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Whole Blood and Serum Copper Levels in Relation to Sex and Age

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The copper content in whole blood and serum was determined in healthy human subjects (240 males and 217 females) by atomic absorption spectrophotometry. The mean level of copper obtained in whole blood was 104.8 ± 20.5 μg/100 ml in males and 117.1 ± 20.1 μg/100 ml in females. The mean level of copper in serum was 102.3 ± 21.7 μg/100 ml and 123.9 ± 30.4 μg/100 ml, in males and females respectively. The copper concentration in whole blood and serum in females proved to be significantly higher than in males ($p < 0.001$). With respect to age, the copper level showed a slightly negative correlation which is only statistically significant in whole blood in females ($p < 0.05$).

Key words: Serum copper level, Whole blood level.

The concentration and distribution of copper in different animal tissues vary greatly from one species to another; however, it is common to all species that the newborn and young animals are normally much richer in copper per unit of body weight than adults of the same species (15). Emphasis should be placed on the lack of evidence concerning copper deficiencies in adults, probably due to the fact that their requirements are not as great as those of children and are satisfactorily met by the normal copper content of foods.

The copper levels in the human organism depends not only on diet, but also on the individual's state of health and physiological factors such as sex and age. Both hypo- and hypercupremia are related to different pathological states (nephrosis, myocardial infarct, Wilson's, Hodgkin's and Addison's disease, etc.) (4, 16).

Copper in blood is distributed between plasma and cellular corpuscles. The corpuscular fraction is smaller compared with that found in plasma and in corpuscles as most copper forms part of an erythroprotein. In plasma, 93 % of the copper is bound to ceruloplasmin and on many occasions the copper level in plasma has been observed to be altered

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by factors affecting that of ceruloplasmin (16).

In this study the copper levels in whole blood and serum were determined taking into consideration the sex and age of the healthy individuals of the community examined. Little information exists in the literature consulted regarding the contents of this metal in whole blood. Certain authors, however, report a different relationship between the copper levels in whole blood and those in serum.

Materials and Methods

The samples of blood belonged to a private laboratory of clinical analysis. The samples chosen were those whose clinical parameters analysed did not reveal the presence of any pathological anomaly in the individual. They are classified according to type of sample and sex, up to a total of 290 of whole blood (167 men and 123 women) and 167 of serum (73 men and 94 women) and are grouped at intervals of 10 years of age, from 10 to 79. The average age of men and women corresponding to each type of sample is compared, and the difference is not statistically significant (table I).

Blood was withdrawn from the individuals while fasting, using disposable syringes and stainless steel needles. The samples of serum and whole blood were refrigerated in polystyrene tubes and 2 drops of a 15 % (w/v) tripotassium EDTA anticoagulant solution (Labex Laboratory, Barcelona, Spain) were added to the tubes.

The reagent solutions were prepared from Merck analytical grade products and with distilled and deionized water from a Milli-Q2 water purifier system (Millipore Iberica Company, Madrid, Spain). Saline solution: 1.4 and 0.5 moles/l of NaCl and KCl. Copper stock solution: Titrisol ampoule. Copper working solutions: prepare by diluting the stock solution with the saline solution, obtaining the following solutions of increasing copper concentrations: 0.5, 1, 2, 3 and 4 ppm.

In order to avoid contamination all material was subjected to a special washing process consisting in rinsing the utensils with a solution of 4 % (v/v) nitric acid and later washing them with distilled and deionized water.

Treatment of whole blood and serum samples was carried out as described in a previous work (3). Copper was measured by an atomic absorption flame technique using a Pye Unicam Atomic Absorption Spectrophotometer Model SP 1900. The instrumental settings were: wavelength 324.8 nm, slit setting 0.10 mm, lamp current 4 mA, air flow 5 l/min, acetylene flow 0.8 l/min, acetylene pressure 0.7 kg/cm^2.

