ZINC NUTRITION IN DEVELOPING COUNTRIES

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INTRODUCTION

Recently the United Nations has urged that priority should be given to developing programmes in less industrialized countries to prevent deficiencies of iodine, vitamin A, and Fe (United Nations, 1991). Nutritional Fe deficiency is associated with plant based diets which contain high levels of dietary fibre and phytate, components known to inhibit non-haem Fe absorption, and low levels of flesh foods, rich sources of readily available haem iron (Monsen, 1988). Such plant based diets will also induce Zn deficiency. The consequences of Zn deficiency on human health in developing countries, however, have not yet been recognized. This is unfortunate because even mild Zn deficiency may contribute to pregnancy complications, low birth weight, impaired immune competence, maternal and infant mortality and morbidity, and growth failure in infancy and childhood (Swanson & King, 1987; Hambidge, 1989; National Academy of Sciences, 1991; United Nations, 1991). Hence Zn deficiency may have far reaching consequences on maternal, infant, and child health in many developing countries.

Table 1. *Zinc*†, *phytic acid*† *and* [*phytate*]:[*zinc*] *molar ratios of some foods and composite dishes consumed in Ghana and Malawi*

Food, and scientific name or recipe	Zn	Phy	Phy:Zn	% H_2O
Cereals				
Maize flour, 95% extraction (*Zea mays* L.)	2·2	792	36	10
Maize flour, 65% extraction	0·9	211	23	10
Maize bran	3·7	1089	29	10
Maize dough	1·4	n.a.	n.a.	50
Sorghum flour (*Sorghum bicolor* (L.) Moench)	1·4	446	32	10
Rice (*Oryza sativa*)	1·6	n.a.	n.a.	10
Legumes				
Ground nuts, boiled (*Arachis hypogaea* L.)	1·4	505	35	49
Ground nuts, flour	2·8	1297	45	8
Pigeon peas, fresh (*Cajanus cajan* (L.) Millsp.)	0·9	255	27	63
Pigeon peas, dry	2·2	727	33	8
Kidney beans, fresh (*Phaseolus vulgaris* L.)	1·5	557	36	52
Cowpeas, boiled (*Vigna unguiculata* (L.) Walp.)	1·0	349	37	68
Lima beans, fresh (*Phaseolus lunatus* L.)	1·5	238	16	66
Bengal beans, fresh (*Stizolobium aterrimum* Piper & Tracey)	1·0	166	17	68
Vegetables (boiled)				
Pumpkin leaf (*Cucurbita maxima* Duch. ex Lam.)	0·7	34	5	89
Chinese cabbage (*Brassica chinensis* L.)	0·7	5	1	94
Okra leaf (*Hibiscus esculentus* (L.))	1·8	97	5	79
Okra (*Hibiscus esculentus* (L.))	0·5	13	3	91
Cassava leaf (*Manihot esculenta* Crantz)	1·2	42	3	78
Cocoyam leaves (*Xanthosoma* sp. Schott.)	0·6	19	3	88
Amaranth leaves (*Amaranth* sp. L.)	0·3	n.a.	n.a.	93
Roots and plantain (boiled)				
Sweet potato (*Ipomoea batatas* L.)	0·2	10	5	70
Yam (*Dioscorea* sp. L.)	0·3	50	13	68
Cocoyam (*Xanthosoma* sp.)	0·5	37	7	60
Cassava (*Manihot* sp.)	0·3	54	18	65
Cassava dough, fermented	0·4	48	12	51
Gari: dry fermented cassava, not boiled	0·7	51	4	12
Plantain, ripe (*Musa paradisiaca* L.)	0·2	0	0	73
Plantain, unripe (*Musa paradisiaca* L.)	0·2	1	1	65
Water yam (*Dioscorea alata* L.)	0·2	26	16	72
Fruits				
Avocado pear (*Persea americana* Mill.)	0·3	11	3	78
Banana (*Musa paradisiaca* L.)	0·2	22	9	72
Mango, raw (*Mangifera indica* L.)	0·1	25	23	82
Composite dishes – home-prepared snacks				
Chitumbuwa (mixture of water, maize flour and pounded bananas formed into a round cake and fried in oil)	1·2	504	42	30
African bread (mixture of water, maize flour and bananas formed into a cake, wrapped in banana leaves and boiled until cooked)	0·3	102	37	70
African cake (mixture of water, maize flour and sugar baked in tin can)	1·2	297	26	45
Composite dishes – staples				
Hausa porridge (thin porridge of corn flour)	0·1	25	25	94
Porridge of corn grits	0·1	23	23	88
Banku (boiled mixture of corn dough and cassava dough)	0·7	107	16	73
Ga kenkey (corn dough made into dumplings and boiled in banana leaves)	0·8	172	19	71
Fanti kenkey (corn dough made into dumplings and boiled in plantain leaves)	0·7	118	21	72
Fufu (pounded boiled cassava and plantain)	0·4	96	24	69
Composite dishes – purchased meals				

Table 1 (*cont.*)

Food, and scientific name or recipe	Zn	Phy	Phy:Zn	% H_2O
Rice and stew (rice and standard ingredients‡)	0·6	118	21	68
Rice and beans (rice, cowpeas and standard ingredients)	0·5	107	18	70
Gari and beans (gari, cowpeas and standard ingredients)	0·9	178	22	59
Composite dishes – soups				
Palmnut soup (water, palmnut cream and standard ingredients)	0·4	n.a.	n.a.	86
Groundnut soup (water, groundnut paste and standard ingredients)	0·8	81	10	88
Composite dishes – stews				
Okra (okra and standard ingredients)	0·4	38	9	90
Bean (cowpeas and standard ingredients)	0·7	n.a.	n.a.	72

†, mg/100 g wet weight. n.a., not analysed.

‡, standard ingredients: tomato, red peppers, salt, onion, fish; palm oil in stews, rice and beans, and gari and beans.

Phy:Zn, [phytate]/[Zn] molar ratios. Phytate was analysed by the standard AOAC method (Harland & Oberleas, 1986).

All data from Ferguson *et al.* (1988, 1989*b*, 1993*a*).

AETIOLOGY OF ZINC DEFICIENCY IN DEVELOPING COUNTRIES

DIETARY FACTORS: LOW INTAKE AND POOR BIOAVAILABILITY OF DIETARY ZINC

The nutritional adequacy of dietary Zn depends on both its amount and bioavailability in the diet. Flesh foods are a rich source of Zn which is readily available because during their digestion certain L-amino acids and cysteine-containing peptides are released, which form soluble ligands with Zn (Sandström *et al.* 1980, 1989). In many developing countries, however, the content of flesh foods in rural diets is often low so that their contribution to total dietary Zn intake is small. Instead, diets are mainly plant based; cereals, starchy roots and/or tubers are often the major sources of Zn in rural diets. Of these staples, starchy roots and tubers generally have a lower Zn content than cereals, as shown by the Ghanaian and Malawian examples shown in Table 1. Hence, diets based on these staples tend to be correspondingly lower in Zn than cereal based diets (Gibson *et al.* 1991*a*; Ferguson *et al.* 1993*a*). Nevertheless, in certain geographical areas where Zn deficient soils exist, cereal staples will have a lower Zn content than when grown on Zn sufficient soils.

Plant based diets often contain high levels of phytic acid (myoinositol hexaphosphate) and dietary fibre, components known to inhibit the absorption of dietary Zn (Sandström, 1989). Of these antinutrients, phytic acid (Phy), the major storage form of phosphorus in cereals, legumes, and oleaginous seeds, is the most potent inhibitor of Zn absorption (Sandström & Lönnerdal, 1989). It forms insoluble chelates at a physiological pH. The lower inositol phosphates (i.e. tetra-, tri-, di-, and mono-inositol phosphates), formed by enzymic or non-enzymic hydrolysis of phytic acid, do not form insoluble complexes with Zn (Lönnerdal *et al.* 1989). The bioavailability of dietary Zn can be predicted from the ratio of phytic acid [Phy] to zinc [Zn] in diets. The critical [Phy]:[Zn] molar ratios associated with risk of Zn deficiency are equivocal; ratios above 15 have been associated with biochemical (Harland & Peterson, 1978; Oberleas & Harland, 1981; Turnlund *et al.* 1984; Bindra *et al.* 1986), and in some cases clinical signs of Zn deficiency in humans (Ferguson *et al.* 1989*a*).

Plant based staples such as unrefined maize flour, brown rice, sorghum and certain legumes (e.g. groundnuts, pigeon peas, kidney beans, and cowpeas) have elevated [Phy]:[Zn] molar ratios (Table 1; Ferguson, 1992). Hence, diets based on cereals and legumes have higher [Phy]:[Zn] molar ratios than those based on starchy roots and/or tubers (Ferguson *et al.* 1993*a*; Fitzgerald *et al.* 1993).

High levels of calcium potentiate the inhibitory effect of phytate on Zn absorption by forming a Ca:Zn:phytate complex that is even less soluble than phytate complexes formed by either ion alone (Wise, 1983). Hence, some authors have proposed that dietary [Phy][Ca]:[Zn] ratios may be a better predictor of Zn bioavailability than [Phy]:[Zn] ratios alone (Davies *et al.* 1985; Fordyce *et al.* 1987). To date, the critical [Ca][Phy]:[Zn] molar ratio that compromises Zn bioavailability in human diets has not been clearly defined. Retrospective calculations of experimental data from Cossack & Prasad (1983) suggest that molar ratios above 0·2 (200 mmol) may be associated with decreased Zn bioavailability in human diets. Most plant based diets are low in Ca, however, with the exception of those based on tortillas (Fitzgerald *et al.* 1993). The latter contain a relatively high concentration of Ca, derived from lime used to soak the maize in the preparation of nixtamal (soaking of corn kernels to liberate the husks) before being milled into masa (raw corn dough). Diets of lacto-ovo vegetarians may also have elevated [Ca][Phy]:[Zn] molar ratios (Bindra *et al.* 1986).

Dietary fibre, notably the insoluble fibres cellulose and lignin, may also inhibit Zn absorption to some degree, although their effects are equivocal, in part because fibre generally occurs concomitantly with phytic acid, making any independent inhibitory effect difficult to establish (Torre *et al.* 1991).

The bioavailability of Zn can also be affected by competitive interactions among certain micronutrients in the intestine, notably between Zn and non-haem Fe, and Zn and copper (Mills, 1985). The Fe and Cu contents of most human diets, however, are generally not high enough to compromise Zn bioavailability, unless high doses of supplemental non-haem iron are used (Solomons, 1986). In some cases, a negative Fe–Zn interaction has not been observed when the Fe is mixed with or is present as an intrinsic part of a food or meal (Valberg *et al.* 1984). Some (Milne *et al.* 1984; Mukherjee *et al.* 1984) but not all (Butterworth *et al.* 1988; Krebs *et al.* 1988) researchers have also observed a negative effect of high doses of folate supplements on Zn status, which could be of significance for pregnant women prescribed both supplemental folate and non-haem iron.

