Deficiency*</th>
<th>Design</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>United States</td>
<td>(38)</td>
<td>+</td>
<td>DB</td>
<td>+</td>
</tr>
<tr>
<td>Canada</td>
<td>(39)</td>
<td>-</td>
<td>DB</td>
<td>-</td>
</tr>
<tr>
<td>Canada</td>
<td>(39)</td>
<td>+</td>
<td>DB</td>
<td>+</td>
</tr>
<tr>
<td>Japan</td>
<td>(32)</td>
<td>+</td>
<td>R</td>
<td>+</td>
</tr>
<tr>
<td>Egypt</td>
<td>(30,42)</td>
<td>+</td>
<td>O</td>
<td>+</td>
</tr>
<tr>
<td>China</td>
<td>(44)</td>
<td>+</td>
<td>O</td>
<td>+</td>
</tr>
<tr>
<td>Guatemala</td>
<td>(45)</td>
<td>-</td>
<td>DB</td>
<td>-</td>
</tr>
<tr>
<td>Gambia</td>
<td>(46)</td>
<td>-</td>
<td>R</td>
<td>-</td>
</tr>
</tbody>
</table>

*Deficiency was indicated by low zinc concentration in hair or serum or by body zinc clearance test (32).

TBLE 4. Effect of oral zinc therapy on growth of stunted children
and an effect could only be shown in a subcohort with low hair zinc. Nakamura et al. (32) studied 21 prepubertal short Japanese children without endocrine abnormalities who were considered to have mild-to-moderate zinc deficiency. Only one child had a serum zinc concentration below 65 mg/l. Ten randomly selected children received 5 mg/kg of zinc sulfate for 6 months. Height (z-score), height velocity (z-score), and energy intake (kcal/day) increased significantly after 6 months of treatment. In the 11 control children, changes were small and statistically nonsignificant. Zinc supplementation also increased plasma levels of IGF-1 and osteocalcin, which indicated greater bone protein synthesis (32).

Zinc supplementation studies in stunted children from nonindustrialized countries with or without biochemical signs of zinc deficiency or low intake of highly bioavailable zinc (Table 4), yield conflicting results. Therapeutic trials in zinc-deficient Egyptian and Iranian boys indicated that oral zinc therapy and adequate intake of other nutrients stimulated linear growth, maturation of the skeleton, and development of the genitalia and secondary sex organs (30,42,43) to a greater extent than treatment with diet alone. Xue-Cun et al. (44) reported significantly lower hair and plasma zinc in 166 children with growth retardation and anorexia. In a subgroup of 11 children who were treated with 1.2–2 mg zinc/kg/day, height gain per month increased significantly during 6 months of supplementation. Two recent studies, however, found no effect of zinc supplementation: (a) Cavan et al. (45) conducted a double-blind vitamin, mineral, and trace element supplementation study with zinc (n = 76; 0.5 mg zinc/kg/day) or without zinc (n = 80) in periurban Guatemalan children (age 81.5 ± 7 months). After 25 weeks, they found no changes in height for age z-scores but some changes in other anthropometric variables such as midarm circumference and triceps skinfold thickness. (b) Bates et al. (46) divided rural Gambian children (n = 110; age range 0.57–2.3 years) with failure to thrive and a low intake of highly bioavailable zinc into two matched groups, one to receive 70 mg zinc twice weekly for 1.25 years and the other to receive a placebo. Of the anthropometric variables reported, only arm circumference was significantly higher in the supplemented group at the end of the study period. These recent well-designed studies indicate that in the two pediatric populations from developing countries studied, zinc is unlikely to be of major overall importance as a cause of stunting and failure to thrive. However, low energy and/or protein intakes, which can also cause type II deficiency (5), were not considered to be growth-limiting factors in those studies and no supplements were offered. In order to study the effect of zinc on growth of stunted children from developing countries, it seems necessary to promote additional well-designed studies where supplements of all nutrients (with or without zinc) known to cause type II deficiency (5) are provided.

WILSON'S DISEASE

Wilson's disease is an autosomal recessive disorder of copper transport, resulting in copper accumulation and toxicity to the liver and brain. Typical features include
neurologic manifestations, hepatocellular disease, Kayser–Fleischer rings of the cornea, and low serum ceruloplasmin. Laboratory diagnosis of Wilson’s disease is usually made by measuring serum ceruloplasmin concentration, urinary copper excretion, and liver copper concentration. The gene has been mapped to chromosome 13q14.3. The Wilson’s disease gene is now known to be a putative copper transporting P-type ATPase (47,48) and shows a particular 76% amino acid homology to the Menkes’ disease gene (48).

