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Assessment of zinc status

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Although the gross skin rash of profound zinc deficiency is well documented in patients with acrodermatitis enteropathica, glucagonoma and severe deprivation, this striking clinical manifestation is rare. Unfortunately, other clinical signs are variable and difficult to assess, and so we seek a quantitative assessment of body Zn stores (Solomons, 1979).

There are two different measurements for assessing the status of a trace element or vitamin in the body; namely, either the total body content, or an indirect measure of an appropriate body content. There is, however, little work on directly measuring the total body content of Zn in man, although nearly 40 years ago Widdowson *et al.* (1951) estimated the content of three adult cadavers. These were of two patients who had died of chronic disease and one emaciated suicide, and so the Zn contents were probably not normal. Nevertheless, the calculated whole-body contents were 0·75, 1·2 and 2·0 g. These macabre studies have not been repeated, but it may be possible to extrapolate less directly from samples of bone and muscle, in which most (90%) of the Zn is contained, after calculating the skeletal and muscle mass. In small animals total content can be obtained directly (Jackson *et al.* 1982), but in man an indirect measure has to be used.

However, demonstration of an estimated body content below normal levels may not indicate deficiency. Thus, in starvation as tissues waste and urinary Zn increases (Elia *et al.* 1984), the body Zn content falls appropriately, and the body is depleted. Deficiency, as indicated by skin rashes or failure to grow, does not occur until refeeding when growth returns and requirements increase, as Golden & Golden (1981) reported for Zn in children with malnutrition in Jamaica, and Paton (1981) for vitamins in adults in Belsen. Deficiency might, therefore, be defined as an inappropriate reduction of whole-body content leading to abnormal physiological or biochemical functions that are reversed simply by supplementation with Zn. Thus, depletion is always associated with deficiency, but not always *vice versa*.

In clinical practice, however, a simple measure is required rapidly and reliably to assess Zn status in an individual. Reduction of the mean levels of groups of patients is useful only for research studies. Many attempts have been made to assess Zn status with static measurement of levels in a body fluid or tissue, but these demonstrate tissue or fluid depletion, which may or may not reflect levels in other tissues. This is particularly so if only a diseased tissue is measured, such as liver biopsy specimens in patients with liver disease (Keeling *et al.* 1981), because infiltration with collagen, fat etc. and loss of hepatocytes dissociate the results from those of other tissues.

STATIC MEASUREMENTS

1. Plasma. Zn is an intracellular element and so only 0·01–0·02% of the body content circulates in plasma where it is highly bound to plasma proteins, particularly albumin. In addition Zn is rapidly drawn into the liver and spleen in response to cytokines released during stress and infection. The levels fall during normal pregnancy (Meadows *et al.*

1981), especially if the plasma volume expands appropriately (Tuttle *et al.* 1985). Hence, it is not surprising that plasma levels, particularly in an individual, give limited information on body Zn status (Solomons, 1979). This is similarly true for potassium, which is also intracellular (Flear *et al.* 1957). In addition, Zn rises after meals, interestingly falls below the baseline 2 h after a meal, rises on short-term starvation (Elia *et al.* 1984) and undergoes a diurnal rhythm. Plasma levels are also correlated with albumin levels, to which Zn is chiefly bound in plasma, in several diseases (Solomons, 1979; Keeling *et al.* 1980; Tuttle *et al.* 1985; Pironi *et al.* 1987; Ainley *et al.* 1988; Goode *et al.* 1989*b*), and this explains the high frequency of Zn deficiency reported in so many chronic diseases. Golub *et al.* (1984) have elegantly demonstrated that plasma Zn levels fall in Zn-deprived pregnant monkeys only if they do not lose weight. If they waste, the Zn released from tissues, probably chiefly muscle, maintains plasma levels. Similarly, plasma levels rise in wasting Zn-deprived rats (Giugliano & Millward, 1984) and in growing marasmic children (Golden & Golden, 1981). Serum levels differ from those of plasma (Hambidge, 1988).

Unfortunately, the avalanche of papers relying entirely on plasma Zn levels continues, and yet their conclusions can only be limited. Nevertheless, in both simple human (Buerk *et al.* 1973; Hess *et al.* 1977; Prasad *et al.* 1978; Baer & King, 1984) and animal (Jackson *et al.* 1982; Crofton *et al.* 1983; Everett & Apgar, 1984; Giugliano & Millward, 1984) experimental Zn deficiency plasma levels fall.