EXCESSIVE LOSSES

Additional factors that may exacerbate suboptimal Zn status in population groups living in developing countries include increased endogenous losses of Zn through perspiration; exfoliation of the skin as a result of the hot, humid climate; chronic haemolysis due to genetic factors (e.g. α-thalassaemia, sickle cell disease) and/or parasite infections (e.g. malaria, hookworm, schistosomiasis), and diarrhoea (Solomons, 1981; Ruz & Solomons, 1990). Ferguson (1992) estimated urinary Zn losses from haemolysis induced by schistosomiasis to range from 0·02 to 0·85 mg/d; faecal losses of Zn in infants with chronic diarrhoea can be as high as 300 μg/kg daily (Rothbaum *et al.* 1982). In areas where geophagia is practised, extensive faecal losses arising from poor absorption of dietary Zn may exacerbate Zn deficiency (Prasad *et al.* 1963).

HIGH PHYSIOLOGICAL REQUIREMENTS

The FAO/WHO/ILEA committee are currently revising the Zn requirements to include estimates to meet both basal and normative requirements (FAO/WHO/ILEA, un-

published observations, 1992). Basal requirements are the amount needed to prevent clinically detectable signs of functional impairment whereas the normative requirement represents the amount needed to maintain tissue stores or reserve capacity.

Physiological requirements of Zn are increased during periods of rapid growth because it has such a critical role in nucleic acid synthesis and protein metabolism. Hence, infants and children are especially vulnerable to Zn deficiency. In infants in developing countries, Zn stores at birth may be small as a consequence of their low birth weight and poor nutritional status of the mothers. Therefore, their dietary requirements for catch-up growth will be higher than those of infants from industrialized countries.

Male infants and children appear to have higher requirements for Zn than females, because of their higher growth rates and greater proportion of muscle/kg body weight; muscle contains a higher content of Zn than fat (Giugliano & Millward, 1984). In several double-blind supplementation studies, males have exhibited greater improvements in rate of linear growth and/or weight gain than their Zn supplemented female counterparts (Walravens & Hambidge, 1976; Walravens *et al.* 1983, 1989; Castillo-Duran *et al.* 1987; Schlesinger *et al.* 1992; M. Ruz, 1993, pers. comm.).

Requirements for Zn are also greater during pregnancy and lactation for the growth and development of the fetus and maternal tissues, and secretion of breast milk. The FAO/WHO/ILEA committee (unpublished, 1992) calculated the average individual physiological requirements for absorbed Zn during each trimester of pregnancy to be 0·8, 1·0, 1·4 mg/d for the basal requirements and 1·1, 1·4, and 2·0 mg/d for the normative requirements. These estimates do not take into account differences in the absorbability of dietary Zn or the varied intakes within the population. During the course of lactation, Zn concentrations in human milk decline (Casey *et al.* 1989). Hence, estimates of the average individual basal requirement range from 1·6 at 0–3 months and 1·5 at 3–6 months to 1·2 mg/d between 6 and 12 months; corresponding estimates for normative requirements are 1·9, 1·8, and 1·5 mg/d respectively (FAO/WHO/ILEA, unpublished observations, 1992).

ZINC INTAKES IN RELATION TO ESTIMATED REQUIREMENTS

In many developing countries, information on intakes and major food sources of Zn in local diets, as well as on the antinutrients dietary fibre and phytate, are limited, in part because of the paucity of data on the content of Zn and antinutrients in local foodstuffs. This is unfortunate because such data are essential for assessing the risk for inadequate intakes of dietary Zn, and for planning dietary strategies to improve its content and bioavailability in traditional diets.

Population groups consuming diets based predominantly on unrefined maize and rice generally have markedly higher intakes of phytate and elevated [Phy]:[Zn] molar ratios compared to those consuming diets based on starchy roots and/or tubers (Table 2) (Mbofung & Atinmo, 1987; Gibson *et al.* 1991*a*; Ferguson *et al.* 1993*b*; Fitzgerald *et al.* 1993). The latter, however, often have lower Zn intakes. Molar ratios of [Ca][Phy]:[Zn] in most of these plant based diets are low with the exception of those based on tortillas (Fitzgerald *et al.* 1993).

The adequacy of dietary Zn intakes can be evaluated by comparison with the newly revised requirements, provided an estimate of the bioavailability of Zn in the diet can be made. Diets can be categorized as high, moderate, or low in terms of Zn bioavailability, based on their content of animal or fish protein, calcium, and [Phy]:[Zn] molar ratios (FAO/WHO/ILEA, unpublished observations, 1992). Alternatively, more direct measure-

Table 2. *Dietary intakes (mean ± SD) of zinc, phytate, phytate:zinc molar ratios, and dietary fibre of children in some developing countries*

Country (n) Age in years Reference	Zinc (mg/day)	Phytate (mg/day)	Phy:Zn	Dietary fibre (g/day)
Papua New Guinea (67) 6–10 Gibson *et al.* 1991*a*	4·4 ± 1·3	646 ± 663	12	37·1 ± 11·4
Malawi (67) 4–6 Ferguson *et al.* 1993*b*	6·6 ± 1·7	1899 ± 590	25	24·9 ± 6·4†
Ghana (148) 3–6 Ferguson *et al.* 1993*b*	4·7 ± 1·1	591 ± 153	13	15·5 ± 3·8†
Egypt (96) 1·5–2·5 Murphy *et al.* 1992	5·2 ± 1·6	796 ± 249	16	17·4 ± 5·9
Kenya (100) 1·5–2·5 Murphy *et al.* 1992	3·7 ± 0·9	1066 ± 324	28	21·6 ± 5·5
Mexico (59) 1·5–2·5 Murphy *et al.* 1992	5·3 ± 1·3	1666 ± 650	30	15·3 ± 4·8
Guatemala (136) 6–8 Cavan *et al.* 1993*a*	9·0‡	962†	11	14·0‡
Canada (106) 4–6 Gibson *et al.* 1991*b*	6·9 ± 2·3	(300)‡	5	11·4 ± 5·5

† Non-starch polysaccharide.
‡ Median.

ments of the bioavailability of Zn in local diets can be made by using radioactive or stable isotope techniques (Sandström & Lönnerdal, 1989).

Some studies report average Zn intakes for a specific population group (Mbofung & Atinmo, 1987), often based on one day's intake per individual. Such data do not take into account the distribution of intakes among individuals and cannot be used to estimate the proportion of individuals within the population at risk for nutrient inadequacy. For the latter, food intake data based on at least two days' intake per person are required. If single days are used, prevalence estimates for risk of inadequacy are always too high (Beaton, 1985). To improve the reliability of the prevalence estimates for dietary inadequacy, they should be determined using the probability approach recommended by the Subcommittee for Criteria for Dietary Evaluation (National Research Council, 1986). When this approach has been used in studies of dietary Zn intakes of children in developing countries, a very high proportion of the children studied from Kenya and Malawi (> 90 %), and more than two-thirds from Mexico and Ghana, were apparently at risk, assuming that the estimates used for both the bioavailability and basal requirements for Zn are valid (Murphy *et al.* 1992; Ferguson *et al.* 1993*b*).

Even the Zn intakes of exclusively breast fed infants may be inadequate during the first 4–6 months in some developing countries, especially if the infants are preterm and/or of low birth weight with high nutrient demands for catch-up growth. Moreover, their supply of Zn from breast milk may be compromised by the poor nutritional status of the lactating mothers, which may result in breast milk with an inherently low Zn content (Butte *et al.* 1992; Dorea, 1993) and/or low volume (Brown *et al.* 1986). To date, studies of the Zn concentrations of breast milk in poorly nourished lactating women with chronically inadequate Zn intakes have revealed inconsistent results. In some, breast milk Zn concentrations have been consistent with those reported for developed countries, and independent of maternal dietary Zn intakes (Kirsten *et al.* 1985; Karra *et al.* 1986; Moser *et al.* 1988; Simmer *et al.* 1990); others dispute this finding (Krebs *et al.* 1985; Shrimpton *et al.* 1985).

In many developing countries, breast milk output may also be compromised by the early introduction of weaning foods which replace rather than complement breast milk (Walker, 1990). Very often these weaning foods are prepared as thin porridges from staples with a low energy and nutrient density which fail to make up the nutrient deficit when breast milk no longer meets the infants' needs. If unrefined and unfermented cereals and/or legumes are used, the weaning foods will have a high phytic acid content; consequently Zn bioavailability will be low. During fermentation, hexa- and penta-inositol phosphates are hydrolysed enzymically to the lower inositol phosphates which do not inhibit Zn absorption (Lönnerdal *et al.* 1989). More work is required in developing countries to evaluate the adequacy of dietary Zn intakes for both exclusively breast fed infants and for weanlings. To date, no recommendations for the Zn content of weaning foods in developing countries exist (Royal Tropical Institute, Amsterdam, 1987). This is unfortunate because Zn deficiency impairs appetite, taste acuity, immune and intestinal function during infancy (Hambidge *et al.* 1972; Krebs *et al.* 1984; Castillo-Duran *et al.* 1987; Roy *et al.* 1992; Schlesinger *et al.* 1993; Tomkins *et al.* 1993) as well as growth (Hambidge, 1989). Such functional disturbances will have a further detrimental effect on the growth and development of the infants.

A high proportion of pregnant women from developing countries are probably also at risk through inadequate intakes of Zn. Although no data based on the probability approach are available in the literature, in a Guatemalan study 94 and 25% of the pregnant women had average Zn intakes below or less than two-thirds of the US Recommended Dietary Allowance for Zn (15 mg) respectively, assuming that 20% of Zn was absorbed from their diets. Mean Zn intakes for pregnant rural and urban women in Nigeria were 6·0 and 6·7 mg/d, respectively, whereas during lactation they ranged from 7·3 to 8·2 mg/d for rural women (Mbofung & Atinmo, 1987). Corresponding mean intakes for Nepalese (Moser *et al.* 1988) and Amazonian (Jackson *et al.* 1988) lactating women were 10·5 and 8·8 mg Zn/d respectively.

Comparison of Zn intakes with the current estimated requirements does not take into account the possibility that humans can adapt to chronically low Zn intakes and achieve Zn balance by increasing Zn absorption (King, 1986). Certainly, Amazonian lactating women maintained normal Zn balance in the presence of low intakes of Zn (and phytate) (Jackson *et al.* 1988), although there was evidence of functional impairment because breast milk Zn and retinol contents were abnormally low. Whether such adaptation also occurs in the presence of very high habitual intakes of phytate seems unlikely. Brune *et al.* (1989) reported that vegetarians did not adapt to their high phytate diet by increased absorption of ^{59}Fe.

Probability estimates for risk of Zn inadequacy do not identify actual individuals in the population who are deficient, or define the severity of the nutrient inadequacy. Such information can only be obtained when the dietary intake data are combined with laboratory and/or clinical indices of Zn status. This is especially important in developing countries where the coexistence of many other multifaceted health problems often confounds the diagnosis of Zn deficiency.

