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The effect of temporary venous occlusion on trace mineral concentrations in plasma[1-4]

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ABSTRACT The effects of short-term venous occlusion on plasma concentration of trace minerals—zinc, iron, and copper—were studied in normal volunteers. In one experiment, antecubital vein blood samples were drawn simultaneously from both arms of 14 subjects while their left arms remained free and their right arms had been occluded from 60 s at 40 mm Hg pressure. Statistical comparison of paired samples showed a significant increase in zinc ($p < 0.05$) and iron ($p < 0.001$), but not copper concentrations in plasma from the occluded extremity. The mean increase in concentration in the right (ligated) arms with respect to the left (unligated) arms was 3.7 and 24.6%, for zinc and iron, respectively. In a second experiment, simultaneous blood samples were drawn from five subjects with neither arm occluded. No significant difference between right and left was observed. Thus, application of tourniquets during the sampling of venous blood for trace mineral analysis introduces a nonrandom factor. Standardization of sampling techniques is essential to overall reliability of trace mineral determinations. *Am J Clin Nutr* 1982;36:354–358.

KEY WORDS Zinc, iron, copper, plasma trace minerals, blood sampling techniques

Introduction

Increasing interest in human metabolism and nutrition with respect to trace elements has been manifested in recent years. Determination of plasma or serum concentrations are the most commonly used index of zinc and copper nutriture (1). Circulating iron levels can play a role, along with measurements of Hb, free-erythrocyte protoporphyrin, and serum ferritin, in the assessment of iron nutriture as well (2). In the routine collection of venous blood, a tourniquet is often applied to the arm to impede venous return. Walker et al. (3) recently examined the effects of venous occlusion on plasma zinc concentration, and found a time-dependent increase in concentration in the occluded arm as compared to the nonoccluded extremity in the same individual. In the present study, we have modified their approach to assess the impact of venous stasis on plasma levels of three important trace mineral nutrients: zinc, iron, and copper.

Methods

Sixteen adult volunteers participated in the experiment. Subjects were studied both fasting and in the postprandial state. In 14 subjects, blood samples were

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taken simultaneously from both arms with a 3 inch pediatric Velcro blood pressure cuff placed around the upper right arm, and venous flow occluded for 60 s at 40 mm Hg with a sphygmomanometer. The left arm was unoccluded. Plastic syringes with a stainless steel needle were introduced simultaneously by separate operators into an antecubital vein of each arm, and 3 ml of blood was drawn into each syringe after 60 s, as measured by a stopwatch, had elapsed. In five subjects, including three individuals from the former group, blood was drawn simultaneously from both arms with neither arm being occluded. All procedures were conducted on the same day.

Blood was transferred into trace mineral-free Falcon tubes containing 0.05 ml of 20% potassium oxalate solution, centrifuged, and the plasma transferred to a dry Falcon tube and stored at 4°C until analyzed. Determination of the concentration of the trace minerals—zinc, copper, and iron—were performed by atomic absorption spectrophotometry on a Varian Techtron AA5 spectrophotometer. A 5:1 dilution of plasma with distilled, deionized water was prepared for aspiration after a modification of the method of Sinha and Gabrieli (4). All analyses were conducted on the same day.

Data were analyzed using the Student's *t* test for paired and unpaired samples.

Results

The tabulated data comparing the trace mineral concentrations of the two arms, when the right arm was ligated for 1 min, are given in **Table 1**. Similar data for the studies with unligated arms are given in **Table 2**. Paired *t* test showed a statistically significant increase of 2.5 μg/dl ($p < 0.05$) and 18.6 μg/dl ($p < 0.001$) for zinc and iron, respectively. No significant differences between left and right arms in copper concentration were detected ($p > 0.05$). None of the minerals showed any statistical changes by extremity when both arms remained free.