Of clinical interest for pediatricians are new developments in the treatment of Wilson’s disease. Standard treatment includes the use of D-penicillamine to promote urinary copper excretion and decreased body stores. Zinc treatment of Wilson’s disease was introduced in the 1960s: oral zinc is effective in controlling copper balance and blocks the intestinal absorption of copper, as demonstrated by uptake of $^{64}$Cu and copper balance measurements (49): Yuzbasiyan-Gurkan et al. found a pronounced increase in intestinal metallothionein levels and a sharp drop in $^{64}$Cu absorption 4–5 days after the initiation of oral zinc treatment. After discontinuation of zinc treatment, metallothionein levels decreased and $^{64}$Cu absorption increased. The authors concluded that intestinal metallothionein induction mediates decreased copper absorption during zinc therapy. In adults and adolescents, $^{64}$Cu absorption studies indicated that a daily oral dose of $2 \times 37.5$ or $3 \times 25$ mg zinc is highly effective (50). Copper balances have indicated similar copper retention in patients treated with zinc alone or with zinc plus penicillamine or trien (51). Thus, it has been speculated that there is no advantage to combining anticopper agents during long-term treatment. However, deterioration of Wilson’s disease during zinc treatment has been reported (52), and our experience with this form of treatment is still limited. Recently, it was proposed that a vegetarian diet could be a management tool for maintenance therapy in Wilson’s disease because of the low copper bioavailability from such diets (53), but there are no studies on long-term outcome in children.

The siblings of patients with newly diagnosed Wilson’s disease are at 25% risk of also having this autosomal recessive disease. Biochemical and DNA markers (54) now enable siblings of patients with Wilson’s disease to be screened to detect asymptomatic cases so that prophylactic treatment can be started before such patients become ill. In 13 patient, prophylactic treatment with oral zinc acetate for 3–9 years preserved liver function, and no signs of zinc toxicity or symptoms related to Wilson’s disease were observed (55).

**TRACE ELEMENTS AND GENE EXPRESSION: NEW PREVENTIVE, DIAGNOSTIC, AND THERAPEUTIC APPROACHES**

Trace elements influence the expression of specific genes. This concept was advanced in the 1960s, and the nutritional implications of transcriptional regulation of gene expression by trace elements have now gained wide appreciation. All organisms require an external supply of essential trace elements in order to satisfy a variety of
FIG. 2. Regulation of transferrin receptor and ferritin by intracellular iron concentration. IRF, iron regulatory factor. Modified from Leibold and Guo (57).

cellular needs, but our present knowledge of metals involved in mammalian gene expression is derived from evidence with iron, zinc, copper, and manganese.

Iron

Ferritin and the transferrin receptor, the major proteins involved in the regulation of iron homeostasis, are proteins which are regulated by intracellular iron (Fig. 2) at the level of translation and mRNA stability. Transferrin receptor brings iron into the cell, where it is transferred to the iron storage protein ferritin or to other cellular proteins requiring iron. A post-transcriptional mechanism (iron regulatory factor) to regulate ferritin and transferrin receptor is needed because of the toxicity of free iron in the cell. This toxicity is due to the ability to form reactive hydroxyls, which can cause peroxidation of lipid membranes and other cellular elements. High free iron concentration in the cell mobilizes ferritin mRNAs onto polysomes for translation but decreases transferrin receptor synthesis, which is controlled at the level of mRNA.
stability. The result of ferritin and transferrin receptor regulation by iron is a decrease in the free iron levels in the cell, thereby preventing iron toxicity. Iron starvation induces the expression of transferrin receptor, thereby increasing iron uptake, and represses the synthesis of proteins involved in iron storage and utilization. During inflammatory diseases, it has been shown that proinflammatory and other cytokines are important mediators of intracellular iron metabolism because they control expression of transferrin receptor and ferritin as well as intracellular iron handling (56–59).

Zinc

Essential trace elements like zinc in the diet can be viewed as determinants of expression because they help maximize cellular potential. The zinc finger proteins, which comprise well over 1% of the genome, are involved in different cellular functions: zinc finger proteins of nutritional interest act as receptors for the retinoic acid, estrogen, progesterone, calcitriol, glucocorticoid hormone, and thyroid hormone (60–62), but the role of diet as a determinant of zinc finger protein functions is still unknown.