For carrying out the recovery assay, a volume containing 2 μg of copper dissolved in saline solution was added to some samples of whole blood and others of serum in which the initial contents of this metal were determined simultaneously. Pools of whole blood and serum were prepared in which successive determinations were carried out after subjecting each pool to the same procedure for the samples and standard solutions. The variation coefficient in table I is the mean value calculated from 7 pools of whole blood and 6 of serum.

All data were analyzed on a Tektronix 4051 computer using the plot 50 Statistics vol. 2 Tektronix computer programs.

Results

Table I shows the mean copper content in whole blood and serum according to sex and independent of age.

For both types of sample the copper concentration is higher in females than in males, showing a very significant dif-

Table I. *Age mean, levels of copper in whole blood and serum (means ± SD) independently of age, recovery and variation coefficient.*
The difference between the age mean of males and females is not statistically significant (N.S.). The copper concentration in whole blood and serum in females proved to be significantly higher than in males ($p < 0.001$). In parenthesis the number of individuals.

	Whole blood	Serum
Age-males (years)	45±16	47±18
Age-females (years)	41±19	44±17
Copper-males (μg/100 ml)	105±20.0 (167)	102±22.0 (73)
Copper-females (μg/100 ml)	117±20.0 (123)	124±30.0 (94)
Recovery (%)	101± 0.9	100± 0.7
Variation Coefficient (%)	2.0± 0.4	0.7± 0.5

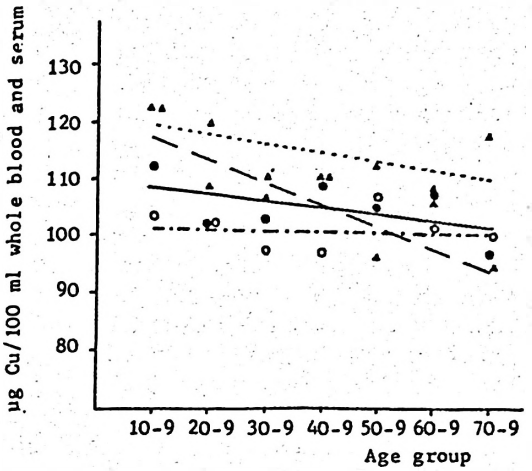

Fig. 1. *Copper levels in whole blood and serum of males and females according to age.* The regression straight line equations and the correlation coefficients are: $y=0.114x \pm 110.5$, $r=-0.507$ (not significant) for whole blood copper males (● — ●) $y=-0.051x \pm 104.7$, $r=-0.274$ (not significant) for serum copper-males (○ — · — ○); $y=-0.368x \pm 132.9$, $r=-0.835$ ($p<0.05$) for whole blood copper-females (▲ — ▲); $y=0.142x \pm 131,2$, $r=-0.525$ (not significant) for serum copper-females (△ - - - △).

Table II. *Comparison between copper levels in whole blood and serum of males (M) and females (F) (μg/100 ml).*
Analyses of variance was used to compare the differences between the copper levels in blood and serum of males and females ($p < 0.001$).

	Whole blood				Serum			
Years	(n)	M	(n)	F	(n)	M	(n)	F
10-19	8	112 ± 14	13	133 ± 25	5	107 ± 20	5	133 ± 22
20-29	22	102 ± 20	31	119 ± 25	12	104 ± 17	23	130 ± 29
30-39	36	103 ± 18	24	117 ± 25	9	99 ± 21	14	120 ± 23
40-49	31	109 ± 20	16	121 ± 27	13	97 ± 22	15	120 ± 22
50-59	41	105 ± 21	13	106 ± 26	17	108 ± 23	18	124 ± 24
60-69	17	108 ± 17	14	115 ± 22	9	101 ± 27	12	118 ± 20
70-79	12	97 ± 16	12	104 ± 18	8	101 ± 16	7	128 ± 32

ference (table II). Though at some age intervals the difference between the copper levels of both sexes does not seem to be significant, by applying the variance analysis or by comparing the two means obtained independently from age (table I), sex proves to influence the level of this element significantly, both in whole blood and in serum.