LABORATORY ASSESSMENT OF ZINC STATUS

BIOCHEMICAL INDICES OF ZINC STATUS

To date, no single, sensitive and specific index of Zn status exists (Golden, 1989). Serum/plasma Zn is the most frequently used index in human studies because it can be easily and accurately measured. Nevertheless, this index has several limitations. It can only be used when the serum samples are not haemolysed or contaminated, and conditions such

as infection are absent. Erythrocytes have a high Zn content and in cases of Zn deficiency red cell fragility is increased (Bettger *et al.* 1978). Parasitaemia is prevalent in many developing countries, and its presence confounds the interpretation of serum Zn concentrations; during infection values are spuriously low because Zn is redistributed from the plasma to other tissues (Aggett, 1991; Filteau & Tomkins, 1994). Other important confounding factors which must be controlled when collecting blood samples for plasma Zn analysis include diurnal variation in circulating Zn level, fasting, meal consumption, the time interval between blood collection and separation of the plasma, and contamination of the blood sample from evacuated tubes with rubber stoppers and non-acid washed glassware (Gibson, 1989; Aggett, 1991; Wallock *et al.* 1993). In general, low plasma/serum Zn levels indicate deficiency or a redistribution of Zn, but normal levels do not necessarily preclude deficiency. For instance, in cases of chronic but mild Zn deficiency states, plasma concentrations are often normal (Gibson *et al.* 1989*b*; Ruz *et al.* 1991), making diagnosis difficult.

Alternative static biochemical indices of Zn status which have been investigated include the concentrations in hair, urine, leucocytes, neutrophils, platelets and saliva. Available evidence suggests that low concentrations in hair samples collected during infancy and childhood probably reflect chronic suboptimal Zn status when the confounding effects of severe protein–energy malnutrition and season are absent (Hambidge *et al.* 1972; Gibson *et al.* 1989*b*; Cavan *et al.* 1993*a*; Ferguson *et al.* 1993*b*). Clinical features of mild Zn deficiency in childhood, such as impairments in linear growth, appetite and taste acuity, have been associated with hair concentrations of less than 1·07 μmol/g (70 μg/g) (Hambidge *et al.* 1972; Krebs *et al.* 1984; Smit Vanderkooy & Gibson, 1987) in the summer, and less than 1·68 μmol/g (110 μg/g) in the winter (Gibson *et al.* 1989*b*; Cavan *et al.* 1993*a*). In some cases, the low hair concentrations have been related to poorly available dietary Zn (Smit Vanderkooy & Gibson, 1987; Ferguson *et al.* 1988; Gibson *et al.* 1991*b*; Cavan *et al.* 1993*a*).

Hair Zn cannot be used in cases of very severe malnutrition when the rate of growth of the hair shaft is often diminished. In such cases, hair Zn concentrations may be normal or even high (Erten *et al.* 1978; Bradfield & Hambidge, 1980). Standardized procedures must be used for sampling, washing, and analysing the hair samples (Hambidge, 1982). Supplementation trials must be undertaken over one year and all the subjects sampled at the same season of the year to minimize the confounding effects of seasonal variation (Gibson *et al.* 1989*a*).

Many investigators have failed to find any positive correlations between the Zn content of hair and serum/plasma Zn concentrations (Hambidge *et al.* 1972; Walravens *et al.* 1983, 1989; Gibson *et al.* 1989*b*). These findings are not unexpected. The Zn content of the hair shaft reflects the quantity of Zn available to the hair follicle over an earlier time interval. Positive correlations between hair and plasma Zn concentrations are only observed in chronic, severe deficiency states, in the absence of confounding factors.

Depletion of body Zn stores causes a reduction in urinary excretion, often before any detectable changes in serum/plasma Zn concentrations (Baer & King, 1984). Twenty-four hour urine collections are recommended because diurnal variation in urinary Zn excretion occurs, although casual urine samples can be used if Zn:creatinine ratios are determined (Zlotkin & Casselman, 1988). Several factors can affect urinary Zn concentrations, however, making interpretation of the results difficult. For example, despite the presence of suboptimal Zn status in sickle cell anaemia, hyperzincuria occurs. The absence of established interpretive criteria for urinary Zn levels further limits their use (Gibson, 1989).

The Zn contents of leucocytes or specific cellular types of leucocytes (e.g. neutrophils) have been used as an index of tissue Zn status; they are said to reflect soft tissue Zn (Jones

et al. 1981) and correlate with retinal dark adaptation. They also have a shorter half-life than erythrocytes and hence should detect changes in Zn status over a shorter time period. Results, however, have been equivocal (Jones *et al.* 1981; Meadows *et al.* 1981; Prasad & Cossack, 1982; Thompson, 1991; Ruz *et al.* 1992). Relatively large volumes of blood are required and isolation of the leucocytes and specific cellular types, as well as their subsequent analysis, is lengthy and technically difficult, limiting their use in some countries. For example, Milne *et al.* (1985) have emphasised that the Zn content of leucocytes is a function of the type of separation used; contamination with Zn from the anticoagulant, reagents, density gradient system, and/or from erythrocytes and platelets may occur. Changes in the relative proportions of leucocyte subsets with physiological state (e.g. pregnancy) and haematological disorders must also be taken into account in the interpretation of the results. Finally, comparison of results among different studies is difficult because no consensus exists as to how to express Zn concentrations in the cell types.

Biochemical functional tests measure changes in the activities of certain enzymes or blood components dependent on Zn. Zinc is a constituent of over 200 metallo-enzymes which vary in their responses to Zn deficiency depending on the tissue examined, their Zn affinity, and rate of turnover of the enzyme. Of the Zn metallo-enzymes, activity of serum alkaline phosphatase has been most widely used to assess Zn status; its response has been inconsistent. In general, its activity is reduced in severe (Rothbaum *et al.* 1982) but not in mild (Ruz *et al.* 1991) Zn deficiency states. No significant changes in activity have been reported in mild Zn depletion–repletion studies of adults (Ruz *et al.* 1991), or in most (Hambidge *et al.* 1972; Walravens & Hambidge, 1976; Walravens *et al.* 1983, 1989; Gibson *et al.* 1989*b*; Cavan *et al.* 1993*b*), but not all (Udomkesmalee *et al.* 1992) of the Zn supplementation studies in infants and children.

The specificity of alkaline phosphatase as an index of Zn status is also poor; its activity is influenced by many factors other than Zn status such as low food intake, type of protein consumed, magnesium or manganese deficiency, season, and in states of increased bone turnover (Chesters & Will, 1978; Koo *et al.* 1989). Measurements of alkaline phosphatase activity in neutrophils (Ruz *et al.* 1991), leucocytes (Schiliro *et al.* 1987), and red blood cell membranes (Ruz *et al.* 1992; Cavan *et al.* 1993*b*) have also been investigated as indices of Zn status; more studies are needed before any definite conclusions can be reached. To date, there is no universally accepted Zn dependent enzyme which can be used to assess mild Zn deficiency.

Levels of the Zn binding protein metallothionein have been investigated in serum, urine, or erythrocytes as indices of Zn status (Golden, 1989). Levels fall in Zn deficiency as a result of impaired synthesis. Specificity is poor; levels are also affected by Fe deficiency, diurnal rhythm, and acute infection. Metallothionein is said to be much less responsive to stress and infection in erythrocytes than in plasma (Grider *et al.* 1990), and hence may provide a useful index of Zn status.

Serum thymulin has also been assessed as a potential index of Zn status. Thymulin is a Zn metallopeptide which controls cell mediated immune function (Prasad *et al.* 1988); its activity falls in mild Zn deficiency. Plasma somatomedin-C, a peptide of low molecular weight which is regulated by growth hormone, nutrition, and insulin, is increased in response to increases in Zn concentration in plasma and tibia of rats. Nevertheless, more work is required to establish the sensitivity, specificity, and validity of erythrocyte metallothionein, serum thymulin and somatomedin-C as indices of Zn status.

PHYSIOLOGICAL FUNCTIONAL INDICES OF ZINC STATUS

Physiological functions dependent on Zn, such as linear and ponderal growth, taste acuity, and immune competence, can also be used to assess Zn status. Such tests have greater biological significance than the biochemical tests because they measure the biological impact of Zn deficiency. Their specificity is low, and hence they must always be used in conjunction with biochemical indices.

Diminished taste acuity is a feature of mild Zn deficiency. Several methods for testing taste acuity have been used. In studies of Canadian (Gibson *et al.* 1989*b*) and Guatemalan (Cavan *et al.* 1993*a*) children, significant inverse relationships between recognition threshold for salt and hair Zn concentrations have been noted. These results suggest that impaired taste acuity can be used as a physiological functional test of suboptimal Zn nutriture in some children, provided a biochemical index of Zn status is also used. The test is not suitable, however, for infants and children less than five years of age.

Some changes in body composition have also been observed after Zn supplementation in some cases of deficiency in children. Specifically, increases in arm circumference were reported in Gambian children (Bates *et al.* 1993), whereas in Zn supplemented Jamaican children recovering from severe malnutrition, accretion of lean tissue was greater. The latter was attributed to an increased efficiency of nutrients for tissue synthesis after Zn supplementation. By contrast, triceps skin folds increased in Guatemalan Zn supplemented children (Cavan *et al.* 1993*b*), probably due to an increase in energy intake concomitant with improved appetite.

From the discussion above, it is evident that diagnosis of Zn deficiency is hampered by the lack of a single, specific, and sensitive index of status. A large number of indices have been proposed, but many are fraught with problems that affect their use and interpretation, especially in mild Zn deficiency states. Hence, it is not surprising that the true magnitude of Zn deficiency in developing countries is not yet known.

ZINC DEFICIENCY THROUGHOUT THE LIFE CYCLE

INFANCY AND CHILDHOOD

Cases of severe Zn deficiency in infancy and childhood are now rare but mild deficiency in infancy and childhood is not uncommon. Growth failure is the most prominent clinical feature of mild Zn deficiency, although impairments in body composition, taste acuity, appetite, immune function, dark adaptation, and delays in secondary sexual maturation have also been described (Hambidge, 1989). Growth failure is also a characteristic feature of childhood growth patterns in many developing countries, which has until recently been attributed to deficits in energy and/or protein. Inadequate Zn intakes are likely to be an important contributing factor because diets low in protein tend to be low in Zn (Golden & Golden, 1981*b*), and Zn has such a critical role in protein synthesis, cell replication, and appetite control.

The first cases of human Zn deficiency were reported in the Middle East among adolescent male dwarfs in the 1960s (Prasad *et al.* 1963). The syndrome was characterized by impaired growth and delayed sexual maturation, which were shown to respond to Zn supplementation in later studies (Ronaghy *et al.* 1969, 1974).