Of special interest are studies on the interactions between zinc and growth. IGF-1 and IGF binding protein–3 (BP-3) are two products involved in mediating the growth-promoting actions of growth hormone. Growth hormone transgenic mice, carrying the ovine metallothionein 1a-ovine growth hormone (oMt1a-oGH) transgene, provide a model for studying hormonal regulation of gene products responsible for efficient growth of lean body mass. Chow et al. (63) activated or inactivated the oMt1a-oGH transgene by addition or removal of zinc sulfate in the drinking water. Transgenic mice receiving zinc had higher plasma IGF-1, hepatic IGF-1 mRNA, plasma BG-3, and hepatic BP-3 mRNA levels than transgenic mice not receiving zinc or control mice receiving or not receiving zinc in their drinking water. This indicates that in the oMt1a-oGH transgenic mouse model system, zinc intake influences growth hormone, which regulates IGF-1 and BP-3 expression. We have already mentioned that in stunted children with biochemical indices of zinc deficiency, low IGF-1 values have been reported (31,32). Oral zinc treatment resulted in an increase in IGF-1 values (5,32).

Zinc can also play a regulatory role for expression of specific genes. It provides signals to the transcription activation process or for translational control of mRNAs. We have already discussed how oral zinc induces intestinal metallothionein (49), which inhibits copper absorption and therefore helps to reduce copper toxicity in patients with Wilson’s disease. Nutritional regulation of metallothionein by zinc and copper in animals has recently been studied in detail (64; Fig. 3). Rats were fed diets with 5, 30, or 180 mg/kg of zinc and 1, 6, or 36 mg/kg of copper. Levels of 30 mg/kg of zinc and 6 mg/kg of copper were considered adequate intakes. From this experiment, it can be concluded that in the rat, dietary zinc has a substantial effect on metallothionein gene expression whereas copper has only a marginal influence. In human subjects, it has been shown that erythrocyte metallothionein can be used
as a measure to differentiate between low and adequate levels of dietary zinc intake over a 6-week period (65).

Copper

Functions which can be influenced by copper include the cross-linking of the collagen in connective tissue, skeletal mineralization, myelin formation, oxidation of ferrous iron, antioxidant protection, oxidative phosphorylation, melanin pigment synthesis, catecholamine metabolism, immune function, glucose regulation, thermal regulation, cholesterol metabolism, anti-inflammatory activity, blood clotting, and cardiovascular system integrity (4,66–69). A deleterious effect of copper deficiency on the myocardium and arteries (67–69) is of special interest because levels of copper intake during childhood that prevent cardiovascular disease later in life are not known. Dietary copper deficiency impairs cardiovascular function by depressing catecholamine metabolism (70–72) and by altering the structure and function of cardiac mitochondria. At the cellular level, several metabolic stressors induce the synthesis of highly conserved proteins, termed heat shock proteins (HSPs). These proteins seem essential for survival during or after stress (73). Matz et al. (74) looked at the effects of dietary copper deficiency on the induction and accumulation of HSPs in several cardiovascular tissues of rats. Stress-induced levels of aortic HSP 70 mRNA were lower in copper-deficient rats than in copper-replete controls. The level of HSP70 mRNA was reduced in the atria of copper-deficient rats, perhaps associated
with altered mitochondrial structure and function. These investigators showed a relation between dietary copper deficiency and the expression of highly conserved cellular stress proteins and speculated that the loss of these homeostatic proteins in vascular tissue might contribute to the impairment of cardiovascular function. It will be of future interest if the level of cellular stress proteins is also an indicator of copper deficiency in human subjects.

CONCLUSIONS

Trace element requirements of children and adolescents are poorly defined. Iron deficiency is still a public health problem, even in industrialized countries. Special target groups at risk are the poor, vegetarians, and children during catch-up growth. New diagnostic indices to establish disturbed trace element metabolism are needed, especially in the case of zinc and copper. Our information on the regulation of genes by trace elements is growing rapidly. This biological function relies on trace elements that enter cells from metabolic compartments which are influenced by dietary supply. Measuring the influence of trace elements on gene expression should allow more precise estimates of the requirements of iron, zinc, and copper in the near future.

REFERENCES


59. Fahmy M, Young SP. Modulation of iron metabolism in monocyte cell line U937 by inflammatory cytokines: changes in transferrin uptake, iron handling and ferritin mRNA. *Biochem J* 1993; 296: 175-81.


**DISCUSSION**

*Dr. Whitehead:* At the very beginning you advised this group that we have very few direct experimental data on which we can set requirements for the micronutrients. You quite correctly said that most of our values for toddlers and adolescents are achieved by interpolation of values between the breast-fed child and the young adult, the university student. We desperately need more direct measurements on children to get a better factual foundation. I was interested at the end about what you said about gene expression. Could you explain exactly what you mean by this?