TABLE 1
Differences in plasma trace mineral concentrations between the right and left arms in 14 subjects with venous occlusion of the right arm

Subjects	Zinc concentration (μg/dl)				Iron concentration (μg/dl)				Copper concentration (μg/dl)			
	Right arm	Left arm	Difference R-L	% Difference	Right arm	Left arm	Difference R-L	% Difference	Right arm	Left arm	Difference R-L	% Difference
I	90	88	+2	2.3	139	124	+15	12.1	88	88	0	0
II	76	73	+3	4.1	133	114	+19	16.7	91	84	+7	8.3
III	72	74	−2	−2.7	85	52	+33	63.5	93	92	+1	1.1
IV	79	76	+3	3.9	144	117	+27	23.1	103	107	−4	−3.7
V	92	92	0	0	199	190	+9	4.7	76	78	−2	−2.6
VI	86	82	+4	4.8	92	85	+7	8.2	92	80	+12	15.0
VII	78	74	+4	5.4	180	147	+33	22.4	90	82	+8	9.8
VIII	90	82	+8	9.7	136	144	−8	−5.6	84	88	−4	−4.5
IX	71	70	+1	1.4	150	123	+27	22.0	90	86	+4	4.7
X	77	76	+1	1.3	106	77	+29	37.7	94	86	+8	9.3
XI	86	88	−2	−2.3	93	91	+2	2.2	100	108	−8	−7.4
XII	63	50	+13	26.0	91	43	+48	111.6	76	56	+20	35.7
XIII	82	87	+5	5.7	114	113	+1	0.9	95	71	+24	33.8
XIV	75	70	−5	−7.1	(95)	QNS*			92	96	−4	−4.2
X	79.8	77.3	+2.5	3.7	127.8†	109.2	+18.6	24.6	90.3	85.9	+4.4	6.8
SD	±8.4	±10.7	±4.5	±7.6	±35.5	±40.1	±15.0	±31.8	±7.6	±13.3	±9.4	±13.5

* Quantity not sufficient.
† Mean, omitting value for subject XIV.

TABLE 2
Differences in plasma trace mineral concentrations between the right and left arms in five subjects without venous occlusion

Subjects	Zinc concentration (μg/dl)				Iron concentration (μg/dl)				Copper concentration (μg/dl)			
	Right arm	Left arm	Difference R-L	% Difference	Right arm	Left arm	Difference R-L	% Difference	Right arm	Left arm	Difference R-L	% Difference
I	77	80	−3	−3.8					88	94	−6	−6.4
II	81	82	−1	−1.2	125	122	+3	2.5	89	86	+3	3.5
III	79	74	+5	6.8	48	56	−8	−14.3	94	97	−3	−3.1
XV	73	82	−9	−11.0	111	125	−14	−11.2	72	77	−5	−6.5
XVI	69	69	0	0	129	78	+51	65.4	70	69	+1	1.4
X	75.8	77.4	−1.6	−1.8	103.2	95.2	+8	10.6	82.6	84.6	−2.0	−2.2
SD	±4.8	±5.7	±5.1	±6.4	±37.6	±33.8	±29.5	±37.2	±10.8	±11.6	±3.9	±4.5

Discussion

It is convenient in the execution of the venipuncture and for the maintenance of an adequate blood flow, using either a syringe or an evacuated rubber-stoppered tube, to ligate the arm with a tourniquet and occlude venous return. Walker et al. (3) compared zinc concentration from an unoccluded arm and an arm occluded with a pressure 10 mm Hg below diastolic pressure in seven subjects, and noted increases in plasma zinc concentrations of approximately 10, 20, and 24%, respectively, after 5, 10, and 15 min of occlusion. Because 15 min is a rather extreme duration for venous occlusion, however, we evaluated a more practical interval, 1 min, approximating more closely the time that a tourniquet might be applied during a routine blood extraction and demonstrated artifactual rises in zinc and iron—but not copper—concentration in the ligated extremity.

The mechanisms for the elevation in apparent concentration of plasma zinc and iron, and the nature of the differential behavior of three circulating trace minerals is largely a matter of speculation. Walker et al. (3) found a closely parallel percentage increase in total plasma proteins and zinc. As zinc is primarily protein bound, the behavior of proteins may explain a large portion of the variance for the rise in its concentration; the relative increase in protein concentration was attributed to the more rapid hydrostatic extravasation of water than proteins from the vasculature with ligation. It is curious, however, that the percentage increase for iron in our study was 5-fold greater than that for zinc. Protein movement, alone, would not explain the differential behavior. Another possibility would be a contribution of microhemolysis, as the concentration of iron in red cells is much greater than that for zinc. Whether venous occlusion would increase or decrease the chances for hemolysis is not intuitively obvious. There was no visible evidence of hemolysis in any of the plasma samples obtained, but Hb concentrations were not measured. Had we determined the concentration of albumin, transferrin, ceruloplasmin, and Hb in our samples, a clearer explanation for the underlying mechanisms of our observed changes in trace metal concentrations might have become apparent.