Since these first reports, nutritional Zn deficiency has been reported in infants and/or children living in some industrialized (Hambidge *et al.* 1972; Walravens & Hambidge, 1976; Arcasoy *et al.* 1978; Buzina *et al.* 1980; Walravens *et al.* 1983, 1989, 1992; Smit Vanderkooy & Gibson, 1987; Gibson *et al.* 1989*b*), and developing (Golden &

Table 3. *Double-blind zinc supplementation studies in infants*

Country, no. of subjects, age of subjects, experimental treatment, reference	Mean plasma zinc levels, (μmol/l)				Growth effects and other responses
	Zinc suppl.		Control		
	Start	End	Start	End	
USA. 68 normal healthy full term male infants at birth studied for 6 months. Double-blind study. Formula with 1·8 mg Zn/l *v.* 5·8 mg Zn/l. Walravens & Hambidge, 1976		11·9		11·0	Improved weight and length in males only.
France. 57 normal healthy infants 5·4 months old studied for 3 months. Double-blind placebo (32), 5 mg Zn/d (25). Walravens *et al.* 1992					Improved weight gain. Improved length gain in males only.
USA. 50 failure to thrive, 8–27 months old studied for 6 months. Randomized double-blind trial pair-matched. 5·7 mg Zn/d as syrup (25) and placebo (25). Walravens *et al.* 1989	10·7	9·8	10·7	10·4	Improved weight especially in boys. Tendency to increased activity of serum alkaline phosphatase in Zn group.
Chile. 32 marasmic infants, 7–8 months old, studied for 90 d. Randomized double-blind trial. 2 mg Zn/kg daily as solution (16). Placebo (16). Castillo-Duran *et al.* 1987	14·7	15·6	16·1	15·6	Weight for length effect. Decrease in % anergic infants, increase in serum IgA in Zn group.
Chile. 39 severely malnourished infants studied for 105 d. Double-blind trial 1·9 mg Zn/kg daily in formula (19) *v.* 0·35 mg Zn/kg daily in formula (20). Schlesinger *et al.* 1992	19·4	18·6	23·4	18·0	Linear length effect. Improved immune function.
Bangladesh. 60 severely malnourished infants 5–60 months old for 3 weeks. Rice based diet *ad lib.* and vitamins and minerals. 10 mg Zn/kg daily if < 6 kg or 50 mg Zn/d for those > 6 kg as $ZnSO_4$. Non-supplemented group (30). Khanum *et al.* 1988	8·2	18·5	7·9	10·6	Improved weight gain and weight for length.
Bangladesh. 65 children with AD 3–24 months old. 152 with PD 3–24 months old supplemented for 2 weeks. Followed for 2 and 3 months in a double-blind randomized study (placebo *v.* 15 mg Zn/kg daily). Roy *et al.* 1993					Improved length gain in AD group, and in PD with < 90% weight/age and < 90% height/age. Reduced no. of episodes of diarrhoea in AD and PD groups and attack rate of respiratory tract infections in AD group only.
Chile. 80 SGA neonates 38–41 weeks gestational age studied from birth for 6 months. Double-blind randomized study with placebo (41), 3 mg Zn/d (39). Rodriguez *et al.* 1991	12·6	10·5	12·1	8·9	Improved linear growth and weight gain. No difference in head circumference.

AD, acute diarrhoea; PD, persistent diarrhoea; SGA, small for gestational age.

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Table 4. *Double-blind zinc supplementation studies in children*

Country, date, number of subjects, age of subjects, experimental treatment, reference	Dietary zinc intake (mg)	Mean plasma zinc levels (μmol/l)				Growth effect and other responses
		Zinc suppl.		Control		
		Start	End	Start	End	
Egypt. 1965–6. 90 growth retarded school boys, 11–18 years old studied for 5·5 months. Randomized trial, placebo (30) and 14 mg Zn (30). Capsules given at school. Carter *et al.* 1969	14	10·7	19·2	11·7	13·3	No weight or height effects. No difference in sexual maturation. No serum alkaline phosphatase effect.
Iran. 1967–8. 60 growth retarded school boys 12–18 years old studied for 17 months (5 months trial, 7 months rest, 5 months trial). Controlled trial. 1st 5 months placebo (20), 28 mg Zn (20), 67 mg Fe (20). 2nd 5 months placebo (20), micronutrients (20), micronutrients + 40 mg Zn (20). Capsules given at school. Ronaghy *et al.* 1969	12	17·2	14·7	11·6	14·1	No weight or height effects. Difference in sexual maturation.
Iran. 1969–71. 50 growth retarded school boys 13 years old studied for 17 months (5 months trial, 7 months rest, 5 months trial). Non-randomized trial placebo (10), micronutrients (20), micronutrients + 40 mg Zn (20). Capsules given at school. Ronaghy *et al.* 1974	12	8·2	10·2	10·5	10·7	Weight and height effects. Difference in bone age. Tendency for faster sexual development. No serum alkaline phosphatase effect.
USA, Colorado. 40 growth retarded, low Zn status children 2–6 years old studied for 1 year. Randomized pair-matched trial with placebo (20) and 10 mg Zn/d (20). Syrup given by parents at home. Walravens *et al.* 1983	4·6	10·7	10·8	11·3	11·3	Height effect (especially boys). Increase in appetite.
Canada. 1986. 60 growth retarded boys 5–7 years old studied for 12 months. Randomized pair-matched trial with placebo (30) and 10 mg Zn/d (30). Fruit juice drink given by parents at home. Gibson *et al.* 1989*b*	6·4	15·6	16·2	16·5	16·4	Height effect only in subjects with low hair Zn ($< 1{\cdot}68$ μmol/g). Increase in appetite perceived by parents.

Thailand. 1989–90. 133 children 6–13 years old with suboptimal Zn and vitamin A nutriture for 6 months. Randomized pair-matched trial with placebo (35), 25 mg Zn/d (33), vit. A + Zn (32). Capsules taken on school days. Udomkesmalee *et al.* 1992	4·3	13·2	19·0	13·2	14·3	No weight or height effect. Increase in serum alkaline phosphatase activity. Improved dark adaption. Improved conjunctival integrity.
The Gambia. 1989–90. 109 apparently healthy children 1/2 to 3 years old for 15 months. Randomized group matched trial with placebo (54), and 70 mg Zn (55) as a drink twice a week at clinic. Bates *et al.* 1993						No weight or height effect. Increase in arm circumference. Less malaria. Improved intestinal permeability.
Guatemala. 1989. 162 school children 6–8 years old for 25 weeks. Randomized pair-matched trial. Micronutrients (82), micronutrients + 10 mg Zn/d (80). Chewable tablet given at school on weekdays. Cavan *et al.* 1993*b*	10	14·2	16·2	14·4	14·9	No weight or height effect. Increase in triceps skinfold. Smaller decrease in mid arm circumference. No increase in serum alkaline phosphatase.
Chile. 1991. 46 short stature school children, 6–12 years old, consuming diets providing 50–60% of normal daily Zn intake. 12 month randomized study involving placebo *v.* 10 mg Zn/d. Castillo-Duran *et al.* 1987						No weight effect. Height effect in males only. No difference in plasma Zn.
Chile. 1993. 98 healthy preschool children studied for 14 months. Placebo *v.* 10 mg Zn/d. Ruz, 1992						Height effect in males. Trend in improved immune function and reduced giardiasis.

Golden, 1981*a*; Xue-Cun *et al.* 1985; Castillo-Duran *et al.* 1987; Khanum *et al.* 1988; Simmer *et al.* 1988; Udomkesmalee *et al.* 1990, 1992; Schlesinger *et al.* 1992; Bates *et al.* 1993; Cavan *et al.* 1993*a*, *b*; Roy *et al.* 1993; M. Ruz, 1993, pers. comm.; Smith *et al.* 1993) countries (Tables 3 & 4). In most of these studies, clinical signs of severe Zn deficiency were not apparent. Instead, mild Zn deficiency existed, characterized by reductions in linear and/or ponderal growth, and/or impairments in taste acuity, appetite, immune and intestinal function, and dark adaptation, some of which have responded positively to Zn supplementation in double-blind studies. Biochemical evidence of Zn deficiency has not been a consistent finding. This is not unexpected; physiological functional consequences (e.g. growth retardation) of mild Zn deficiency are often apparent before the Zn concentrations in plasma and/or tissues are significantly reduced (Gibson *et al.* 1989*b*; Ruz *et al.* 1991), emphasizing the importance of confirming mild Zn deficiency by a positive response to a supplement in double-blind studies.

PREGNANCY

Animal studies have clearly demonstrated the teratogenic effects of Zn deficiency (Hurley & Swenerton, 1966), but results of human studies have been inconsistent. In severe Zn deficiency in humans arising from acrodermatitis enteropathica, abortions and gross congenital malformations (e.g. anencephaly) have been reported (Hambidge *et al.* 1975). The existence of mild Zn deficiency during pregnancy and its effect on pregnancy outcome is less clear, in part because of difficulties in establishing the existence of marginal Zn status during pregnancy and/or inadequacies in the experimental designs. No double-blind Zn supplementation studies during pregnancy have been carried out in developing countries.

Serum Zn has been the most frequently used index of Zn status during pregnancy; it declines during pregnancy even in the presence of optimal maternal Zn nutriture (Swanson & King, 1987), attributed in part to expansion in plasma volume. Nevertheless, in women with inadequate Zn intakes, the decline in serum Zn during pregnancy may be abnormally large (Hambidge *et al.* 1983; Cherry *et al.* 1989).

Relationships between maternal plasma Zn and pregnancy outcome have been inconsistent, and have varied with both the stage of gestation and the outcome variable measured (Swanson & King, 1987). For example, plasma Zn correlated weakly with birth weight when sampled at mid pregnancy (McMichael *et al.* 1982), more strongly in early rather than in later pregnancy, i.e. third trimester (Neggers *et al.* 1990), or not at all (Arcasoy *et al.* 1978; Buzina *et al.* 1980; Hambidge *et al.* 1983; Campbell-Brown *et al.* 1985; Hunt *et al.* 1985; Tuttle *et al.* 1985; Mahomed *et al.* 1989). Plasma Zn has also been reported to correlate with pregnancy complications such as prolonged labour, hypertension, postpartum haemorrhage, spontaneous abortions, and/or congenital malformations by some (Jameson, 1976; Çavdar *et al.* 1980, 1991; Cherry *et al.* 1981; McMichael *et al.* 1982; Soltan & Jenkins, 1982; Hunt *et al.* 1985) but not all (Breskin *et al.* 1983; Mukherjee *et al.* 1984) investigators. In some double-blind Zn supplementation studies, significant reductions in pregnancy complications have been observed in the Zn treated compared to the placebo group (Hunt *et al.* 1984; Cherry *et al.* 1989; Jameson *et al.* 1990; Simmer *et al.* 1991) (Table 5), associated in some cases with alterations in prostaglandin metabolism (O'Dell *et al.* 1977).