*Dr. Haschke:* The influence of trace elements on gene expression is in my opinion a diagnostic tool for the future, and I have addressed just one example. If it is possible with an established method, i.e., polymerase chain reaction (PCR), to measure iron regulating factors, we can establish values for intracellular iron needs in an efficient way. PCR will be cheap in the future and the tests can be repeated.

Copper deficiency in premature infants results in hypochromic anemia, but it has not been reported in children and adolescents. Since we have the possibility to measure the influence of copper on gene expression of the heat shock proteins, we learn more about copper deficiency and impaired heart function. Heat shock proteins regulate catecholamine metabolism during stress. Animal experiments have shown that during experimental copper deficiency, the expression of heat shock proteins is reduced, catecholamine production is lowered, and the heart is therefore not protected during stress.

*Dr. Gracey:* Zinc deficiency has been shown to be very prevalent in Aboriginal children in the north of western Australia. This was described in reports by Professor Donald Cheek and his colleagues (1–3); they did some supplementation experiments which were unsuccessful and were reported briefly in *Lancet* (4). This deficiency was associated with growth failure but, of course, that may have been multifactorial and associated with repeated infections and parasitic infestations. I have always suspected that the lack of success of the zinc supplementation was due to undetected malabsorption in these children. I wonder if you have a comment about that.

*Dr. Haschke:* This is one possibility. The other possibility is that in the population which was studied by Cheek, other micronutrient deficiencies were present which could not be identified. There are several micronutrient deficiencies which can interfere with growth.

*Dr. Gracey:* Could I just add that Cheek also found that excess copper was present in many of these individuals (1,5). Would that be significant in this association between those micronutrients?
Dr. Haschke: Excess copper causes other diseases but I am not aware that it negatively affects growth.

Dr. Baudon: In clinical practice, what do you consider the best biological marker for zinc deficiency?

Dr. Haschke: In clinical practice, usually we try to measure zinc in serum or plasma. This tells us something about zinc status if the serum protein levels are adequate. In case of protein deficiency with low serum protein, a low serum zinc level does not necessarily indicate zinc deficiency. So serum zinc is helpful only in a state where protein is normal. Hair zinc is not a good indicator in the individual but helps to identify subgroups with possible deficiency in populations with a clear cutoff value. Alkaline phosphatase, of course, is reduced during zinc depletion states but is also influenced by other factors. Recently, a publication suggested the use of erythrocyte metallothionein level as marker for zinc depletion (6). It was shown to be low in 13 adults who were on an experimental low-zinc diet, and it reflected the depletion state accurately. However, this has not been evaluated in clinical practice.

Dr. Kleinman: I want to reiterate one point, which is that for patients who have Wilson's disease in the later stages, the problem is not so much that they absorb too much copper but that they don't get rid of the copper that they have. The defect is probably one in which the gene for copper receptor on the biliary epithelial cell is defective and so they can't move copper into the bile. In this situation, zinc treatment may in fact be dangerous and allow the disease to progress.

Dr. Haschke: The gene was mapped recently to chromosome 13, and a copper carrying ATPase was identified in 1994. Zinc is not very helpful in eliminating copper from the body. However, in asymptomatic cases, different groups have shown that there is no deterioration during 8–10 years of zinc treatment. Zinc treatment doesn't work in all patients, and severe deterioration has been observed in a few. The main reason why zinc therapy has been proposed is because people think it is less toxic than the other drugs.

Dr. Kleinman: The issue of zinc treatment raises again the issue of the interaction of trace elements in the gut and the recommendations that we make for supplementation, e.g., what effects such recommendations may have on the absorption of other trace elements when we make them for a single trace element. Could you comment on that?

Dr. Haschke: Of course there are interactions between trace element absorptions; we have established this for iron, zinc, and copper, but mainly during infancy, and this was one of the reasons why the European Union is proposing lower levels of iron in infant formulas than are recommended in the USA. In Europe, there is concern that zinc and copper retention might be negatively affected if iron intake is high.

Dr. Rey: You mentioned that the incidence of iron deficiency is higher in blacks than in whites. This could be linked to the worse socioeconomic status of blacks in general, but there are also genetic factors. Black people have lower hemoglobin levels than white people, in spite of a similar or higher plasma ferritin, and it is probable that the lower hemoglobin values in blacks are not related at all to iron status (7). I don’t know what the term “iron requirement” means because iron status is self-regulated within broad limits. When iron intake decreases, iron absorption increases and vice versa, and iron status is probably more important than iron bioavailability for iron absorption (8). So I am not convinced that we need more data to evaluate precisely the range of iron intake for toddlers and adolescents. For infancy, I agree, but for toddlers and adolescents, I am not sure. I believe there is no health problem relating to the other trace elements in healthy toddlers and adolescents, apart from zinc and iodine perhaps, and I am convinced that we are wasting time and money by
efforts to obtain more precise data on molybdenum, chromium, copper, germanium, and so on.