The analytical variability (coefficients of error) for repeated determinations of a quality control serum pool by atomic absorption spectrophotometry within a single day run of samples in our laboratory is 3.2, 5.8, and 3.3% for zinc, iron, and copper, respectively. Thus, the variability of sampling both extremities simultaneously appears to be equivalent to (zinc, copper) or greater than (iron) that introduced by the analytical method.

Our data also bear on the inherent intraindividual variation in mineral determinations (Tables 1 and 2). We have previously reported the stability of fasting zinc concentrations at hourly intervals over a 6-h fast (5). Rubber tourniquets were applied during the extraction of blood and analytical methods were identical to those described here. Copper concentrations had been simultaneously determined in those samples, but not previously reported. We have disaggregated the data for both zinc and copper concentrations from that earlier study in **Table 3**. The mean coefficient of variation for seven repeat determinations of zinc in five fasting subjects was 8.3% (range 6.0 to 11.0%) while that for copper was 15.1% (range 3.9 to 16.6%). It appears that plasma zinc concentration, in this setting, is more inherently stable than that of plasma copper. The reason for this difference is not readily apparent. Unfortunately, iron concentrations were not determined in that earlier study.

Our results may have practical implications for field nutrition surveys. The data from our 14 subjects give us an estimation of the variance in plasma zinc and iron concentration. Using the formula for calculating sample size for the Student's t test (6), we have calculated the minimum number of individuals that would have to be in each of two identical populations for a difference in blood extraction technique (a tourniquet used in one group but not in the other) for a statistically significant difference at the 5% probability level to emerge. For plasma zinc, this difference would be seen with 145 subjects in each subpopulation; for iron, only 50 subjects in each group would be required for a difference due soley to technique to be seen.

In conclusion, with increasing interest in and proliferation of scientific investigation into human trace mineral nutriture, it behooves us to understand the diagnostic pro-

TABLE 3
Sequential fasting concentrations of plasma zinc and copper over a 6-h period in five subjects

Subject		Zinc (hourly sample)							Copper (hourly sample)						
		1st	2nd	3rd	4th	5th	6th	7th	1st	2nd	3rd	4th	5th	6th	7th
	Concentration*	79	78	80	72	78	86	73	102	95	96	98	100	101	92
1	Δ†		−1	+1	−7	−1	+7	−6		−7	−6	−4	−2	−1	−10
	%‡		−1.3	1.3	−8.9	−1.3	8.9	−7.6		−6.9	−5.9	−3.9	−2.0	−1.0	−9.8
	Concentration	98	68	88	81	85	87	88	69	93	95	84	94	90	84
2	Δ		−24	−4	−11	−7	−5	−4		+24	+26	+15	+25	+21	+15
	%		−26.1	−4.3	−12.0	−7.6	−5.4	−4.3		34.8	37.7	21.7	36.2	30.4	21.7
	Concentration	57	54	52	63	64	66	62	102	136	100	99	128	90	100
3	Δ		−3	−5	+6	+7	+9	+5		+34	−2	−3	+26	−12	−2
	%		−5.3	−8.8	10.5	12.3	15.8	8.8		33.3	−2.0	−2.9	25.5	−11.8	−2.0
	Concentration	72	67	50	67	66	68	70	123	101	115	84	98	116	86
4	Δ		−5	−22	−5	−6	−4	−2		−22	−8	−39	−25	−7	−37
	%		−6.9	−30.6	−6.9	−8.3	−5.6	−2.8		−17.9	−6.5	−31.7	−20.3	−5.7	−30.1
	Concentration	79	78	80	72	78	86	73	142	110	147	101	102	102	124
5	Δ		−1	+1	+7	−1	+7	−6		−32	−5	−41	−40	−40	−18
	%		−1.3	1.3	−8.9	−1.3	8.9	−7.6		−22.5	−3.5	−28.9	−28.2	−28.2	−12.7

* Plasma concentration of the metal in μg/dl.
† Absolute change in metal concentration with respect to the 1st h sample, in μg/dl.
‡ Corresponding percentage change in concentration with respect to the 1st h sample.

cedures in ample detail. The present findings demonstrate a substantial degree of intrinsic variability in circulating levels of iron, zinc, and copper. However, these findings also show that with application of venous occlusion for as brief a period as 1 min there is a nonrandom but consistent factor introduced in measures of concentrations of iron and zinc by tourniquet placement.

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