

ZINC IN EPIDERMIS AND DERMIS IN HEALTHY SUBJECTS

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Abstract. Thin tangential skin biopsies were obtained from 40 healthy subjects by a shaving technique. The zinc content in these samples was determined by atomic absorption spectrophotometry. The mean concentration of zinc in epidermis was about 60 µg/g and in upper dermis 40 µg/g dry weight. The concentration was much higher in the upper than in the lower layer of dermis. There was no correlation between the tissue and serum concentration of zinc. Full awareness of the great risk for contamination is essential and special precautions are mandatory if reproducible results are to be obtained.

Key words: Zinc; Skin; Epidermis; Dermis

Zinc is an essential component of many enzyme systems and it probably plays a central role in nucleic acid metabolism (3, 7). In severe zinc deficiency, the clinical picture is now characterized and in these cases the serum zinc level is low.

A marginal zinc deficiency—without any characteristic clinical symptoms—may be common both in apparently healthy individuals with an inadequate daily intake of zinc and also in a number of clinical conditions associated with diminished absorption or increased losses. The determination of zinc in serum has been the main parameter used for evaluating the zinc status but this probably provides insufficient information about the zinc nutriture (2, 4). The serum level may not reflect the zinc content of the main tissue stores which are located in the skin, muscle, bone and liver. We have developed a method for determining zinc in small samples of epidermis and dermis, in order to gain additional information about zinc nutriture in various groups of patients. Evaluation of the results from zinc determinations is complicated by problems of contamination. The method and the results are therefore to be presented in detail. In this paper we report data on epidermal and dermal zinc from healthy subjects.

MATERIAL AND METHODS

Control subjects

Skin biopsies were obtained from 40 apparently healthy medical students and members of the staff. There were 18 males with a mean age of 31 (range 21-52) years and 22 females with a mean age of 32 (range 18-53) years. Seven of the 22 women used oral contraceptive agents (OCA) and were therefore considered as a separate group.

Procedures for taking and preparing skin biopsies

During each manipulation particular care was taken to avoid contamination. Before the biopsies were taken, the skin was thoroughly cleansed with gauze pads moistened with 70% ethanol. An intradermal anaesthetic, 1% lidocaine, was injected, so that the injected skin area became slightly raised. Thin, tangential shavings about 0.7×1.5 cm were taken with platinum razor blades (Gillette) from two symmetrical areas on the lateral sides of the upper shoulder and/or the upper gluteal region. Pigmented spots were avoided. The shavings were placed on gauze pads moistened with saline. As soon as possible, and within 2 hours, the biopsies were placed on aluminium foil in a drop of saline and heated to 52°C for 5 sec, whereafter they were separated into epidermis and dermis with stainless steel tweezers. The samples were left on the foil and dried at 52°C for 2 hours under a glass cover weighed on an automatic electro-balance (Cahn 4700) and transferred to acid-washed glass tubes Milli-6 vials, Biotec, Stockholm, Sweden). To each sample was added 50 µl of Millipore®-treated water and the mixture was left for 24 hours, whereafter 250 µl of 65% nitric acid was added for dissolution and the samples left for a further 96 hours.

Determination of zinc

The zinc content was determined, after dilution with 250 µl Millipore®-treated water, by atomic absorption spectrophotometry (AAS) (Perkin-Elmer AA 460) at 214 nm. Titrissol (Merck) zinc standard solutions were used for the preparation of standard solutions.

Zinc in serum was analysed by AAS at the Department of Clinical Chemistry. The blood samples were drawn in the morning, when the individual was fasting.

Procedure for minimizing zinc contamination

The skin should not have been treated with any topical preparations. Gauze pads, ethanol and anaesthetics

