

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.* 1989b), 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.* 1983a) 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.* 1985a; 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.* 1985a; 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, 1983b; Simmer & Thompson, 1985; Wells *et al.* 1987) and the elderly (Goode *et al.* 1989c; 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.* 1989a), since monocytes are heterogeneous, are more difficult to separate, are more easily contaminated with Zn-rich platelets (Milne *et al.* 1985b; Wallwork, 1987) and have longer and variable half-lives. Since monocytes contain more Zn than polymorphonuclear leucocytes (Simmer & Thompson, 1985; Goode *et al.* 1989a), 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.* 1985a; 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.* 1989b). 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 α 2-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.

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