2. Urine. In humans about 0·5 mg Zn is normally excreted in urine daily. This falls during Zn deprivation (Prasad *et al.* 1978; Baer & King, 1984), presumably as the level of non-protein-bound Zn in plasma falls, and hence, together with increased intestinal absorption, healthy adults on bioavailable diets can equilibrate even on an intake of only 2–3 mg/d (Buerk *et al.* 1973). However, since urinary Zn is greatly increased, for instance, in some patients with cirrhosis, possibly due to decreased hepatic extraction of the surge of absorbed Zn after a meal (Keeling *et al.* 1981), or in patients who are wasting (Fell *et al.* 1973; Jackson *et al.* 1981), or in diabetes mellitus (Kinlaw *et al.* 1983) or due to ethambutol (King & Schwartz, 1987) or during refeeding (Elia *et al.* 1984) or receiving intravenous Zn, its level is not reliable. There are also further problems in obtaining complete urinary collection, and in preventing contamination (Solomons, 1979). Urinary excretion may depend on the concentration of plasma and urine free amino acids (Yunice *et al.* 1978).

3. Liver. Zn levels in liver fall in experimental Zn deficiency (Jackson *et al.* 1982; Giugliano & Millward, 1984; Keen *et al.* 1988), as do the levels and hepatic extraction of Zn in liver disease in man (Keeling *et al.* 1981), but measurement of small biopsy samples, the effects of fat infiltration or frank liver disease, and the invasive nature of the test will always limit its use.

4. Muscle. About 60% of body Zn is in skeletal muscle. In man Zn levels are reduced in biopsy specimens in pregnancy (Meadows *et al.* 1983*a*) and liver disease (Jones *et al.* 1981). However, muscles are not homogeneous (Jackson *et al.* 1982; Giugliano & Millward, 1984). In the commonly used laboratory rat, experimental deficiency does not reduce levels in muscle (Jackson *et al.* 1982; Giugliano & Millward, 1984; Senapati, 1986), nor the pig (Crofton *et al.* 1983), although they fall in the cat (Jacobson *et al.* 1986). In any case, many clinicians find muscle biopsy needles rather invasive!

5. Bone. Bone contains about 30% of body Zn. Based on experimental deficiency in the rat (Giugliano & Millward, 1984; Milne *et al.* 1985*a*; Senapati, 1986) low levels in

bone should indicate depletion of 'stores' of Zn, when bone becomes avid for Zn (Senapati, 1986). This has been little studied in man, although the uptake of Zn may be increased in cirrhosis (Gvozdanovic *et al.* 1982). Bone structure is heterogeneous and so the core of tissue obtained with the needle may not be uniform. Nevertheless, needle bone biopsy specimens are routinely taken to diagnose osteomalacia, and this method needs further study.

6. Erythrocytes. These are readily available and contain a large amount of Zn, which is chiefly fixed within carbonic anhydrase (*EC* 4.2.1.1). Not surprisingly, therefore, erythrocyte Zn concentrations do not reliably change in experimental (Milne *et al.* 1985*a*; Apgar & Fitzgerald, 1987) and clinical deficiency (Prasad *et al.* 1978; Solomons, 1979; Keeling *et al.* 1980; Baer & King, 1984). It is possible that the small quantities of Zn in the erythrocyte membrane may reflect levels in other tissues, and this is being explored in our laboratory.

7. Leucocytes. Leucocytes are nucleated and their Zn content should reflect the levels of other tissues (Lindh & Johansson, 1987). Mixed leucocytes, and more recently, polymorphonuclear leucocytes (neutrophils) have, therefore, been used to measure Zn status in man. Given the heterogeneity of the cells, the results have been surprisingly consistent, and seem to reflect deficiency in a variety of diseases, ranging from experimental human deprivation (Prasad *et al.* 1978) to intra-uterine growth retardation (Meadows *et al.* 1981, 1983*b*; Simmer & Thompson, 1985; Wells *et al.* 1987) and the elderly (Goode *et al.* 1989*c*; Senapati *et al.* 1989). Leucocytes are easily obtained, but the separation procedure is difficult and lengthy. Haematological disorders can affect levels (Fredricks *et al.* 1964).