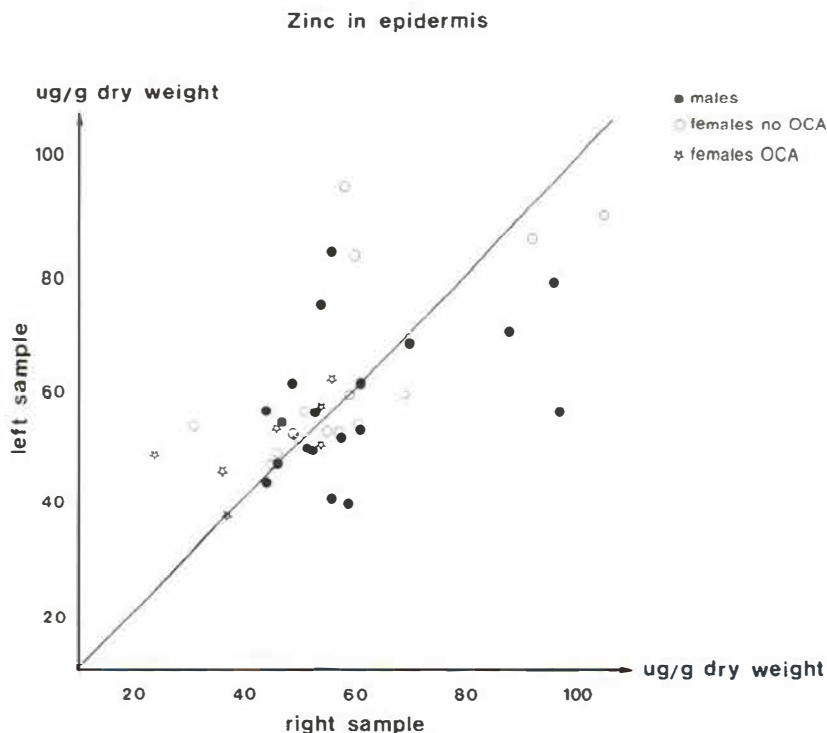


Fig. 1. Paired determinations for zinc in epidermis. Two persons are represented with two paired values each, the samples from one person were mislaid. The other persons are represented by a single dot.

should be checked for zinc contamination. Razor blades and stainless tweezers were soaked in 50% EDTA solution and after rinsing repeatedly in zinc-free deionized water were kept in acid-washed glass jars. The rinse solution from cleaned test tubes was checked for zinc by AAS before the test tubes were used. Surgical gloves were not used as they contaminate the skin with zinc.

RESULTS

Methodological aspects

Repeated weighing ($n=10$) of samples of 0.5, 1.0, 1.5 and 2.0 mg gave a coefficient of variation of 1.4–2.0% for each sample.

In repeated analyses ($n=10$) of standard zinc solutions of 0.100 $\mu\text{g/ml}$ and 0.050 $\mu\text{g/ml}$, the coefficient of variation was 2.3 and 3.2% respectively.

The dry weight of the samples was between 1 and 3 mg. Larger shavings were divided in order to obtain similar weights. Smaller shavings were analysed together; in these cases only one value was obtained. Constant weight was obtained within one hour of drying.

The temperature employed for splitting the epidermis from the dermis is critical: if too low, epidermal fragments may remain attached to the dermis with consequently high dermal values.

The epidermis and dermis samples were degraded by treatment with nitric acid for 3 days. Small, barely visible, whitish flakes are often still present after this time.

The concentration of zinc varies with the depth of dermis. In three shavings from the upper layer of dermis taken from mammary skin in the same way as for the controls, the concentration was 28, 23, 25 $\mu\text{g/g}$ whereas in four shavings from the lower dermis (free of subcutaneous tissue) it was 5, 7, 8, 11 $\mu\text{g/g}$.

Reproducibility: In nine shavings from the mammary skin of one patient, the coefficient of variation for the epidermis zinc was 16% and for the dermis 13%. The mean zinc concentration in epidermis was $110 \pm 18 \mu\text{g}$ (mean $\pm$ S.D.) and in the dermis $45 \pm 6 \mu\text{g/g}$. This skin was obtained during an operation for mammary hyperplasia and the high epidermal values are probably due to contamination by routine surgical cleansing and manipulation.

The intra-individual reproducibility was studied in two healthy subjects by taking two shavings from the shoulder region and two from the gluteal region. The concentrations in the four epidermis samples were 56 ± 4 and $62 \pm 15 \mu\text{g/g}$ respectively, and in the four dermis samples 24 ± 2 and $25 \pm 1 \mu\text{g/g}$, re-

Zinc in upper dermis

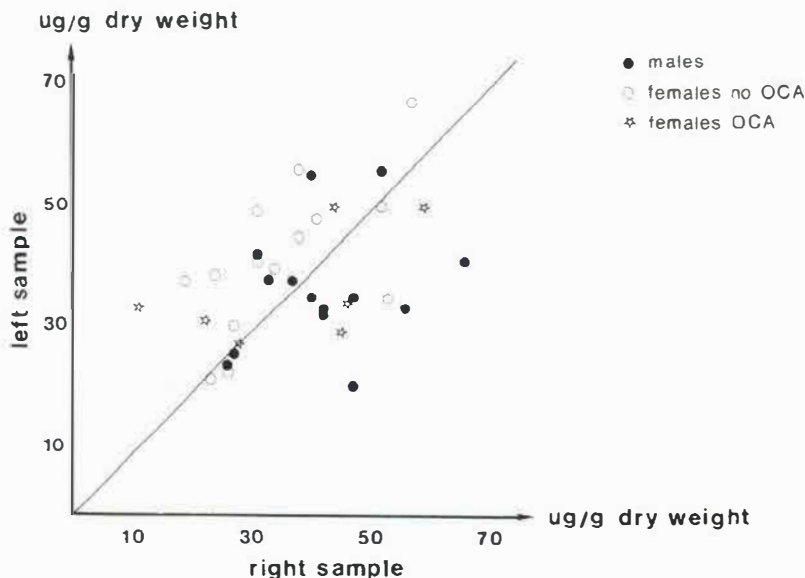


Fig. 2. Paired determinations for zinc in upper dermis. Two persons are represented with two paired values each.

spectively. No differences between the gluteal and shoulder regions were observed.

