

Albumin bound and α_2 -macroglobulin bound zinc concentrations in the sera of healthy adults

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SUMMARY Reference ranges for albumin bound and α_2 -macroglobulin bound zinc concentrations have been determined in a study of sera obtained from 134 healthy adults. The concentrations of zinc bound to α_2 -macroglobulin were remarkably constant with a mean (\pm SD) of $2.4 \pm 0.6 \mu\text{mol/l}$; the variations in total serum zinc concentrations were almost entirely accounted for by variations in the zinc associated with albumin. There were no sex related differences in the transport of zinc in serum; neither was this sensitive to the use of oral contraceptives. These data provide a baseline for further investigations into the effects of zinc deficiency on the serum transport of the metal.

There is increasing recognition that secondary zinc deficiency states may complicate a wide variety of clinical conditions.¹ Research in this field has been inhibited, however, by difficulties associated with the assessment of body zinc availability, which is commonly attempted using analyses for the total zinc in samples of plasma or serum. Only the most florid deficiencies of zinc can be reliably diagnosed by such means since equivocally reduced plasma zinc concentrations may be found in many circumstances in which no deficiency of the metal is suspected.² On some occasions these findings are the result of a transfer of zinc from the plasma to intracellular sites; this commonly occurs acutely after operations or after other similarly stressful events.³ Even in more stable clinical circumstances difficulties may arise because almost all of the plasma zinc is bound to proteins, which are themselves subject to changes of concentration in response to factors unrelated to the availability of the metal.

Because of these latter problems attempts have been made to characterise the distribution of zinc between its plasma protein ligands, both in health and disease. Unfortunately, the results of these studies have often been contradictory, not only with regard to the identities of the protein species which bind zinc in plasma, but also with regard to the distribution of zinc between them. The disparity of

these findings almost certainly results from analytical errors arising from three principal sources: incomplete protein separation, disruption of metal-protein interactions during the process of protein fractionation, and contamination with exogenous zinc.

We have recently shown that the zinc contained in human serum is bound almost exclusively to only two proteins—albumin and α_2 -macroglobulin^{4,5}—and we have developed a rapid and reliable micro-method for the determination of these two zinc-protein species.⁴ The present study was undertaken to investigate the concentrations of albumin bound and α_2 -macroglobulin bound zinc in the sera of healthy human subjects to provide control data for use in subsequent studies of zinc deficiency states.

Subjects and methods

SUBJECTS

Whole blood was obtained from 134 normal healthy volunteers: 86 men aged 18–62 years and 48 women aged 19–60 years. Eleven of the women who participated, aged 19–31 years, were taking combined oral contraceptives (30 μg of ethinylloestradiol and 125–250 μg of levonorgestrel daily). All of the subjects studied gave their informed consent for venepuncture, and the study was performed with the approval of the joint ethical subcommittee of the Hampshire Area Health Authority (Southampton District) and the Faculty of Medicine of Southampton University.

METHODS

Disposable plastic syringes and stainless steel needles were used to obtain venous blood, which was transferred to plain glass bottles; these had been made free of zinc by immersion in dilute (1 + 19) nitric acid for 2 h before being rinsed six times with deionised water and then dried. Serum was separated by centrifugation and stored in zinc free polycarbonate tubes at -20°C until required for analysis.

Previously described methods were used to determine the serum albumin, α_2 -macroglobulin, and total zinc concentrations together with the concentrations of albumin bound zinc and α_2 -macroglobulin bound zinc.^{4,6} Further details relating to these analytical methods are given in Table 1.

STATISTICAL ANALYSIS

Sets of data were compared by means of Student's unpaired *t* test. All of the data given in this paper are expressed as mean \pm 1 standard deviation except where otherwise indicated. In tests of significance, 0.05 was taken to be the critical value of *p*.

Results

The data resulting from the analyses of the sera obtained from the 86 men and the 34 women who were not using oral contraceptives are summarised in Table 2.

RECOVERY OF ZINC

The summation of the concentrations of zinc found

in association with albumin and α_2 -macroglobulin were generally in good agreement with the total zinc concentration in the case of each sample of serum examined. The total recovery of zinc was $100.0 \pm 6.4\%$ with a range of 88–114%.

DISTRIBUTION OF ZINC

The total zinc, α_2 -macroglobulin bound zinc, and albumin bound zinc concentrations were $14.8 \pm 2.4 \mu\text{mol/l}$, $2.4 \pm 0.6 \mu\text{mol/l}$, and $12.4 \pm 2.2 \mu\text{mol/l}$, respectively. None of these parameters showed a significant difference when data relating to the male and female sub-populations were analysed separately. In percentage terms, the distribution of the recovered zinc was as follows: $16.3 \pm 4.1\%$ (range 8.9–27.1%) with α_2 -macroglobulin, and $83.7 \pm 4.1\%$ (range 72.9–91.1%) with albumin.