Polymorphonuclear leucocytes are probably now the preferred subpopulations to analyse (Goode *et al.* 1989*a*), since monocytes are heterogeneous, are more difficult to separate, are more easily contaminated with Zn-rich platelets (Milne *et al.* 1985*b*; Wallwork, 1987) and have longer and variable half-lives. Since monocytes contain more Zn than polymorphonuclear leucocytes (Simmer & Thompson, 1985; Goode *et al.* 1989*a*), changes in their relative numbers can affect levels in mixed cells as, for instance, occurs in normal pregnancy as the proportion of polymorphs falls (Meadows *et al.* 1981). In addition, in some experimental animals Zn deficiency does not lower levels (Crofton *et al.* 1983; Milne *et al.* 1985*a*; Apgar & Fitzgerald, 1987), although it does so in the cat (Jacobson *et al.* 1986). Since in the rat, muscle Zn levels also do not fall in deficiency, the good correlations with levels in muscle in man (Jones *et al.* 1981) were initially unexpected. In the housebound elderly, leucocyte Zn levels correlate with Zn balance (Bunker *et al.* 1987). Recent work has shown muscle and leucocyte Zn levels to be correlated in surgical patients (Goode *et al.* 1989*b*). Finally, the reduction in mean levels in diseases such as polycythaemia (Simmer *et al.* 1987) and diabetes mellitus (Pai & Prasad, 1988) suggests that leucocyte levels may not always simply reflect whole-body levels. The reasons for levels falling in the leucocyte, therefore, need further study.

8. Non-protein-bound Zn. On analogy with calcium, the portion of plasma Zn that is not bound to albumin and α2-macroglobulin should be in equilibrium with the much larger Zn pools in tissues, and should fall during deprivation as depleted tissues take up more Zn than plasma. This increased avidity has been demonstrated in the Zn-deficient rat (Senapati, 1986). The levels of plasma protein should not affect this fraction, and so potentially its measurement in a fasting blood sample could be useful (Whitehouse *et al.* 1983). Unfortunately, the non-protein-bound Zn is a tiny proportion of the whole, so its

accurate measurement is easily affected by contamination or binding of Zn to filters and containers. Nevertheless this method has potential. The latest careful estimates are that this fraction is only 0·2% of total plasma Zn (Bloxam *et al.* 1984).

9. Hair and nails. Although levels of toxic metals in hair and skin can indicate body burdens, most agree that measurement of levels of Zn in the integument are of little value (Solomons, 1979; Dormandy, 1986; Klevay *et al.* 1987; Hambidge, 1988). The hair is easily contaminated in vivo, cleaning removes intrinsic Zn (Buckley & Dreosti, 1984; Mikasa *et al.* 1988), and the content depends on the rate of growth (Erten *et al.* 1978) and its site (McKenzie, 1978). Nails are no better (Lavis *et al.* 1986). Unfortunately, the purveyors of commercial assessments of mineral status continue to use hair measurements.

10. Saliva. Similar to measurement of phenytoin levels, levels of Zn in saliva might reflect levels in plasma. Samples are easily obtained, but are liable to contamination in the mouth and may depend on the protein level and cellular content of saliva (Freeland-Graves *et al.* 1981) and flow rates. Salivary Zn cannot be recommended (Solomons, 1979; Baer & King, 1984; Hambidge, 1988).

FUNCTION TESTS

1. Electroretinogram. The highest tissue level of Zn is in the retina, partly because retinol dehydrogenase (*EC* 1.1.1.105) is a Zn metalloenzyme. Both vitamin A and Zn depletion, therefore, impair dark adaptation by the cones. The electroretinogram when carefully performed is, therefore, a sensitive physiological measure of Zn function, in which the speed and magnitude of the gradual increasing electrical response to short flashes of light is measured in the dark. The electroretinogram is abnormal in alcoholic cirrhosis (Morrison *et al.* 1978) when it correlates with leucocyte Zn levels (Keeling *et al.* 1982), and in experimental Zn depletion in the cat (Jacobson *et al.* 1986). Unfortunately, the measurements are not easily performed, and it is difficult to be sure that concomitant vitamin A deficiency is not also affecting the results.

2. Taste acuity. Tissue Zn deficiency impairs taste and food uptake, so there has been a campaign, chiefly outside scientific literature, to put forward the ability to detect small quantities of zinc sulphate on the tongue as a commercial test of Zn deficiency (Bryce-Smith & Hodgkinson, 1986). Most would agree that taste is difficult to measure objectively and that so far the findings are at best unconvincing (Solomons, 1979; Hambidge, 1988).

*3. Alkaline phosphatase (*EC *3.1.3.1).* Alkaline phosphatase is a Zn metalloenzyme and, therefore, its activity in blood has been measured and related to Zn levels (Weismann & Hoyer, 1985). It has not, however, proved useful (Solomons, 1979; Hambidge, 1988), probably because the enzyme is preserved in the face of depletion.

4. Metallothionein I. The metal-binding protein metallothionein is present in most tissues. A small amount circulates in plasma and erythrocytes, and their levels fall in experimental Zn depletion (Sato *et al.* 1984). It can be measured by radioimmunoassay (Garvey & Chang, 1981), but since it is affected by, for instance, a diurnal rhythm and iron intake (Robertson *et al.* 1989), more work is needed fully to assess its potential in detecting Zn deficiency in man.