Zinc in the epidermis and the upper dermis of healthy controls

All "paired" values for epidermis and upper dermis are shown in Figs. 1 and 2 and the distribution of the individual mean values in Figs. 3 and 4. Mean values of the groups are shown in Table I. In 7 cases only one dermal value was obtained from each subject as the samples were small and therefore analysed together. These values were included in Figs. 3 and 4 as well as in Table I.

The zinc concentration was significantly higher in epidermis than in the upper part of dermis ($p < 0.001$). Women not using OCA had about the same epidermal and dermal zinc concentrations as men. However, the women using OCA had significantly lower epidermal zinc than the men ($p < 0.05$). The correlation between zinc in epidermis and in dermis was poor.

As can be seen from Fig. 5 there was no correlation between the serum zinc and the epidermal zinc concentration. Nor was there any correlation between zinc in serum and zinc in the upper dermis.

The concentration of zinc in serum was significantly lower in women than in men ($p < 0.05$ for both groups of women).

DISCUSSION

Few reliable reports have appeared on zinc concentration in epidermis and dermis in healthy controls or in patients with various diseases. One reason for this is probably the problems of contami-

Zinc in epidermis

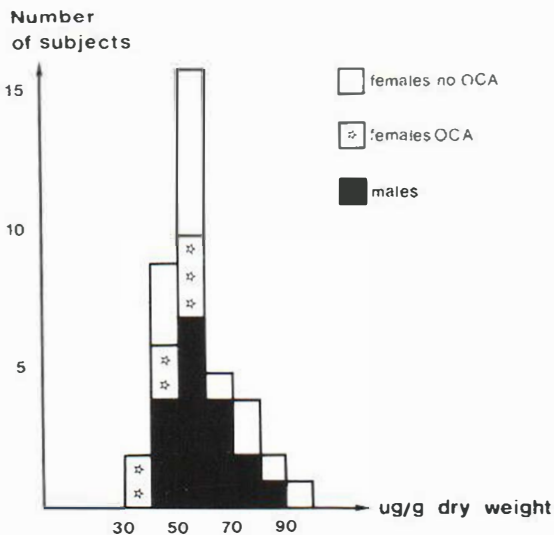


Fig. 3. Distribution of individual mean values for zinc in epidermis

Table I. Zinc concentration (mean values $\pm$ S.D.) in serum, epidermis and upper dermis of healthy subjects

Figures in parentheses denote numbers of subjects studied

	Serum ($\mu\text{mol/l}$)	Epidermis ($\mu\text{g/g}$)	Dermis ($\mu\text{g/g}$)
Males	13.6 $\pm$ 2.0 (15)	59 $\pm$ 12 (18)	41 $\pm$ 10 (18)
Females: no OCA	12.2 $\pm$ 1.6 (14)	61 $\pm$ 17 (14)	40 $\pm$ 10 (15)
Females: OCA	11.5 $\pm$ 0.8 (6)	47 $\pm$ 9 (7)	37 $\pm$ 12 (7)
All subjects	12.7 $\pm$ 1.9 (35)	57 $\pm$ 14 (39)	40 $\pm$ 10 (40)

nation. Full awareness of this risk is essential for reproducible results. The few previously reported values on zinc in skin are controversial and in some reports very high concentrations are given. In our study, the mean epidermal concentration of zinc was about 60 $\mu\text{g/g}$ dry tissue. Portnoy & Molokhia (5) used neutron activation analysis to determine zinc in the epidermis and dermis of abdominal skin from 36 cadavers. They gave no details as to how zinc contamination was avoided. Although their mean epidermal value (71 $\pm$ 26 $\mu\text{g/g}$) was higher than that in our study and their maximum value was as high as 154 $\mu\text{g/g}$, their minimum value was the same. From their histogram a skew distribution to high values is evident and such a tendency is also seen in our histograms, although much less marked. In mammary skin epidermis, obtained at plastic surgery, we have often found levels around 100–

200 $\mu\text{g/g}$ but we strongly suspect that these high levels are due to contamination. Mammary epidermis obtained in the same way as our routine shavings showed concentrations around 45 $\mu\text{g/g}$. It is conceivable that some of our samples might also have been contaminated. We prefer to repeat shavings when high values, such as >70 $\mu\text{g/g}$, are obtained. So far we have not recorded any cases where initially high values have been confirmed. Thus new shavings from the two re-shaved control subjects had a mean value of 70 compared with an initial value of around 90 $\mu\text{g/g}$. This does not mean that contamination can be ruled out in the samples with low values, although it seems less likely.