PROTEIN-ZINC STOICHIOMETRIES

The molar ratios of albumin to albumin bound zinc and α_2 -macroglobulin to α_2 -macroglobulin bound zinc were calculated in the case of each serum studied. Although the binding ratio between albumin and its associated zinc varied considerably, there was always a remarkable excess of albumin relative to zinc. Only 1.1–2.6% of the albumin present in the samples of serum studied was engaged in zinc transport. The binding ratio between α_2 -macroglobulin and its associated zinc was also subject to considerable variation. Even with a fourfold difference between the highest and the lowest points obtained, however, the ratio of α_2 -macroglobulin to α_2 -macroglobulin bound zinc taken over the entire population was close to unity.

Table 1 Analytical methods

Analysis	Method	Worst case relative standard deviation (%)
Albumin	Kinetic immunoturbidimetry ^a	3.1
α_2 -macroglobulin		2.9
Total serum zinc	Electrothermal atomic absorption spectrophotometry ^b	3.6
Albumin bound zinc	Affinity chromatography (for albumin) with electrothermal atomic absorption spectrophotometry ^a	4.5
α_2 -macroglobulin bound zinc		5.9

Table 2 Zinc fractions and zinc binding proteins in normal sera

	Men + Women (n = 123)	Men* (n = 86)	Women† (n = 37)
Total serum zinc ($\mu\text{mol/l}$)	14.8 ± 2.4	14.9 ± 2.4	14.7 ± 2.4
α_2 -macroglobulin bound zinc ($\mu\text{mol/l}$)	2.4 ± 0.6	2.4 ± 0.6	2.5 ± 0.6
Albumin bound zinc ($\mu\text{mol/l}$)	12.4 ± 2.2	12.5 ± 2.1	12.1 ± 2.4
α_2 -macroglobulin (g/l)	1.9 ± 0.5	1.8 ± 0.4	2.1 ± 0.5
Albumin (g/l)	45.7 ± 4.0	45.8 ± 4.0	45.6 ± 3.7
α_2 -macroglobulin: α_2 -macroglobulin bound zinc	1.1 ± 0.4	1.1 ± 0.4	1.2 ± 0.3
Albumin: albumin bound zinc	56.7 ± 10.7	56.1 ± 10.1	58.2 ± 12.0

*Age range 18–62 years.

†Age range 19–60 years (none was taking oral contraceptives)

CORRELATIONS

No significant correlations were found between the concentrations of either albumin or α_2 -macroglobulin and the concentrations of their associated zinc ($r = 0.110$ and 0.101 , respectively; $n = 123$).

REFERENCE RANGES

Table 3 shows reference ranges limited by the 5th and 95th percentile points of the relevant cumulative frequency distribution curves for the total serum zinc, α_2 -macroglobulin bound zinc, and albumin bound zinc concentrations. Reference ranges for the binding ratios between zinc and the two carrier proteins are also given.

EFFECTS OF ORAL CONTRACEPTIVES

The results arising from the analyses of the serum samples taken from the 11 women taking oral contraceptives were compared with similar data relating to 12 women, aged 40 years or less, who were not taking any drugs (Table 4). There were no significant differences between these two populations for any of the parameters shown in Table 4.

Discussion

About 98% of the zinc in human serum is bound to proteins.⁷ Although it is widely accepted that this zinc is bound principally to albumin and α_2 -macroglobulin, there are conflicting reports about the relative distributions of the metal between these proteins, and also about its association with

other protein species such as transferrin, immunoglobulin G, and histidine rich glycoprotein.⁸⁻¹⁶

It is possible that the apparent associations between zinc and these latter proteins that have been reported by some workers may be artefacts resulting from the displacement of the metal from its true serum ligands. Zinc-protein interactions are known to be disrupted by electrophoretic, anion exchange chromatographic, and some gel filtration procedures for protein separation.^{10, 17, 18} We have recently shown that the zinc present in normal human serum is almost exclusively bound to albumin and α_2 -macroglobulin and that these two protein-zinc species may be completely separated by affinity chromatography (for albumin) using Blue Sepharose CL-6B without any appreciable displacement of zinc from its serum protein carriers.^{4, 5}