5. Thymulin. The level of this thymic hormone falls in experimental human Zn deficiency (Prasad *et al.* 1988), presumably because it is Zn-dependent, and this may prove to be a relatively simple measure of deficiency.

6. Ethanol clearance. There has been a surprising report that the oral-plasma bioavailability curve of ethanol is increased in human experimental Zn deficiency so mild that even the levels in leucocytes only slightly fell (Milne *et al.* 1987). Although this could hardly be used as a clinical test, the results emphasize that Zn deficiency can affect many functions that could be usefully measured.

Zn KINETICS

1. Balance studies. Since intestinal Zn absorption increases in response to Zn deprivation, so that in the rat nearly 100% of Zn can be absorbed from the diet (Senapati, 1986), it should be possible to measure intake and excretion in urine and faeces on a small oral dose of Zn (Solomons, 1979; Bunker *et al.* 1987) and conclude whether patients are in positive or negative balance. This is impractical, however, in individual patients outside metabolic units, and results will depend on whether the cause of Zn deficiency, such as from intestinal malabsorption or increased urinary loss, is corrected. Nevertheless, retention of Zn was increased in patients with alcoholic cirrhosis (Blendis *et al.* 1978), suggesting that they were deficient.

3. Plasma bioavailability. The area under the plasma time *v.* concentration curve of Zn has been used as a proxy for intestinal absorption. On analogy with experimental Zn deficiency in animals, absorption and hence the plasma curves, should be increased. Surprisingly, therefore, reduced curves have been found in patients with Crohn's disease (Nakamura *et al.* 1988) and cirrhosis (Sullivan *et al.* 1979). However, such a Zn tolerance test presupposes a normal rate of plasma clearance, and recently this has been shown to be increased in patients with active Crohn's disease (Nakamura *et al.* 1988). This could be due either to increased avidity of depleted tissues for Zn, or to the disease itself, which greatly complicates interpretation of the curves. In liver disease, reduced hepatic extraction of Zn (Keeling *et al.* 1981) should increase plasma levels, while the increased urinary excretion (Keeling *et al.* 1980) will increase plasma clearance and reduce the area under the curves. Diabetes mellitus (Kinlaw *et al.* 1983) and ethambutol (King & Schwartz, 1987) may do the same.

An interesting method is to administer a mass dose of isotopic Zn and measure the plasma curves of not only total Zn, but also of the specific activity. This increases with the cold peak, suggesting that the exogenous Zn is diluted with endogenous Zn entering the plasma (Van den Hamer *et al.* 1987). It was suggested that this was derived from Zn in the mucosal cells, but it might also be due to exchange with Zn in any tissue. Such calculations in Zn-deficient subjects will be interesting.

2. Plasma clearance. The rate of clearance of cold or isotopic Zn might measure the avidity of tissues depleted of Zn, with the provisos mentioned previously (Nakamura *et al.* 1988), although over-analysis of curves (Prasad *et al.* 1963) should be resisted. Again, however, it would be difficult to use this as a simple test in individual patients. Furthermore, recent work suggests that the distribution of Zn among tissues can be altered. Thus, valproic acid increases the retention of Zn in liver (Keen *et al.* 1989). In women, oral Fe supplementation decreases plasma but not tissue Zn levels (Bloxam *et al.* 1989), and ethambutol may increase intestinal absorption and urinary excretion (King & Schwartz, 1987). In animals, cadmium induces metallothionein and traps Zn in tissues (Simmer *et al.* 1986). Similar shifts might explain unexpected reduced leucocyte Zn levels, such as in polycythaemia (Simmer *et al.* 1987).

4. Whole-body turnover. It would be expected that oral or intravenously administered isotopic Zn would turn over more slowly if Zn were depleted, because it would be retained more avidly in tissues. However, Zn has a very long half-life in some tissues, and it will also depend on whether there continues to be increased losses of Zn in urine (cirrhosis, sickle cell anaemia, tissue metabolism) or faeces (intestinal disease). A sensitive counter is needed (Lykken, 1983) to detect the small amount (5–10 μCi) Zn that can safely be administered to man. It is unlikely that all the pools of Zn in muscle and bone will fully equilibrate with the isotope for many weeks. Nevertheless, studies in man have shown Zn retention might be used as a proxy for absorption and then whole-body turnover calculated, which may be slower in Zn deficiency. These are lengthy studies (Aamodt *et al.* 1982). Our own studies unfortunately suggest that turnover is best related to urinary excretion rates.