In a later report on copper and zinc in vitiligo, moles and seborrhoeic warts, Portnoy & Molokhia (6) included, for comparison, 14 control specimens with a mean value of 57 $\pm$ 9 $\mu\text{g/g}$ for epidermis. These biopsies were taken from interscapular skin of white volunteers. They did not comment on the difference between the results in their reports, which can presumably be ascribed to contamination. The fact that they used abdominal skin from cadavers in their first study and interscapular skin from living subjects in their later studies might also have influenced their results. We found no significant differences between samples from scapular and gluteal skin, however. Our results with smaller skin biopsies are in agreement with their later findings, although our mean value may be slightly too high. The lowest normal level as well as optimal levels cannot yet be defined.

Zinc in serum is often moderately low in those using OCA (2, 3). In our small group of women on oral contraceptives, epidermal values tended to be low.

In the uppermost layer of dermis, closest to the epidermis, we have found mean values of around 40 $\mu\text{g/g}$, whereas the concentration was much lower in the deeper dermis. The values for upper dermis are

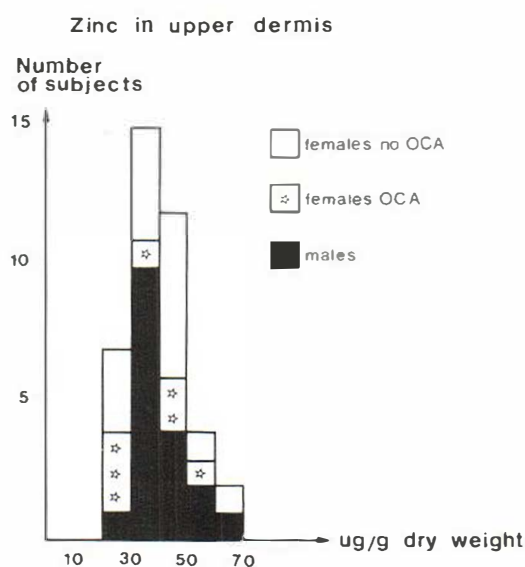


Fig. 4. Distribution of individual mean values for zinc in upper dermis.

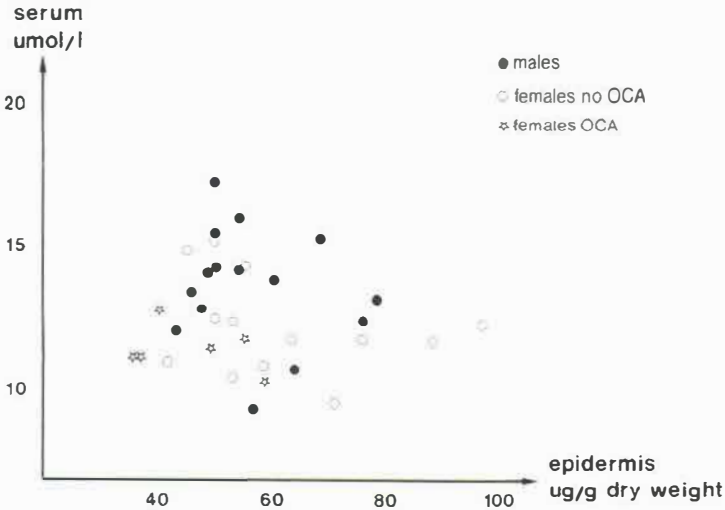


Fig. 5. Comparison between individual epidermal mean values and serum values for zinc.

higher than those reported for dermis by Portnoy & Molokhia (5). They took biopsies which evidently included the whole dermis down to the subcutaneous layer and reported dermal mean values of around $13 \mu\text{g/g}$ and a ratio of 6:1 for epidermis:dermis in their first study and 4:1 in their later study. It therefore seems likely that the concentration is lower in the lower dermis than in the upper. Our findings would appear to confirm this assumption. This is also consistent with the suggestion that dermis forms two compartments: an upper, thin zone immediately underlying the epidermis (the papillary dermis and periadnexal dermis) and a lower zone comprising the thick reticular dermis. Epidermis and the upper dermis is considered as a functional unit (1). Our results show that the ratio of zinc is here 6:4, whereas the ratio for epidermis: lower dermis is about 6:1.

As can be seen from Fig. 2 the correlation between the pairs of dermal samples is not as good as that for the epidermal samples. This might be accounted for by incomplete separation of dermis from epidermis or inclusion of fragments of the lower dermis. When taking dermal samples, the differences between the zinc concentrations in the upper and lower dermis must be taken into consideration. Further studies are planned to establish whether the information obtained from the epidermal shavings ought to be sufficient.

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