Some relationships have also been reported between low maternal Zn concentrations in leucocytes and/or lymphocytes and intrauterine growth retardation (Meadows *et al.* 1981; Simmer & Thompson, 1985), low birth weight (Wells *et al.* 1987; Malhotra *et al.* 1990), and neural tube defects (Hinks *et al.* 1989). In two double-blind Zn supplementation studies

Table 5. *Zinc supplementation studies in pregnant women*

Country, date, no. of subjects, type of subjects, experimental treatment, reference	Dietary zinc intake (mg)	Responses
UK. 1985–6. 494 middle class women studied for last 4 months of pregnancy. Randomized double-blind trial with placebo (248) and 20 mg Zn/d (246). Capsules taken at home. Mahomed *et al.* 1989	9	No effect on birth weight. No difference in leucocyte Zn in supplemented and placebo group.
USA, New Orleans. 556 low income adolescent women studied for last 3 months of pregnancy. Randomized double-blind trial with placebo (288) and 30 mg Zn/d (268). Tablets taken at home. Cherry *et al.* 1989	?	No effect on birth weight. Reduced rates of prematurity and neonatal morbidity.
USA, Los Angeles. 1981–2. 138 Hispanic teenagers studied for last 4 months of pregnancy. Randomized double-blind trial with micronutrients (68) and micronutrients + 20 mg Zn/d (70). Capsules taken at home. Hunt *et al.* 1985	9·8	No effect on birth weight.
USA, Los Angeles. 213 Hispanic low income women enrolled < 27 week gestation age. Randomized double-blind trial with micronutrients (106) and micronutrients + 20 mg Zn/d (107). Hunt *et al.* 1984	9·3	No effect on birth weight. Reduced incidence of pregnancy induced hypertension.
UK. 56 pregnant females at risk of SGA infants. Studied last 15–25 weeks. Randomized double-blind trial with placebo (26) and 22·5 mg Zn/d (30). Simmer *et al.* 1991	?	Lower incidence of IUGR. Labour induced less often. C-section less often.
USA. 46 pregnant middle income females studied for 7–9 months. Not randomized double-blind study. Placebo (36) *v.* 15 mg Zn/d (10). Tablet taken 2 h after dinner. Hambidge *et al.* 1983	11	No effect on birth weight. No other effects observed.
Sweden. 1983–6. 1231 pregnant women. Not randomized double-blind study. 15–90 mg Zn/d (depending on serum Zn) given to 598 subjects; 633 given no Zn supplement. Jameson *et al.* 1990	9·4	Fewer preterm deliveries before 33rd week of gestation. Reduction in perinatal deaths; fewer spontaneous abortions.

C-section, Caesarian section; IUGR, intra-uterine growth retardation; SGA, small for gestational age.

(Mahomed *et al.* 1989; Thauvin *et al.* 1992), however, no differences in leucocyte Zn concentrations between the two groups were observed.

Several adaptive mechanisms exist during pregnancy to help meet the increased demands for Zn, including an increase in absorption, a reduction in endogenous losses, redistribution of tissue Zn, and an efficient maternal–fetal transfer (Swanson & King, 1987). Although such adaptive mechanisms may be adequate to prevent Zn deficiency in women in developed countries, they may not be sufficient for pregnant women from developing countries, whose Zn status may be especially low because of frequent reproductive cycling, excessive losses of endogenous Zn, combined with diets low in readily available Zn. Unfortunately, however, investigations of the Zn status of pregnant women in developing countries are limited (Çavdar *et al.* 1980; Prema, 1980; Okonofua *et al.* 1989, 1990); none has involved double-blind Zn supplementation trials.

In view of the inconsistencies noted above, the precise nature of the association between Zn status and pregnancy outcome remains unclear. Existing evidence suggests that the prevalence of deficiency in women during pregnancy in developing countries is likely to be

Table 6. *Double-blind zinc supplementation studies in lactating women*

Country, no. of subjects, type of subjects, experimental treatment, reference	Dietary zinc intake (mg)	Response
Brazil, Amazon region. 65 poor urban women studied for first 5 months of lactation. Randomized trial with placebo (28) and group consuming 15 mg Zn/d (37). Capsules taken at home. Shrimpton *et al.* 1983 and Shrimpton *et al.* 1985	23	No effect on milk Zn levels. Milk vitamin A levels increased. Less diarrhoea in infants.
USA, Colorado. 53 middle income lactating women, for varying durations up to 9 months. Controlled (8) trial with placebo (39) and group consuming 15 mg Zn/d (14). Tablets taken at home. Krebs *et al.* 1985	12·2	Decreased fall in milk Zn levels.
USA, Indiana. 49 middle income mothers studied during first 6 months of lactation. Controlled trial with groups consuming micronutrients (25) and micronutrients + 25 mg Zn (24). Different commercial supplements taken at home. Karra *et al.* 1986	11·2	Higher milk Zn levels.
USA, Maryland. 40 middle income women studied during the first 6 months of lactation. Randomized double-blind trial with groups consuming micronutrients (20) and micronutrients + 25 mg Zn/d (20). Tablets taken at home. Moser-Veillon & Reynolds, 1990	12	No effect on milk Zn levels.

higher than that in developed countries, but large, well designed, double-blind Zn supplementation trials are required to confirm the existence of nutritional deficiency and its precise impact on pregnancy outcome.

LACTATION

Studies of maternal Zn status during lactation are limited (Table 6). Some have documented low plasma concentrations in the presence of normal concentrations in hair, urine (Jackson *et al.* 1988), and/or breast milk, even in poorly nourished lactating women with chronically inadequate intakes of dietary Zn (Kirsten *et al.* 1985; Karra *et al.* 1986; Simmer *et al.* 1990). Two Zn supplementation studies during lactation (Krebs *et al.* 1985; Shrimpton *et al.* 1985) documented a reduction in the abnormally steep decline in breast milk Zn content during late lactation, although the numbers of subjects in these studies were small. Furthermore, the incidence of diarrhoea in the infants decreased, and milk retinol content was maintained at a higher level throughout lactation in the Zn supplemented Amazonian women (Shrimpton *et al.* 1983, 1985).

By contrast, in a US study in Indiana (Karra *et al.* 1986) in which 25 mg Zn/d were given, Zn levels of breast milk apparently increased. Such increases were not observed by Moser-Veillon & Reynolds (1990), despite a comparable daily Zn supplement to US Maryland lactating women. The study of Karra *et al.* (1986), unlike the Maryland study (Moser-Veillon & Reynolds, 1990), was not a double-blind randomized trial.

Even in malnourished women from developing countries whose breast milk Zn concentrations are not compromised, their volume of breast milk may be reduced (Brown

et al. 1986), thus contributing to growth failure in early infancy. Traditional weaning foods used in many developing countries are often based on unrefined cereals and/or legumes, low in bioavailable Zn. If these weaning foods are not processed to reduce their phytic acid content, their use may further compromise infant growth, especially if they replace rather than complement breast milk (Walker, 1990). Strategies which can be used in developing countries to reduce the phytic acid content of traditional staple foods, including weaning foods, are outlined below.

NUTRITION INTERVENTION STRATEGIES TO PREVENT ZINC DEFICIENCY IN DEVELOPING COUNTRIES

Both short term and long term nutrition intervention strategies can be used to prevent Zn deficiency in developing countries: (1) supplementation; (2) fortification; and (3) dietary modification/diversification using traditional household techniques. For pregnant women, supplementation or fortification is appropriate because a relatively short term response is required to improve their Zn status before the end of pregnancy. Moreover, requirements for Zn during pregnancy, like Fe, cannot be met from dietary sources alone. Such approaches can also be used to provide several micronutrients simultaneously. They do, however, rely on a stable infrastructure and require financial support on a long standing economic basis if they are to be successful. All too often such programmes have been suspended for economic, political, and logistical reasons.

The third approach, dietary modification/diversification, involves changes in food selection patterns and/or traditional household methods for preparing and processing indigenous foods. It is a more economically feasible, culturally acceptable, and sustainable intervention for alleviating Zn deficiency in developing countries. Possible dietary changes to improve both the content and bioavailability of Zn include increasing the consumption of flesh foods, rich sources of readily available Zn, when economically feasible, and making modifications to food preparation and processing practices to reduce the level of the higher inositol phosphates in plant based staples. Higher inositol phosphates can be hydrolysed to lower inositol phosphates enzymically *via* fermentation and/or germination (Svanberg & Sandberg, 1988). Alternatively, in some cases non-enzymic hydrolysis of the higher inositol phosphates can be achieved by thermal processing, or soaking, provided the phytic acid is present as the soluble potassium salt (Reddy *et al.* 1989). The extent of the hydrolysis of the higher inositol phosphates can be monitored using the HPLC method for phytic acid analysis (Lehrfeld, 1989). The latter, unlike the AOAC method (Harland & Oberleas, 1986), differentiates the hexaphosphate and pentaphosphate from the lower inositol phosphates. Only the former inhibit the bioavailability of Zn (Tao *et al.* 1986; Lönnerdal *et al.* 1989).

To be successful, these dietary modifications/diversifications must be introduced using well designed educational and social marketing projects aimed to change attitudes and dietary behaviours. To enhance their effectiveness and sustainability, they should be integrated into ongoing national health and nutrition programmes in developing countries which emphasize the broader health consequences of micronutrient deficiencies. This approach has been highly successful in the Philippines for controlling vitamin A deficiency (Solon, 1986). Implementation of these dietary strategies could have far reaching consequences for both maternal and infant health in many developing countries, decreasing morbidity and complications in pregnancy, reducing mortality during childbirth, risk of prematurity and low birth weight, and enhancing growth and development in infancy and childhood.