5. Specific activity in urine. Following the administration of a small quantity of isotopic Zn, the urinary Zn specific isotope ratio will rise and then fall to a plateau when urinary Zn is in equilibrium with plasma Zn, which itself may eventually come into equilibrium with tissue Zn. Using these assumptions the normal whole-body Zn has been estimated (Mills *et al.* 1983) to be much lower (<1 g) than previous estimates, even as low as 100 mg! These results are difficult to understand. Perhaps urinary isotopic excretion remains too high because it does not fully equilibrate with all the metal in muscle and particularly in bone.

Thus in the rat, endotoxin can alter the size of Zn pools with which isotopic Zn equilibrates (Lowe & Jackson, 1989), and this could be relevant. The estimated whole-body content was increased in the patients with alcoholic cirrhosis (Mills *et al.* 1983), in spite of hyperzincuria, and was combined with increased absorption (amount retained at 10 d) and a normal turnover rate (measured up to 32 weeks). These results are best explained by increased avidity for the isotope in a depleted tissue, such as bone (Gvozdanovic *et al.* 1982) or muscle (Senapati, 1986). With the persisting high urinary loss of non-isotopic Zn, whole-body content, calculation of which depends on urinary specific activity, would then be inaccurate.

In conclusion, a simple, reliable clinical measure of Zn deficiency is lacking. Polymorphonuclear leucocyte Zn levels are probably at present the most reliable measurement and are increasingly used, but are difficult to measure. If we agree with Solomons (1979) that the response of clinical variables to Zn supplementation is the best test for detecting deficiency, then Zn deficiency is worldwide in apparently healthy children (Hambidge *et al.* 1985; Gibson *et al.* 1989), in malnutrition (Castillo-Duran *et al.* 1987; Simmer *et al.* 1988), and in pregnancy (Simmer & Thompson, 1985). Supplementation carries the risk of causing Cu depletion (Anon, 1985) and so selection of individuals needing extra Zn will be required, but for this a simpler and better test is urgently needed.

REFERENCES

Aamodt, R. L., Rumble, W. F., Babcock, A. K., Foster, D. M. & Henkin, R. I. (1982). Effects of oral zinc loading on zinc metabolism in humans. I. Experimental studies. *Metabolism* **31**, 326–334.

Ainley, C. C., Cason, J., Carlsson, L. K., Slavin, B. M. & Thompson, R. P. H. (1988). Zinc status in inflammatory bowel disease. *Clinical Science* **75**, 277–283.

Anon (1985). Copper deficiency induced by megadoses of zinc. *Nutrition Reviews* **43**, 148–149.

Apgar, J. & Fitzgerald, J. A. (1987). Measure of zinc status in ewes given a low zinc diet throughout pregnancy. *Nutrition Research* **7**, 1281–1290.

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.

Blendis, L. M., Wesson, D., Doody, M., Allen, L. C., Dietrich, L. & Goldberg, E. M. (1978). Zinc deficiency in cirrhosis. *Gastroenterology* **75**, 956.

Bloxam, D. L., Tan, J. C. Y. & Parkinson, C. E. (1984). Non-protein bound zinc concentration in human plasma and amniotic fluid measured by ultrafiltration. *Clinica Chimica Acta* **144**, 81–93.

Bloxam, D. L., Williams, N. R., Waskett, R. J. D., Pattinson-Green, P. M., Morarji, Y. & Stewart, S. G. (1989). Maternal zinc during oral iron supplementation in pregnancy: a preliminary study. *Clinical Science* **76**, 59–65.

Bryce-Smith, D. & Hodgkinson, L. (1986). *The Zinc Solution*. London: Century Arrow.

Buckley, R. A. & Dreosti, I. E. (1984). Radioisotopic studies concerning the efficacy of standard washing procedures for the cleaning of hair before zinc analysis. *American Journal of Clinical Nutrition* **40**, 840–846.

Buerk, C. A., Chandy, M. G., Pearson, E., MacAuly, A. & Soroff, H. S. (1973). Zinc deficiency: effect on healing and metabolism in man. *Surgical Forum* **24**, 101–102.

Bunker, V. W., Hinks, L. J., Stansfield, M. F., Lawson, M. S. & Clayton, B. E. (1987). Metabolic balance studies for zinc and copper in housebound elderly people and the relationship between zinc balance and leukocyte zinc concentrations. *American Journal of Clinical Nutrition* **46**, 353–359.

Castillo-Duran, C., Heresi, G., Fisberg, M. & Oauy, 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.