Figure 1 shows the relationship between the copper levels in whole blood and serum and the age of the individuals examined. The correlation coefficient of these straight lines was negative, suggesting that copper tends to decrease with age. This slight tendency was greater in females than in males and greater in whole blood than in serum and was only statistically significant in the whole blood of females ($p < 0.05$). In serum, the negative correlation was also greater in females than in males, but in no case was it statistically significant. In males the correlation coefficient was so small and so close to zero that the copper level may be considered independent of age.

Discussion

According to the results of the precision and recovery assays carried out, the method employed is valid for determining the copper content in whole blood and serum.

In this study strong measures are adopted to avoid the contamination of the samples by copper during the analytical process. These proved to be valid because the absorbance of the lowest standard solution was out of the $\pm$ 3 SD range, defined around the absorbance value of the blank.

The results obtained in this study of copper in serum in males and females agree in general with the values found by other authors who also differentiate between the serum values corresponding to each sex (4, 8, 9, 13, 18). The comparison of copper levels in whole blood according to sex is difficult since various authors in the literature consulted do not distinguish between the content in males and females. Taking this aspect into account, the mean value of copper in whole blood of both sexes obtained in this study (table I), is either similar (2, 14) or differs appreciably from the results obtained by other authors (11, 12).

The mean copper values obtained in serum and whole blood in males and females suggest that sex affects the content of this element in these biological fluids, since the difference found is statistically significant with the same degree of significance in both types of samples. Other authors (4, 8, 9, 13, 18) have also found higher serum copper levels in females than in males; however, only HARTOMA (8) and YUNICE (18) report a statistically significant difference.

Many authors (5-7, 15, 17) have reported that estrogens considerably raise the copper level in serum and that women who regularly take oral contraceptives or who are pregnant show a marked increase in the serum concentration of this element, with a mean level of 200 μg per 100 ml. It does not appear possible to account for the difference observed between the copper levels in blood in each sex simply with the possible intake of estrogens on the part of many of the women included in this study, although estrogen intake probably does participate in obtaining a significance level of the order of one per one thousand. This is supported by the work of YUNICE (18) who, in spite of excluding women taking oral contraceptives, obtained a statistically significant difference in the serum copper concentration between males and females.

The decrease in copper levels in whole blood and serum with age is only statistically significant in the whole blood of females. In addition, the line corresponding to the relationship between

serum copper levels in males and age is practically parallel to the axis of the abcissa and, therefore, both variables may be considered to be independent. These results differ substantially from those found by YUNICE (18) who studied the possible relationship between serum copper and age and found a positive correlation for males but no significant variation with age in females; in contrast, in this study the copper/age correlation was always negative for both sexes and types of sample.

However, according to the results of this work, the copper concentration seems to be greater on comparing the levels corresponding to adolescence with those of the adult, as is the case of copper levels in numerous tissues (10, 16), and is independent of the age of the individuals once adulthood is reached. In children from 4 to 16 years of age, LEWIS *et al.* (10) found a negative correlation between the copper level in whole blood and age, and showed that adults (20-70 years old) have lower levels than children.

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Resumen

Se determina el contenido de cobre en sangre total y en suero de 240 hombres y 217 mujeres sanas, por espectrofotometría de absorción atómica. El nivel medio de cobre obtenido en sangre total de hombre es de $104,8 \pm 20,5$ μg/100 ml, y en la mujer de $117,1 \pm 20,1$ μg/100 ml. En suero, el nivel medio de cobre es de $102,3 \pm 21,7$ μg/100 ml y $123,9 \pm 30,4$ μg/100 ml, en hombres y mujeres respectivamente. La diferencia entre el contenido de cobre de ambos sexos, tanto en sangre total como en suero, es estadísticamente significativa ($p < 0,001$). Con respecto a la edad, el nivel de cobre muestra una correlación negativa que sólo es estadísticamente significativa en sangre total de mujer ($p < 0,05$).

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