REFERENCES

Aggett, P. J. (1991). The assessment of zinc status: a personal view. *Proceedings of the Nutrition Society* **50**, 9–17.
Arcasoy, A., Çavdar, A. O. & Babacan, E. (1978). Decreased iron and zinc absorption in Turkish children with iron deficiency and geophagia. *Acta Hematologica* **60**, 76–84.
Baer, M. T. & King, J. C. (1984). Tissue zinc levels and zinc excretion during experimental zinc depletion in young men. *American Journal of Clinical Nutrition* **39**, 556–570.
Bates, C. J., Evans, P. H., Dardenne, M., Prentice, A., Lunn, P. G., Northrop-Clewes, C. A., Hoare, S., Cole, T. J., Horan, S. J., Longman, S. C., Stirling, D. & Aggett, P. J. (1993). A trial of zinc supplementation in young rural Gambian children. *British Journal of Nutrition* **69**, 243–255.
Beaton, G. H. (1985). Uses and limits of the use of the Recommended Dietary Allowances for evaluating dietary intake data. *American Journal of Clinical Nutrition* **41**, 155–164.
Bettger, W. J., Fish, T. J. & O'Dell, B. L. (1978). Effects of copper and zinc status of rats on erythrocyte stability and superoxide dismutase activity. *Proceedings of the Society for Experimental Biology and Medicine* **158**, 279–282.
Bindra, G. S., Gibson, R. S. & Thompson, L. U. (1986). [Phytate][calcium]/[zinc] ratios in Asian immigrant lacto-ovo vegetarian diets and their relationship to zinc nutriture. *Nutrition Research* **6**, 475–483.
Bradfield, R. B. & Hambidge, K. M. (1980). Problems with hair zinc as an indicator of body zinc status. *Lancet* **i**, 363.
Breskin, M. W., Worthington-Roberts, B. S., Knopp, R. H., Brown, Z., Plovie, B., Mottet, N. K. & Mills, J. L. (1983). First trimester serum zinc concentrations in human pregnancy. *American Journal of Clinical Nutrition* **38**, 943–953.
Brown, K., Akhtar, N. A., Robertson, A. D. & Ahmed, M. G. (1986). Lactational capacity of marginally nourished mothers: relationships between maternal nutritional status and quantity and proximate composition of milk. *Pediatrics* **78**, 909–919.
Brune, M., Rossander, L. & Hallberg, L. (1989). Iron absorption: no intestinal adaptation to a high-phytate diet. *American Journal of Clinical Nutrition* **49**, 542–545.
Butte, N. F., Villalpando, S., Wong, W. W., Flores-Huerta, S., Hernandez-Beltran, M. de J., O'Brian Smith, E. & Garza, C. (1992). Human milk intake and growth faltering of rural Mesoamerindian infants. *American Journal of Clinical Nutrition* **55**, 1109–1116.
Butterworth, C. E., Hatch, K., Cole, P., Sauberlich, H. E., Tamura, T., Cornwell, P. E. & Soong, S.-J. (1988). Zinc concentration in plasma and erythrocytes of subjects receiving folic acid supplementation. *American Journal of Clinical Nutrition* **47**, 484–486.
Buzina, R., Jušić, M., Sapunar, J. & Milanović, N. (1980). Zinc nutrition and taste acuity in school children with impaired growth. *American Journal of Clinical Nutrition* **33**, 2262–2267.
Campbell-Brown, M., Ward, R. J., Haines, A. P., North, W. R. S., Abraham, R. & McFadyen, I. R. (1985). Zinc and copper in Asian pregnancies – is there evidence for a nutritional deficiency? *British Journal of Obstetrics and Gynaecology* **92**, 875–885.
Carter, J. P., Grivetti, L. E., Davis, J. T., Nasiff, S., Mansour, A., Mousa, W. A., Atta, A., Patwardhan, V. N., Moneim, M. A., Abdou, I. A. & Darby, W. J. (1969). Growth and sexual development of adolescent Egyptian village boys. Effects of zinc, iron, and placebo supplementation. *American Journal of Clinical Nutrition* **22**, 59–78.
Casey, C. E., Neville, M. C. & Hambidge, K. M. (1989). Studies in human lactation: secretion of zinc, copper, and manganese in human milk. *American Journal of Clinical Nutrition* **49**, 773–785.
Castillo-Duran, C., Heresi, G., Fisberg, M. & Uauy, R. (1987). Controlled trial of zinc supplementation during recovery from malnutrition: effects on growth and immune function. *American Journal of Clinical Nutrition* **45**, 602–608.
Cavan, K. R., Gibson, R. S., Grazioso, C. F., Isalgue, A. M., Ruz, M. & Solomons, N. W. (1993*a*). Growth and body composition of periurban Guatemalan children in relation to zinc status: a cross-sectional study. *American Journal of Clinical Nutrition* **57**, 334–343.
Cavan, K. R., Gibson, R. S., Grazioso, C. F., Isalgue, A. M., Ruz, M. & Solomons, N. W. (1993*b*). Growth and body composition of periurban Guatemalan children in relation to zinc status: a zinc intervention trial. *American Journal of Clinical Nutrition* **57**, 344–352.
Çavdar, A. O., Babacan, E. & Arcasoy, A. (1980). Effect of nutrition on serum zinc concentration during pregnancy in Turkish women. *American Journal of Clinical Nutrition* **33**, 542–544.
Çavdar, A. O., Bahceci, M., Akar, N., Erten, J. & Yavuz, H. (1991). Effect of zinc supplementation in a Turkish woman with two previous anencephalic infants. *Gynecologic and Obstetric Investigation* **32**, 123–125.
Cherry, F. F., Bennett, E. A., Bazzano, G. S., Johnson, L. K., Fosmire, G. J. & Batson, H. K. (1981). Plasma zinc in hypertension/toxemia and other reproductive variables in adolescent pregnancy. *American Journal of Clinical Nutrition* **34**, 2367–2375.
Cherry, F. F., Sandstead, H. H., Rojas, P., Johnson, L. K., Batson, H. K. & Wang, X. B. (1989). Adolescent pregnancy: associations among body weight, zinc nutriture, and pregnancy outcome. *American Journal of Clinical Nutrition* **50**, 945–954.
Chesters, J. K. & Will, M. (1978). The assessment of zinc status of an animal from the uptake of ^{65}Zn by the cells of whole blood in vitro. *British Journal of Nutrition* **38**, 297–306.

Cossack, Z. T. & Prasad, A. S. (1983). Effect of protein source on the bioavailability of zinc in human subjects. *Nutrition Research* **3**, 23–31.

Davies, N. T., Carswell, A. J. P. & Mills, C. F. (1985). The effect of variations in dietary calcium intake on the phytate-zinc interaction in rats. In *Trace Elements in Man and Animals* (*5th International Symposium*), pp. 456–457 [C. F. Mills, I. Bremner and J. K. Chesters, editors]. Farnham Royal: Commonwealth Agricultural Bureaux.

Dorea, J. G. (1993). Is zinc a first limiting nutrient in human milk? *Nutrition Research* **13**, 659–666.

Erten, J., Arcasoy, A., Çavdar, A. O. & Cin, S. (1978). Hair zinc levels in healthy and malnourished children. *American Journal of Clinical Nutrition* **31**, 1172–1174.

Ferguson, E. L. (1992). A comparison of food consumption patterns and zinc status of preschool children from Southern Malawi and Ghana. Ph.D. thesis, University of Guelph.

Ferguson, E. L., Gibson, R. S., Opare-Obisaw, C., Osei-Opare, F., Stephen, A. M., Lehrfeld, J. & Thompson, L. U. (1993*a*). The zinc, calcium, copper, manganese, nonstarch polysaccharide and phytate content of seventy-eight locally grown and prepared African foods. *Journal of Food Composition and Analysis* **6**, 87–99.

Ferguson, E. L., Gibson, R. S., Opare-Obisaw, C., Ounpuu, S., Thompson, L. U. & Lehrfeld, J. (1993*b*). The zinc nutriture of preschool children living in two African countries. *Journal of Nutrition* **123**, 1487–1496.

Ferguson, E. L., Gibson, R. S., Thompson, L. U. & Ounpuu, S. (1989*a*). Dietary calcium, phytate, and zinc intakes and the calcium, phytate, and zinc molar ratios of the diets of a selected group of East African children. *American Journal of Clinical Nutrition* **50**, 1450–1456.

Ferguson, E. L., Gibson, R. S., Thompson, L. U., Ounpuu, S. & Berry, M. (1988). Phytate, zinc, and calcium contents of 30 East African foods and their calculated phytate:Zn, Ca:phytate, and [Ca][phytate]/[Zn] molar ratios. *Journal of Food Composition and Analysis* **1**, 316–325.

Ferguson, E. L., Gibson, R. S., Weaver, S. D., Heywood, P., Heywood, A. & Yaman, C. (1989*b*). The mineral content of commonly consumed Malawian and Papua New Guinean foods. *Journal of Food Composition and Analysis* **2**, 260–272.

Filteau, S. M. & Tomkins, A. M. (1994). Micronutrients and tropical infections. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **88**, 1–3.

Fitzgerald, S. L., Gibson, R. S., Quan de Serrano, J., Portocarrero, L., Vasquez, A., de Zepeda, E., Lopez-Palacios, C. Y., Thompson, L. U., Stephen, A. M. & Solomons, N. W. (1993). Trace element intakes and dietary phytate/Zn and Ca × phytate/Zn millimolar ratios of periurban Guatemalan women during the third trimester of pregnancy. *American Journal of Clinical Nutrition* **57**, 195–201.

Fordyce, E. J., Forbes, R. M., Robins, K. R. & Erdman, J. W. (1987). Phytate × calcium/zinc molar ratios: are they predictive of zinc bioavailability? *Journal of Food Science* **52**, 440–444.

Gibson, R. S. (1989). Assessment of trace element status in humans. *Progress in Food and Nutrition Science* **13**, 67–111.

Gibson, R. S., Ferguson, E. F., Smit Vanderkooy, P. D. & MacDonald, A. C. (1989*a*). Seasonal variations in hair zinc concentrations in Canadian and African children. *Science of the Total Environment* **84**, 291–298.

Gibson, R. S., Heywood, A., Yaman, C., Sohlström, A., Thompson, L. U. & Heywood, P. (1991*a*). Growth in children from the Wosera subdistrict, Papua New Guinea, in relation to energy and protein intakes and zinc status. *American Journal of Clinical Nutrition* **53**, 782–789.

Gibson, R. S., Smit Vanderkooy, P. D., MacDonald, A. C., Goldman, A., Ryan, B. A. & Berry, M. (1989*b*). A growth-limiting, mild zinc-deficiency syndrome in some Southern Ontario boys with low height percentiles. *American Journal of Clinical Nutrition* **49**, 1266–1273.

Gibson, R. S., Smit Vanderkooy, P. D. & Thompson, L. U. (1991*b*). Dietary phytate × calcium/zinc millimolar ratios and zinc nutriture in some Ontario preschool children. *Biological Trace Element Research* **30**, 87–94.

Giugliano, R. & Millward, D. J. (1984). Growth and zinc homeostasis in the severely Zn-deficient rat. *British Journal of Nutrition* **52**, 545–560.

Golden, M. H. N. (1989). The diagnosis of zinc deficiency. In *Zinc in Human Biology* (*International Life Sciences Institute of Human Nutrition Reviews*), pp. 324–333 [C. F. Mills, editor]. Berlin: Springer-Verlag.

Golden, M. H. N. & Golden, B. E. (1981*a*). Effect of zinc supplementation on the dietary intake, rate of weight gain, and energy cost of tissue deposition in children recovering from severe malnutrition. *American Journal of Clinical Nutrition* **34**, 900–908.

Golden, M. H. N. & Golden, B. E. (1981*b*). Trace elements: potential importance in human nutrition with particular reference to zinc and vanadium. *British Medical Bulletin* **37**, 31–36.

Grider, A., Bailey, L. B. & Cousins, R. J. (1990). Erythrocyte metallothionein as an index of zinc status in humans. *Proceedings of the National Academy of Sciences, USA* **87**, 1259–1262.

Hambidge, K. M. (1982). Hair analyses: worthless for vitamins, limited for minerals. *American Journal of Clinical Nutrition* **36**, 943–949.

Hambidge, K. M. (1989). Mild zinc deficiency in children. In *Zinc in Human Biology* (*International Life Sciences Institute of Human Nutrition Reviews*), pp. 285–295 [C. F. Mills, editor]. Berlin: Springer-Verlag.

Hambidge, K. M., Hambidge, C., Jacobs, M. & Baum, J. D. (1972). Low levels of zinc in hair, anorexia, poor growth, and hypogeusia in children. *Pediatric Research* **6**, 868–874.

Hambidge, K. M., Krebs, N. F., Jacobs, M. A., Favier, A., Guyette, L. & Ikle, D. N. (1983). Zinc nutritional status during pregnancy: a longitudinal study. *American Journal of Clinical Nutrition* **37**, 429–442.

Hambidge, K. M., Neldner, K. H. & Walravens, P. A. (1975). Zinc, acrodermatitis enteropathica, and congenital malformations. *Lancet* **i**, 577–578.

Harland, B. F. & Oberleas, D. (1986). Anion-exchange method for determination of phytate in foods: collaborative study. *Journal of the Association of Official Analytical Chemists* **69**, 667–670.

Harland, B. F. & Peterson, M. (1978). Nutritional status of lacto-ovo vegetarian Trappist monks. *Journal of the American Dietetic Association* **72**, 259–264.

Hinks, L. J., Ogilvy-Stuart, A., Hambidge, K. M. & Walker, V. (1989). Maternal zinc and selenium status in pregnancies with a neural tube defect or elevated plasma α-fetoprotein. *British Journal of Obstetrics and Gynaecology* **96**, 61–66.