Crofton, R. W., Clapham, M., Humphries, W. R., Aggett, P. J. & Mills, C. F. (1983). Leucocyte and tissue zinc concentrations in the growing pig. *Proceedings of the Nutrition Society* **42**, 128A.

Dormandy, T. L. (1986). Trace element analysis of hair. *British Medical Journal* **293**, 975–976.

Elia, M., Crozier, C. & Neale, G. (1984). Mineral metabolism during short-term starvation in man. *Clinica Chimica Acta* **139**, 37–45.

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

Everett, G. & Apgar, J. (1984). Effect of low zinc intake on plasma and leukocyte zinc concentration in pregnant ewes. In *Trace Element Analytical Chemistry in Medicine & Biology*, vol. 3, pp. 695–702 [P. Bratter and P. Schramel, editors]. Berlin: Walter de Gruyter.

Fell, G. S., Fleck, A., Cuthbertson, D. P., Queen, K., Morrison, D. P., Bessent, R. G. & Husain, S. L. (1973). Urinary zinc levels as an indication of muscle catabolism. *Lancet* **i**, 280–282.

Flear, C. T. G., Cooke, W. T. & Quinton, A. (1957). Serum potassium levels as an index of body content. *Lancet* **i**, 458–459.

Fredricks, R. E., Tanaka, K. R. & Valentine, W. N. (1964). Variations of human blood cell zinc in disease. *Journal of Clinical Investigation* **43**, 304–315.

Freeland-Graves, J. H., Hendrickson, P. J., Ebangit, M. L. & Snowden, J. Y. (1981). Salivary zinc as an index of zinc status in women fed a low-zinc diet. *American Journal of Clinical Nutrition* **34**, 312–321.

Garvey, J. S. & Chang, C. C. (1981). Detection of circulating metallothionein in rats injected with zinc or cadmium. *Science* **214**, 805–807.

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

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

Golden, B. E. & Golden, M. H. N. (1981). Plasma zinc, rate of weight gain and the energy cost of tissue deposition in children recovering from severe malnutrition on a cow's milk or soya protein based diet. *American Journal of Clinical Nutrition* **34**, 892–899.

Golub, M. S., Gershwin, M. E., Hurley, L. S., Baly, D. L. & Hendrickx, A. G. (1984). Studies of marginal zinc deprivation in rhesus monkeys. I. Influence on pregnant dams. *American Journal of Clinical Nutrition* **39**, 265–280.

Goode, H. F., Kelleher, J. & Walker, B. E. (1989*a*). Zinc concentrations in pure populations of peripheral blood neutrophils, lymphocytes and monocytes. *Annals of Clinical Biochemistry* **26**, 89–95.

Goode, H. F., Kelleher, J., Walker, B. E., Hall, R. I. & Guillou, P. J. (1989*b*). Neutrophil zinc is related to the severity of hepatic damage in patients with liver disease. *Proceedings of the Nutrition Society* **49**, 73A.

Goode, H. F., Penn, N. D., Kelleher, J. & Walker, B. E. (1989*c*). A critical assessment of leucocyte zinc as an index of Zn status in chronically ill hospitalized elderly patients. *Proceedings of the Nutrition Society* **49,** 71A.

Gvozdanovic, S., Gvozdanovic, D., Crofton, R. W., Aggett, P. J., Mowatt, N. A. G. M. & Brunt, P. W. (1982). Study of zinc kinetics in liver and skeleton in patients with cirrhosis. *Nuclear Medicine Communications* **3,** 127.

Hambidge, K. M. (1988). Assessing the trace element status of man. *Proceedings of the Nutrition Society* **47,** 37–44.

Hambidge, K. M., Krebs, N. F. & Walravens, P. A. (1985). Zinc-deficiency. In *Proceedings of the XIII International Congress of Nutrition*, pp. 513–516 [T. G. Taylor and N. K. Jenkins, editors]. London: John Libbey.

Hess, F. M., King, J. C. & Margen, S. (1977). Zinc excretion in young women on low zinc intakes and oral contraceptive agents. *Journal of Nutrition* **107,** 1610–1620.

Jackson, M. J., Jones, D. A. & Edwards, R. H. T. (1981). Zinc excretion as an index of muscle catabolism. *Clinical Science* **61,** 7.

Jackson, M. J., Jones, D. A. & Edwards, R. H. T. (1982). Tissue zinc levels as an index of body zinc status. *Clinical Physiology* **2,** 333–343.

Jacobson, S. G., Meadows, N. J., Keeling, P. W. N. & Thompson, R. P. H. (1986). Rod-mediated retinal dysfunction in cats with zinc depletion: comparison with taurine depletion. *Clinical Science* **71,** 559–564.