Dr. Haschke: Although genetic factors might partly explain the hemoglobin difference between blacks and whites, no epidemiologic study has fully examined the influence of differences in food intake or in socioeconomic variables. So the question is still open. Your point about the self-regulation of iron intake and iron absorption is an important one. It is my opinion that we must consider this carefully and this is one of the reasons why I am promoting these diagnostic tools for iron metabolism so vigorously.

Dr. Agostoni: Do you think we have enough data now to suggest a weekly supplement of iron instead of daily supplementation to avoid the possible negative effects? There are certainly data in pregnant women and in children showing that such periodic supplementation is as effective as a daily supplement.

Dr. Haschke: We don't yet have enough data to propose a weekly supplement. There might be some target groups that would benefit, like pregnant women, but I am not sure whether this can be said at present for the general population. I am strongly against any supplementation of the childhood population or even the adolescent population. I think iron should be provided in adequate form with food and one should be aware that iron absorption is influenced by whether this iron comes from the heme or the nonheme iron pool, and this is our way for dietetic counseling.

Dr. Soriano: You mentioned the difficulty in diagnosing micronutrient deficiency. For some micronutrients like magnesium, a loading test may be useful to diagnose the deficiency because the amount excreted after a loading dose decreases when the body is deficient in the ion you are loading. Can this loading test be useful in other micronutrients, and could we explore this way of diagnosing deficiency?

Dr. Haschke: I don't have any personal experience with loading tests. I think a lot of work was done in the 1970s with the zinc loading test, but in more recent publications, it is no longer used. I guess it was not really effective at identifying subclinical deficiency. The main problem is that cases with marginal zinc deficiency are missed with the tests which we have available at present. Patients with severe deficiency are identified by loading tests or even by measuring the serum values. So the problem with stunting is to identify marginal deficiency in time, and so far nobody has solved the problem, especially in the third world where the situation is very complex.

Dr. Guesry: Many articles have been published showing the deleterious effect of phytic acid on both iron and zinc absorption. On the other hand, Hallberg published a paper about 4 years ago (9) showing that increased calcium intake also reduced iron absorption. I think we need a more holistic approach and that the different lobbies—the calcium lobbies, the iron lobbies, fresh food lobbies, and so on—should speak to each other in order to try to make sense of the recommendations.

Dr. Haschke: I fully agree. I think we should address the cow's milk lobby first. We have data now on the factors which are of importance for iron nutritional status at 1 year of age in European children, and we found that the use of cow's milk during late infancy is the strongest variable with a negative effect on iron nutritional status. It is much more important in European children than socioeconomic status or other factors which we have evaluated. So, at least during late infancy, I think the avoidance of cow's milk is very important.

Dr. Garza: I would like to emphasize the lack of available information on trace mineral toxicity, especially when animal data show that the margin of safety between the requirement level and the toxicity level is so much narrower than it is for most other of the water-soluble nutrients. This is particularly important at a time when the nutrition community is
continuing—perhaps inappropriately, as Dr. Rey points out—to heighten the role of trace minerals in normal nutrition. We see them increasingly added to foods, included in fortification schemes, and individuals appear to rely increasingly on supplements to meet their needs. This may be especially true for children. When we look at the literature, there are few data from which we can determine toxic thresholds and thus formulate a fortification policy more rationally.

Dr. Haschke: I agree.

Dr. Alatas: I would like to have your comment about the supplementation of zinc in chronic renal failure patients who are undergoing dialysis; some people say it should be supplemented, others not.

Dr. Haschke: It is not only a matter of zinc; there is uncertainty about supplementation of many trace elements in this situation. In the case of children with renal failure undergoing hemodialysis, we have to be very careful because we don't have adequate measures of deficiency, or in fact of toxicity, and that is very dangerous if renal function is impaired. I think at present there is not sufficient evidence to advocate supplementation. Children on hemodialysis receive trace elements through contamination of fluids; this has been pointed out by several investigators, so one has to be very careful.

Dr. Whitehead: Toxicity is a very important issue. Those of you who have read the British report on dietary reference values will know that it suggests ranges of requirements. We also wanted to define toxic limits, but by and large we were unable to do so because the data were inadequate, particularly for young people. We had no idea at all as to what the toxic limits were for young people in various stages of active growth and this is a major gap in our knowledge. I am sorry to keep on emphasizing these gaps in our knowledge but they will remain until the appropriate research is done.

REFERENCES