Hunt, I. F., Murphy, N. J., Cleaver, A. E., Faraji, B., Swendseid, M. E., Browdy, B. L., Coulson, A. H., Clark, V. A., Settlage, R. H. & Smith, J. C. (1985). Zinc supplementation during pregnancy in low-income teenagers of Mexican descent: effects on selected blood constituents and on progress and outcome of pregnancy. *American Journal of Clinical Nutrition* **42**, 815–828.

Hunt, I. F., Murphy, N. J., Cleaver, A. E., Faraji, B., Swendseid, M. E., Coulson, A. H., Clark, V. A., Browdy, B. L., Cabalum, M. J. & Smith, J. C. (1984). Zinc supplementation during pregnancy: effects on selected blood constituents and on progress and outcome of pregnancy in low-income women of Mexican descent. *American Journal of Clinical Nutrition* **40**, 508–521.

Hurley, L. S. & Swenerton, H. (1966). Congenital malformations resulting from zinc deficiency in rats. *Proceedings of the Society for Experimental Biology and Medicine* **123**, 692–696.

Jackson, M. J., Giugliano, R., Giugliano, L. G., Oliveira, E. F., Shrimpton, R. & Swainbank, I. G. (1988). Stable isotope metabolic studies of zinc nutrition in slum-dwelling lactating women in the Amazon valley. *British Journal of Nutrition* **59**, 193–203.

Jameson, S. (1976). Effects of zinc deficiency in human reproduction. *Acta Medica Scandinavica* Suppl. 593, 5–89.

Jameson, S., Burtsröm, M. & Hellsing, K. (1990). Zinc status in pregnancy. The effect of zinc therapy on perinatal mortality. In *International Symposium on Trace Elements in Man and Animals VII*, pp. 4.8–4.9 [B. Momcilovic, editor].

Jones, R. B., Keeling, P. W. N., Hilton, P. J. & Thompson, R. P. H. (1981). The relationship between leucocyte and muscle zinc in health and disease. *Clinical Science* **60**, 237–239.

Karra, M. V., Udipi, S. A., Kirksey, A. & Roepke, J. L. B. (1986). Changes in specific nutrients in breast milk during extended lactation. *American Journal of Clinical Nutrition* **43**, 495–503.

Khanum, S., Alam, A. N., Anwar, I., Akbar Ali, M. & Mujibur Rahaman, M. (1988). Effect of zinc supplementation on the dietary intake and weight gain of Bangladeshi children recovering from protein-energy malnutrition. *European Journal of Clinical Nutrition* **42**, 709–714.

King, J. C. (1986). Assessment of techniques for determining human zinc requirements. *Journal of the American Dietetic Association* **86**, 1523–1528.

Kirsten, G. F., Heese, H. de V., Watermeyer, S., Dempster, W. S., Pocock, F. & Varkvisser, H. (1985). Zinc and copper levels in the breast-milk of Cape Town mothers. *South African Medical Journal* **68**, 402–405.

Koo, W. W. K., Succop, P. & Hambidge, K. M. (1989). Serum alkaline phosphatase and serum zinc concentrations in preterm infants with rickets and fractures. *American Journal of Diseases of Children* **143**, 1342–1345.

Krebs, N. F., Hambidge, K. M., Hagerman, R. J., Peirce, P. L., Johnson, K. M., English, J. L., Miller, L. L. & Fennessey, P. V. (1988). Effects of pharmacologic doses of folate on zinc absorption and zinc status. In *Nutrient Availability: Chemical and Biological Aspects* (*Royal Society of Chemistry Special Publication No. 72*), pp. 226–228 [D. A. T. Southgate, I. T. Johnson and G. R. Fenwick, editors]. Cambridge: Royal Society of Chemistry.

Krebs, N. F., Hambidge, K. M., Jacobs, M. A. & Rasbach, J. O. (1985). The effects of a dietary zinc supplement during lactation on longitudinal changes in maternal zinc status and milk zinc concentrations. *American Journal of Clinical Nutrition* **41**, 560–570.

Krebs, N. F., Hambidge, K. M. & Walravens, P. A. (1984). Increased food intake of young children receiving a zinc supplement. *American Journal of Diseases of Children* **138**, 270–273.

Lehrfeld, J. (1989). High-performance liquid chromatography analysis of phytic acid on a pH-stable, macroporous polymer column. *Cereal Chemistry* **66**, 510–515.

Lönnerdal, B., Sandberg, A.-S., Sandström, B. & Kunz, C. (1989). Inhibitory effects of phytic acid and other inositol phosphates on zinc and calcium absorption in suckling rats. *Journal of Nutrition* **119**, 211–214.

McMichael, A. J., Dreosti, I. E., Gibson, G. T., Hartshorne, J. M., Buckley, R. A. & Colley, D. P. (1982). A prospective study of serial maternal serum zinc levels and pregnancy outcome. *Early Human Development* **7**, 59–69.

Mahomed, K., James, D. K., Golding, J. & McCabe, R. (1989). Zinc supplementation during pregnancy: a double blind randomised controlled trial. *British Medical Journal* **299**, 826–830.

Malhotra, A., Fairweather-Tait, S. J., Wharton, P. A. & Gee, H. (1990). Placental zinc in normal and intra-uterine growth-retarded pregnancies. *British Journal of Nutrition* **63**, 613–621.

Mbofung, C. M. F. & Atinmo, T. (1987). Trace element nutriture of Nigerians. *World Review of Nutrition and Dietetics* **51**, 105–139.

Meadows, N. J., Ruse, W., Smith, M. F., Day, J., Keeling, P. W. N., Scopes, J. W., Thompson, R. P. H. & Bloxam, D. L. (1981). Zinc and small babies. *Lancet* **ii**, 1135–1137.

Mills, C. F. (1985). Dietary interactions involving the trace elements. *Annual Review of Nutrition* **5**, 173–193.
Milne, D. B., Canfield, W. K., Mahalko, J. R. & Sandstead, H. H. (1984). Effect of oral folic acid supplements on zinc, copper, and iron absorption and excretion. *American Journal of Clinical Nutrition* **39**, 535–539.
Milne, D. B., Ralston, N. V. C. & Wallwork, J. C. (1985). Zinc content of cellular components of blood: methods for cell separation and analysis evaluated. *Clinical Chemistry* **31**, 65–69.
Monsen, E. R. (1988). Iron nutrition and absorption: dietary factors which impact iron bioavailability. *Journal of the American Dietetic Association* **88**, 786–791.
Moser, P. B., Reynolds, R. D., Acharya, S., Howard, M. P., Andon, M. B. & Lewis, S. A. (1988). Copper, iron, zinc, and selenium dietary intake and status of Nepalese lactating women and their breast-fed infants. *American Journal of Clinical Nutrition* **47**, 729–734.
Moser-Veillon, P. B. & Reynolds, R. D. (1990). A longitudinal study of pyridoxine and zinc supplementation of lactating women. *American Journal of Clinical Nutrition* **52**, 135–141.
Mukherjee, M. D., Sandstead, H. H., Ratnaparkhi, M. V., Johnson, L. K., Milne, D. B. & Stelling, H. P. (1984). Maternal zinc, iron, folic acid, and protein nutriture and outcome of human pregnancy. *American Journal of Clinical Nutrition* **40**, 496–507.
Murphy, S. P., Beaton, G. H. & Calloway, D. H. (1992). Estimated mineral intakes of toddlers: predicted prevalence of inadequacy in village populations in Egypt, Kenya, and Mexico. *American Journal of Clinical Nutrition* **56**, 565–572.
National Academy of Sciences. (1991). *Nutrition During Pregnancy*. Washington, DC: National Academy Press.
National Research Council. (1986). *Nutrient Adequacy: Assessment Using Food Consumption Surveys*. Washington, DC: National Academy Press.
Neggers, Y. H., Cutter, G. R., Acton, R. T., Alvarez, J. O., Bonner, J. L., Goldenberg, R. L., Go, R. C. P. & Roseman, J. M. (1990). A positive association between maternal serum zinc concentration and birth weight. *American Journal of Clinical Nutrition* **51**, 678–684.
O'Dell, B. L., Reynolds, G. & Reeves, P. G. (1977). Analogous effects of zinc deficiency and aspirin toxicity in the pregnant rat. *Journal of Nutrition* **107**, 1222–1228.
Oberleas, D. & Harland, B. F. (1981). Phytate content of foods: effect on dietary zinc bioavailability. *Journal of the American Dietetic Association* **79**, 433–436.
Okonofua, F. E., Amole, F. A., Emofurieta, W. O. & Ugwu, N. C. (1989). Zinc and copper concentration in plasma of pregnant women in Nigeria. *International Journal of Gynecology and Obstetrics* **29**, 19–23.
Okonofua, F. E., Isinkaye, A., Onwudiegwu, U., Amole, F. A., Emofurieta, W. A. & Ugwu, N. C. (1990). Plasma zinc and copper in pregnant Nigerian women at term and their newborn babies. *International Journal of Gynecology and Obstetrics* **32**, 243–245.
Prasad, A. S. & Cossack, Z. T. (1982). Neutrophil zinc: an indicator of zinc status in man. *Transactions of the Association of American Physicians* **95**, 165–176.
Prasad, A. S., Meftah, S., Abdallah, J., Kaplan, J., Brewer, G. J., Bach, J. F. & Dardenne, M. (1988). Serum thymulin in human zinc deficiency. *Journal of Clinical Investigation* **82**, 1202–1210.
Prasad, A. S., Miale, A., Farid, Z., Sandstead, H. H. & Schulert, A. R. (1963). Zinc metabolism in patients with syndrome of iron deficiency anemia, hepatosplenomegaly, dwarfism, and hypogonadism. *Journal of Laboratory and Clinical Medicine* **61**, 537–549.
Prema, K. (1980). Predictive value of serum copper and zinc in normal and abnormal pregnancy. *Indian Journal of Medical Research* **71**, 554–560.
Reddy, N. R., Pierson, M. D., Sathe, S. K. & Salunkhe, D. K. (1989). *Phytates in Cereals and Legumes*. Boca Raton, FL: CRC Press.
Rodriguez, A., Venegas, G. V. & Torres, S. (1991). Zinc supplementation of infants fetal malnourished. In *Reunión de la Sociedad Latinoamericana de Oncologia Pediatrica VIII*.
Ronaghy, H. S., Reinhold, J. G., Mahloudji, M., Ghavami, P., Spivey Fox, M. R. & Halsted, J. A. (1974). Zinc supplementation of malnourished schoolboys in Iran: increased growth and other effects. *American Journal of Clinical Nutrition* **27**, 112–121.
Ronaghy, H. S., Spivey Fox, M. R., Garn, S. M., Israel, H., Harp, A., Moe, P. G. & Halsted, J. A. (1969). Controlled zinc supplementation for malnourished school boys: a pilot experiment. *American Journal of Clinical Nutrition* **22**, 1279–1289.
Rothbaum, R. J., Maur, P. R. & Farrell, M. K. (1982). Serum alkaline phosphatase and zinc undernutrition in infants with chronic diarrhea. *American Journal of Clinical Nutrition* **35**, 595–598.
Roy, S. K., Behrens, R. H., Haider, R., Akramuzzaman, S. M., Mahalanabis, D., Wahed, M. A. & Tomkins, A. M. (1992). Impact of zinc supplementation on intestinal permeability in Bangladeshi children with acute diarrhoea and persistent diarrhoea syndrome. *Journal of Pediatric Gastroenterology and Nutrition* **15**, 289–296.
Roy, S. K., Tomkins, A. M., Haider, R., Behrens, R. H. & Akramuzzaman, S. M. (1993). The importance of zinc deficiency in stunting and morbidity. *International Congress of Nutrition XV*, p. 713 (Abstr.)
Royal Tropical Institute, Amsterdam. (1987). *Weaning Food – a New Approach to Small-scale Weaning Food Production from Indigenous Raw Materials in Tropical Countries*, 2nd edn. Amsterdam: Royal Tropical Institute.
Ruz, M., Cavan, K. R., Bettger, W. J. & Gibson, R. S. (1992). Erythrocytes, erythrocyte membranes, neutrophils and platelets as biopsy materials for the assessment of zinc status in humans. *British Journal of Nutrition* **68**, 515–527.