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.

Keeling, P. W. N., Jones, R. B., Hilton, P. J. & Thompson, R. P. H. (1980). Reduced leucocyte zinc in liver disease. *Gut* **21,** 561–564.

Keeling, P. W. N., O'Day, J., Ruse, W. & Thompson, R. P. H. (1982). Zinc deficiency and photoreceptor dysfunction in chronic liver disease. *Clinical Science* **62,** 109–111.

Keeling, P. W. N., Ruse, W., Bull, J., Hannigan, B. & Thompson, R. P. H. (1981). Direct measurement of the hepatointestinal extraction of zinc in cirrhosis and hepatitis. *Clinical Science* **61,** 441–444.

Keen, C. L., Golub, M. S., Gershwin, M. E., Lonnerdal, B. & Hurley, L. S. (1988). Studies of marginal zinc deprivation in rhesus monkeys. III. Use of liver biopsy in the assessment of zinc status. *American Journal of Clinical Nutrition* **47,** 1041–1045.

Keen, C. L., Peters, J. M. & Hurley, L. S. (1989). The effect of valproic acid on ^{65}Zn distribution in the pregnant rat. *Journal of Nutrition* **119,** 607–611.

King, A. B. & Schwartz, R. (1987). Effects of the antituberculous drug ethambutol on zinc absorption, turnover and distribution in rats fed diets marginal and adequate in zinc. *Journal of Nutrition* **117,** 704–708.

Kinlaw, W. B., Levine, A. S., Morley, J. E., Silvis, S. E. & McClain, C. J. (1983). Abnormal zinc metabolism in type II diabetes mellitus. *American Journal of Medicine* **75,** 273–277.

Klevay, L. M., Bistrian, B. R., Fleming, C. R. & Neumann, C. G. (1987). Hair analysis in clinical and experimental medicine. *American Journal of Clinical Nutrition* **46,** 233–236.

Lavis, G. J., Ofei, V. & Bender, D. A. (1986). Differences in the zinc content of different regions of the toe-nail. *Proceedings of the Nutrition Society* **46,** 59A.

Lindh, U. & Johansson, E. (1987). Trace-element determination in individual peripheral blood cells and possible diagnostic applications. *Biological Trace Element Research* **12,** 351–362.

Lowe, N. M. & Jackson, M. J. (1989). Plasma ^{65}zinc kinetics in zinc-deficient and endotoxin-treated rats. *Proceedings of the Nutrition Society* **49,** 69A.

Lykken, G. I. (1983). A whole body counting technique using ultralow doses of ^{59}Fe and ^{65}Zn in absorption and retention studies in humans. *American Journal of Clinical Nutrition* **37,** 652–662.

McKenzie, J. M. (1978). Alteration of the zinc and copper concentration of hair. *American Journal of Clinical Nutrition* **31,** 470–476.

Meadows, N. J., Cunnane, S. C., Keeling, P. W. N. & Thompson, R. P. H. (1983*a*). The diagnosis of nucleated tissue zinc depletion in man and its effect upon pregnancy. In *Zinc Deficiency in Human Subjects*, [A. S. Prasad, A. O. Cavdar, G. J. Brewer and P. J. Aggett, editors]. New York: Alan R. Liss.

Meadows, N., Ruse, W., Keeling, P. W. N., Scopes, J. W. & Thompson, R. P. H. (1983*b*). Peripheral blood leucocyte zinc depletion in babies with intrauterine growth retardation. *Archives of Diseases in Childhood* **58,** 807–809.

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.

Mikasa, H., Suzuki, Y., Fujii, N. & Nishiyama, K. (1988). Adsorption and elution of metals on hair. *Biological Trace Element Research* **16,** 59–66.

Mills, P. R., Fell, G. S., Bessent, R. G., Nelson, L. M. & Russell, R. I. (1983). A study of zinc metabolism in alcoholic cirrhosis. *Clinical Science* **64,** 527–535.

Milne, D. B., Canfield, W. K., Gallagher, S. K., Hunt, J. R. & Klevay, L. M. (1987). Ethanol metabolism in postmenopausal women fed a diet marginal in zinc. *American Journal of Clinical Nutrition* **46,** 688–693.

Milne, D. B., Ralston, N. V. C. & Wallwork, J. C. (1985*a*). Zinc content of blood cellular components and lymph node and spleen lymphocytes in severely zinc-deficient rats. *Journal of Nutrition* **115,** 1073–1078.

Milne, D. B., Ralston, N. V. C. & Wallwork, J. C. (1985*b*). Zinc content of cellular components of blood: Methods for cell separation and analysis evaluated. *Clinical Chemistry* **31,** 65–69.