The data presented here indicate that albumin is the major zinc transport protein in human serum; about 85% of the zinc present in the sera examined was associated with this protein. The most striking feature of the results of this study was, however, the stability of the α_2 -macroglobulin bound zinc concentration, which was closely regulated within a range of only $1.2-3.9 \mu\text{mol/l}$ with a mean \pm SD of $2.4 \pm 0.6 \mu\text{mol/l}$. This result is in good agreement with that of Giroux *et al.*,⁹ who found a mean α_2 -macroglobulin bound zinc concentration of $2.5 \pm 0.5 \mu\text{mol/l}$ in 28 normal sera which were separated by means of polyethylene glycol precipitation. It is also comparable with the mean α_2 -macroglobulin bound zinc concentration of $3.3 \pm 0.1 \mu\text{mol/l}$ obtained by Song and Adham,¹⁰ who isolated this protein-zinc species from 10 serum samples using sucrose density gradient centrifugation. The stability of the concentration of zinc associated with α_2 -macroglobulin found here was not the indirect result of physiological influences on the serum concentration of the protein itself, because the correlation between the concentration of α_2 -macroglobulin and its associated zinc was poor ($r = 0.101$, $n = 123$), there being a fourfold variation in the binding ratio between the protein and the metal (from 0.5 to 2.2). These findings suggest that the α_2 -macroglobulin bound zinc fraction is itself subject to metabolic control, but the biological importance of this feature is obscure. There is no clear relation between the binding of zinc by α_2 -macroglobulin under physiological conditions and its role as a plasma proteinase inhibitor.¹⁹

The albumin bound zinc fractions of the sera studied were subject to a remarkable degree of variation, which accounted for virtually all of the differences in the total zinc concentrations found in these samples. Zinc may bind at any one of 16 sites where imidazole groups are located on the albumin

Table 3 Transport of zinc in serum: reference ranges

	Range
Total serum zinc ($\mu\text{mol/l}$)	11.0-19.0
α_2 -macroglobulin bound zinc ($\mu\text{mol/l}$)	1.5- 3.5
Albumin bound zinc ($\mu\text{mol/l}$)	9.5-16.5
α_2 -macroglobulin: α_2 -macroglobulin bound zinc	0.6- 1.9
Albumin: albumin bound zinc	40.0-75.0

Table 4 Zinc fractions and zinc binding proteins in serum: effect of oral contraceptives

	Women aged <40 yr not taking oral contraceptives	Women taking oral contraceptives
No of subjects	12	11
Total serum zinc ($\mu\text{mol/l}$)	14.9 ± 2.5	15.5 ± 2.0
α_2 -macroglobulin bound zinc ($\mu\text{mol/l}$)	2.5 ± 0.7	2.9 ± 0.8
Albumin bound zinc ($\mu\text{mol/l}$)	12.1 ± 2.6	12.0 ± 2.2
α_2 -macroglobulin: α_2 -macroglobulin bound zinc	1.2 ± 0.3	1.3 ± 0.4
Albumin: albumin bound zinc	58.1 ± 10.0	57.2 ± 10.9

molecule;²⁰ so it would therefore appear that the total zinc binding capacity of this protein is approximately 10–11 $\mu\text{mol/l}$ per litre of serum, if we assume a serum albumin concentration of 45 g/l. It would seem that under normal physiological conditions zinc occupies less than 0.2% of this capacity. Even when allowance is made for any possible competition between zinc and other divalent metallic species present in serum, such as calcium or magnesium, it is difficult to envisage naturally occurring circumstances in which the binding capacity of albumin for zinc might even approach saturation. Several groups of workers have found apparently significant correlations between the concentrations of zinc and albumin in the sera of patients with gastrointestinal or hepatic disease,^{9,21} and it has been generally assumed that the hypozinaemia that is often found in such patients is in many instances the simple consequence of coexisting hypoalbuminaemia, just as reduced total serum calcium concentrations may occur as a result of hypoalbuminaemic states. It is also possible, however, that the reduced serum albumin concentrations described in these reports^{9,21} were themselves a reflection of zinc deficiency because zinc losses from patients with gastrointestinal and hepatic disease are often excessive^{22,23} and the synthesis of albumin is itself dependent on the availability of zinc.²⁴ The independence of the concentrations of albumin and the albumin bound zinc in the sera of the patients with apparently normal body zinc availability who were investigated in the present study is not surprising in view of the excessive binding capacity for the metal which is exhibited by human albumin.

The zinc which is associated with the free amino acids that are present in human serum has not been considered in this report. Zinc-amino acid complexes might be expected to coelute with α_2 -macroglobulin from Blue Sepharose CL-6B columns under the conditions for chromatography used here. This is unlikely to lead to any serious error because the fraction of the serum zinc bound to these low molecular weight species is seldom more than 1–2% of the total zinc in normal samples.^{7,25} Whether this is true when pathological sera are analysed is not certain, however, and the determination of the ultrafiltrable serum zinc fraction in disease states clearly warrants further research.

The data obtained in the present study did not show any significant effect of combined oral contraceptives on the distribution of zinc among its serum protein ligands. Although early reports indicated that total serum zinc concentrations were reduced as a result of the use of oral contraceptives,^{26,27} this has not been confirmed by recent studies.^{28,29} It is possible that these conflicting

reports reflect the tendency to reduce the oestrogen content of modern contraceptive preparations.

The reference ranges for the protein bound zinc species present in human serum that have been established should facilitate studies of the serum transport of the metal in patients who are zinc deficient. It is possible that zinc deficiency states may be reflected more sensitively by disturbances in the concentrations of these zinc protein species than by the total serum zinc concentration itself.

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