Ruz, M., Cavan, K. R., Bettger, W. J., Thompson, L. U., Berry, M. & Gibson, R. S. (1991). Development of a dietary model for the study of mild zinc deficiency in humans and evaluation of some biochemical and functional indices of zinc status. *American Journal of Clinical Nutrition* **53**, 1295–1303.

Ruz, M. & Solomons, N. W. (1990). Mineral excretion during acute dehydrating diarrhea treated with oral rehydration therapy. *Pediatric Research* **27**, 170–175.

Sandström, B. (1989). Dietary pattern and zinc supply. In *Zinc in Human Biology* (*International Life Sciences Institute Human Nutrition Reviews*), pp. 351–363 [C. F. Mills, editor]. Berlin: Springer-Verlag.

Sandström, B., Almgren, A., Kivistö, B. & Cederblad, Å. (1989). Effect of protein level and protein source on zinc absorption in humans. *Journal of Nutrition* **119**, 48–53.

Sandström, B., Arvidsson, B., Cederblad, Å. & Björn-Rasmussen, E. (1980). Zinc absorption from composite meals. 1. The significance of wheat extraction rate, zinc, calcium, and protein content in meals based on bread. *American Journal of Clinical Nutrition* **33**, 739–745.

Sandström, B. & Lönnerdal, B. (1989). Promoters and antagonists of zinc absorption. In *Zinc in Human Biology* (*International Life Sciences Institute Human Nutrition Reviews*), pp. 57–78 [C. F. Mills, editor]. Berlin: Springer-Verlag.

Schiliro, G., Russo, A., Azzia, N., Mancuso, G. R., Di Gregorio, F. D., Romeo, M. A., Fallico, R. & Sciacca, S. (1987). Leucocyte alkaline phosphatase (LAP): a useful marker of zinc status in β-thalassemic patients. *American Journal of Pediatric Hematology/Oncology* **9**, 149–152.

Schlesinger, L., Arevalo, M., Arredondo, S., Diaz, M., Lönnerdal, B. & Stekel, A. (1992). Effect of a zinc-fortified formula on immunocompetence and growth of malnourished infants. *American Journal of Clinical Nutrition* **56**, 491–498.

Shrimpton, R., Alencar, F. H., Vasconcelos, J. C. & Rocha, Y. R. (1985). Effect of maternal zinc supplementation on the growth and diarrhoeal status of breast fed infants. *Nutrition Research* Suppl. 1, 338S–342S.

Shrimpton, R., Marinho, H. A., Rocha, Y. S. & Alencar, F. H. (1983). Zinc supplementation in urban Amazonian mothers: concentrations of Zn and retinol in maternal serum and milk. *Proceedings of the Nutrition Society* **42**, 122A.

Simmer, K., Ahmed, S., Carlsson, L. & Thompson, R. P. H. (1990). Breast milk zinc and copper concentrations in Bangladesh. *British Journal of Nutrition* **63**, 91–96.

Simmer, K., Khanum, S., Carlsson, L. & Thompson, R. P. H. (1988). Nutritional rehabilitation in Bangladesh – the importance of zinc. *American Journal of Clinical Nutrition* **47**, 1036–1040.

Simmer, K., Lort-Phillips, L., James, C. & Thompson, R. P. H. (1991). A double-blind trial of zinc supplementation in pregnancy. *European Journal of Clinical Nutrition* **45**, 139–144.

Simmer, K. & Thompson, R. P. H. (1985). Maternal zinc and intrauterine growth retardation. *Clinical Science* **68**, 395–399.

Smit-Vanderkooy, P. D. & Gibson, R. S. (1987). Food consumption patterns of Canadian preschool children in relation to zinc and growth status. *American Journal of Clinical Nutrition* **45**, 609–616.

Smith, J. C., Udomkesmalee, E. & Dhanamitta, S. (1993). Effect of vitamin A and zinc supplementation of children in Thailand and Belize, Central America. *International Congress of Nutrition XV*, p. 618 (Abstr.).

Solomons, N. W. (1981). Zinc and copper in human nutrition. In *Nutrition in the 1980s: Constraints on Our Knowledge* (*Progress in Clinical and Biological Research* Vol. 67), pp. 97–127 [N. Selvey and P. L. White, editors]. New York: Alan R. Liss.

Solomons, N. W. (1986). Competitive interaction of iron and zinc in the diet: consequences for human nutrition. *Journal of Nutrition* **116**, 927–935.

Solon, M. A. (1986). Control of vitamin A deficiency by education and the public health approach. In *Vitamin A Deficiency and its Control*, pp. 285–318 [J. C. Bauernfeind, editor]. New York: Academic Press.

Soltan, M. H. & Jenkins, D. M. (1982). Maternal and fetal plasma zinc concentration and fetal abnormality. *British Journal of Obstetrics and Gynaecology* **89**, 56–58.

Svanberg, U. & Sandberg, A. S. (1988). Improved iron availability in weaning foods. In *Improved Young Child Feeding in Eastern and Southern Africa: Household Level Food Technology*, pp. 366–373 [D. Alnwick, S. Moses and O. G. Schmidt, editors]. Ottawa: International Development Research Center.

Swanson, C. A. & King, J. C. (1987). Zinc and pregnancy outcome. *American Journal of Clinical Nutrition* **46**, 763–771.

Tao, S.-H., Spivey Fox, M. R., Phillippy, B. Q., Fry, B. E., Johnson, M. L. & Johnston, M. R. (1986). Effects of inositol phosphates on mineral utilization. *Federation Proceedings* **45**, 819.

Thauvin, E., Fusselier, M., Arnaud, J., Faure, H., Favier, M., Coudray, C., Richard, M.-J. & Favier, A. (1992). Effects of a multivitamin mineral supplement on zinc and copper status during pregnancy. *Biological Trace Element Research* **32**, 405–414.

Thompson, R. P. H. (1991). Assessment of zinc status. *Proceedings of the Nutrition Society* **50**, 19–28.

Tomkins, A., Behrens, R. & Roy, S. (1993). The role of zinc and vitamin A deficiency in diarrhoeal syndromes in developing countries. *Proceedings of the Nutrition Society* **52**, 131–142.

Torre, M., Rodriguez, A. R. & Saura-Calixto, F. (1991). Effects of dietary fiber and phytic acid on mineral availability. *CRC Critical Reviews in Food Science and Nutrition* **30**, 1–22.

Turnlund, J. R., King, J. C., Keyes, W. R., Gong, B. & Michel, M. C. (1984). A stable isotope study of zinc absorption in young men: effects of phytate and α-cellulose. *American Journal of Clinical Nutrition* **40**, 1071–1077.

Tuttle, S., Aggett, P. J., Campbell, D. & MacGillivray, I. (1985). Zinc and copper in human pregnancy: a longitudinal study in normal primigravidae and in primigravidae at risk of delivering a growth retarded baby. *American Journal of Clinical Nutrition* **41**, 1032–1041.

Udomkesmalee, E., Dhanamitta, S., Sirisinha, S., Charoenkiatkul, S., Tuntipopipat, S., Banjong, O., Rojroongwasinkul, N., Kramer, T. R. & Smith, J. C. (1992). Effect of vitamin A and zinc supplementation on the nutriture of children in Northeast Thailand. *American Journal of Clinical Nutrition* **56**, 50–57.

Udomkesmalee, E., Dhanamitta, S., Yhoung-Aree, J., Rojroongwasinkul, N. & Smith, J. C. (1990). Biochemical evidence suggestive of suboptimal zinc and vitamin A status in schoolchildren in Northeast Thailand. *American Journal of Clinical Nutrition* **52**, 564–567.

United Nations. (1991). Some options for improving nutrition in the 1990s. *SCN News* No. 7, Suppl. 16–18.

Valberg, L. S., Flanagan, P. R. & Chamberlain, M. J. (1984). Effects of iron, tin, and copper on zinc absorption in humans. *American Journal of Clinical Nutrition* **40**, 536–541.

Walker, A. F. (1990). The contribution of weaning foods to protein–energy malnutrition. *Nutrition Research Reviews* **3**, 25–47.

Wallock, L. M., King, J. C., Hambidge, K. M., English-Westcott, J. E. & Pritts, J. (1993). Meal-induced changes in plasma, erythrocyte, and urinary zinc concentrations in adult women. *American Journal of Clinical Nutrition* **58**, 695–701.

Walravens, P. A., Chakar, A., Mokni, R., Denise, J. & Lemonnier, D. (1992). Zinc supplements in breastfed infants. *Lancet* **340**, 683–685.

Walravens, P. A. & Hambidge, K. M. (1976). Growth of infants fed a zinc supplemented formula. *American Journal of Clinical Nutrition* **29**, 1114–1121.

Walravens, P. A., Hambidge, K. M. & Koepfer, D. M. (1989). Zinc supplementation in infants with a nutritional pattern of failure to thrive: a double-blind controlled study. *Pediatrics* **83**, 532–538.

Walravens, P. A., Krebs, N. F. & Hambidge, K. M. (1983). Linear growth of low income preschool children receiving a zinc supplement. *American Journal of Clinical Nutrition* **38**, 195–201.

Wells, J. L., James, D. K., Luxton, R. & Pennock, C. A. (1987). Maternal leucocyte zinc deficiency at start of third trimester as a predictor of fetal growth retardation. *British Medical Journal* **294**, 1054–1056.

Wise, A. (1983). Dietary factors determining the biological activities of phytate. *Nutrition Abstracts and Reviews* **53**, 791–806.

Xue-Cun, C., Tai-An, Y., Jin-Sheng, H., Qui-Yan, M., Zhi-Min, H. & Li-Xiang, L. (1985). Low levels of zinc in hair and blood, pica, anorexia, and poor growth in Chinese preschool children. *American Journal of Clinical Nutrition* **42**, 694–700.

Zlotkin, S. H. & Casselman, C. (1988). Diurnal variation in urinary zinc excretion and the use of zinc/Cr ratio from random urine samples to monitor zinc status. *Canadian Federation of Biological Sciences*, Quebec, p. 624 (Abstr.).

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