Morrison, S. A., Russell, R. M., Carney, E. A. & Oaks, E. V. (1978). Zinc deficiency: a cause of abnormal dark adaptation in cirrhotics. *American Journal of Clinical Nutrition* **31,** 276–281.

Nakamura, T., Higashi, A., Takano, S., Akagi, M. & Matsuda, I. (1988). Zinc clearance correlates with clinical severity of Crohn's disease. A kinetic study. *Digestive Diseases and Sciences* **33,** 1520–1524.

Pai, L. H. & Prasad, A. S. (1988). Cellular zinc in patients with diabetes mellitus. *Nutrition Research* **8,** 889–897.

Paton, A. (1981). Mission to Belsen 1945. *British Medical Journal* **283,** 1656–1659.

Pironi, L., Miglioli, M., Cornia, G. L., Ursitti, M. A., Tolomelli, M., Piazzi, S. & Barbara, L. (1987). Urinary zinc excretion in Crohn's disease. *Digestive Diseases and Sciences* **32,** 358–362.

Prasad, A., 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., Miale, A., Farid, Z., Sandstead, H. H. & Schulert, A. R. (1963). Zinc metabolism in patients with the syndrome of iron deficiency anemia, hepatosplenomegaly, dwarfism, and hypogonadism. *Journal of Laboratory and Clinical Medicine* **61,** 537–549.

Prasad, A. S., Rabbani, P., Abbasii, A., Bowersox, E. & Fox, M. R. S. (1978). Experimental zinc deficiency in humans. *Annals of Internal Medicine* **89,** 483–490.

Robertson, A., Morrison, J. N., Wood, A. M. & Bremner, I. (1989). Effects of iron deficiency on metallothionein-I concentrations in blood and tissues of rats. *Journal of Nutrition* **119,** 439–445.

Sato, M., Mehra, R. K. & Bremner, I. (1984). Measurement of plasma metallothionein-I in the assessment of the zinc status of zinc-deficient and stressed rats. *Journal of Nutrition* **114,** 1683–1689.

Senapati, A. (1986). Zinc in growth and healing. PhD Thesis, London.

Senapati, A., Jenner, G. & Thompson, R. P. H. (1989). Zinc in the elderly. *Quarterly Journal of Medicine* **70,** 81–87.

Simmer, K., Carlsson, L. & Thompson, R. P. H. (1986). Interaction of cadmium and zinc in pregnancy. *Clinical Science* **70,** 50–51P.

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

Simmer, K., Pearson, T. C., Wheeler, M. J. & Thompson, R. P. H. (1987). Zinc status in polycythaemia. *European Journal of Haematology* **38,** 433–436.

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

Solomons, N. W. (1979). On the assessment of zinc and copper nutriture in man. *American Journal of Clinical Nutrition* **32,** 856–871.

Sullivan, J. F., Jetton, M. M. & Burch, R. E. (1979). A zinc tolerance test. *Journal of Laboratory and Clinical Medicine* **93,** 485–492.

Tuttle, S., Aggett, P. J., Campbell, D. & MacGillivray, I. (1985). Zinc and copper nutrition 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.

Van den Hamer, C. J. A., Kroon, J. J. & Tjioe, P. S. (1987). An oral zinc loading test using an enriched stable zinc isotope. In *Trace Element Analytical Chemistry in Medicine & Biology*, vol. 4, pp. 247–253 [P. Bratter and P. Schramel, editors]. Berlin: Walter de Gruyter.

Wallwork, J. C. (1987). Appraisal of the methodology and applications for measurement of the zinc content of blood components as indicators of zinc status. *Biological Trace Element Research* **12,** 335–350.

Weismann, K. & Hoyer, H. (1985). Serum alkaline phosphatase and serum zinc levels in the diagnosis and exclusion of zinc deficiency in man. *American Journal of Clinical Nutrition* **41,** 1214–1219.

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.

Whitehouse, R. C., Prasad, A. S. & Cossack, Z. T. (1983). Determination of ultrafiltrable zinc in plasma by flameless atomic absorption spectrophotometry. *Clinical Chemistry* **29,** 1974–1977.

Widdowson, E. M., McCance, R. A. & Spray, C. M. (1951). The chemical composition of the human body. *Clinical Science* **10**, 113–125.

Yunice, A. A., King, R. W., Kraikitpanitch, S., Haygood, C. C. & Lindeman, R. D. (1978). Urinary zinc excretion following infusions of zinc sulfate, cysteine, histidine, or glycine. *American Journal of Physiology* **235**, F40–45.

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