STUDIES ON PLASMA COPPER AND PROTEIN LEVELS OF HEALTHY LACTOVEGETARIAN MEDICAL STUDENTS

By

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The total plasma copper, albumin bound copper, copper in ceruloplasmin and paraphenylene diamine oxidase activity have been determined in the plasma samples of about one hundred normal healthy lactovegetarian medical students. These values resemble closely to the normal values determined by other workers. The plasma copper was found to be significantly higher in females. It was also found that plasma copper level increased after ingestion of food.

Estimation of total plasma proteins and their fractionation have also been carried out in the plasma samples of the same subjects. The total albumin and *beta*-globulin fractions of plasma proteins have been equated with the corresponding values of loosely or firmly bound copper fractions. Observations made by Lahey *et al.* (20) that *alpha*-2 globulin fraction of plasma proteins present in ceruloplasmin has a direct relationship with the concentration of plasma copper has been substantiated.

The trace element copper is one of the numerous nutritional factors required for the normal erythropoesis in human beings. Hart and his colleagues (12), Elvehjem *et al.* (9) Josephs (17), and Parsons (27) have confirmed experimentaly the mechanism of adjuvant action of this element in the process of synthesis of haemoglobin and red cell maturation. Schultze (34) has shown that copper is also concerned with the cytochrome oxidase activity of the bone marrow while Holmberg and Laurell (15) and Cartwright *et al.* (5) have demonstrated the use of this metal as a constituent of certain enzymes or in some of the enzymic activities. Nielson (26) has reported variations in plasma copper level in pregnency.

The nutritional status of an individual has an important effect upon the synthesis of plasma proteins also. Copper circulates in blood in combination with some components of plasma proteins. Holmberg and Laurell (15) have pointed out that under normal conditions almost all of the copper present in the plasma circulates as an integral part of the blue *alpha*-2 globulin, ceruloplasmin, while the remainder is loosely bound to albumin. Lahey *et al.* (20) have shown the relationship between *alpha*-2 and *beta* fractions of the globulins aud the concentrations of total copper in human plasma.

The conditions of hypercupremia and hypocupremia also prevail in certain pathological conditions. The changes in the total copper content of the plasma and the ceruloplasmin content have been reported by Cartwright *et al.* (5), Lahey *et al.* (21), Gobler *et al.* (10), Brendstrup (2), Cumming *et al.* (8) and Muller and Kahler (23).

In view of all these observations it was considered of interest to assess the values of total copper content and its different proportions combined with various fractions of

plasma protiens together with copper oxidase activity in the samples of plasma of healthy medical students of Gujarat consuming a Gujarati type of vegetarian diet with milk and milk products.

MATERIALS AND METHODS

One hundred apparently healthy medical students who were the premanent residents of Gujarat and Saurashtra were selected for this study. There were sixty five males and thirty five females within the age limit of 19—23 years in this group. Their dietary habits and clinical history were carefully recorded. The usual criteria for normality as suggested by Patel (28) were applied to each student for selection for this study. Each selected student was free from any symptom; could carry out his/her duty well and was apparently normal. None had suffered from any recent acute infection or prolonged chronic illness. The selected students consumed vegetarian diet with a fairly good quota of milk and vegetables.

The investigations were carried out in the morning hours during the preabsorptive period, in order to eliminate, as far as possible, the physiological diurnal variarions, if any. Blood samples in females were not collected during the menstural period. 5.0 c.c. of blood was withdrawn from anticubital vein with a sterile dry syringe, after minimal stasis, to avoid haemo-concentration and other changes in blood composition. The blood was collected in vials containing heparin which contained very negligible amount of copper. Only in cases of finding the relationship between plasma copper level and ingestion of vegetarian diet the blood samples were collected 2 hours after the lunch.

Total copper, direct reacting copper and ceruloplasmin copper i.e. copper bound to *alpha*-2 globulin fractions of the plasma were determined by the method of Gobler *et al.* (11) Plasma paraphenylene diamine activity was estimated by the method of Revin (30) and the total plasma protein concentration by the method of Kingsley (18).

For the fractionation of proteins by electrophoresis method, Whatman No. 1 Filter paper strips (4 x 36 cm) were used in a vertical closed strip type cell with barbital buffer of pH 8.6 and ionic strength of 0.75. Separation was effected at a constant current of 1.0 *ma.* per strip for 16 hours at 26°C. Duplicate strips were run for each subject. The staining and evaluation of separated fractions of plasma proteins were carried out as described by Jencks *et al.* (16). No correction factor for globulin fraction was employed.

RESULTS

The values for total plasma copper, albumin bound copper, copper present in ceruloplasmin molecule bound with *alpha*-2 globulin of the plasma and the copper oxidase activity of the plasma with standard deviation and coefficient of variation per cent in the cases of sixty five male and thirty five female medical students have been recorded in Table I. A perusal to this will reveal that these values are higher in females than in the males.

The statistical constants of plasma proteins and their fractions in normally healhy lactovegetarian students have been presented in Table II. This table brings forth the fact that the plasma electrophoretic pattern of males differ slightly from that of the females. The former have high total protein content and slightly higher albumin content,

TABLE I

Total copper content, albumin bound copper, copper in ceruloplasmin and copper oxidase activity in plasma samples of healthy Medical Students of Gujarat.

	Males	Females.
Number of Subjects.	65	35
Age group in years	19—23	20—22
Total copper content		
Mean values.	110 ∓ 13.5	131 ∓ 14.5
Range.	92—140	100—150
C. V.	12.2%	11.6%
Albumin bound copper		
Mean values.	6.4 ∓ 0.97	7.2 ∓ 1.26
Range.	5.1—8.0	5.2—9.0
C. V.	15.1%	17.5%
Copper in ceruloplasmin		
Mean values.	106.2 ∓ 17.0	126.0 ∓ 17
Range.	85 — 133.	80 —142.
C. V.	16.0%	13.5%
Paraphenelenediamine oxidase activity.		
Mean value.	67% ∓ 10%	66% ∓ 9.0
Range.	59.0—75%	68.0—74%
C. V.	23.2%	43.0%

1. Copper values are expressed in micrograms per 100 c.c. of plasma.
2. Copper oxidase activity has been expressed in terms of per cent transmission at the wave length 530 μ, black having 100% transmission.
3. C.V.=Coefficient of variation.

The *alpha*-2 globulin and *beta* globulin fractions of the total plasma globulin on an average are higher in females than in males.

The values of plasma copper and its fractions with the copper binding fractions of plasma proteins have been shown in the cases of both the sexes in the Table III. It can be observed that in cases of females on an average 7.2 μg of copper, (the transport copper), is present in loose combination with a fraction of albumin out of the total 3.85 *gms* of albumin present in 100 c.c. of plasma. Whereas 126.0 μg of copper are in combination with a portion of the *alpha*-2 globulin out of an average of 0.63 *gms* of total *beta* globulin per cent. Similarly in the cases of a sixty five healthy young male medical students, on an average 6.4 μg of copper have been found to be bound with a fraction of albumin out of the total 4.1 gms in 100 c.c. of plasma in loose combination as transport copper. Whereas some of the *alpha*-2 globulin from 0.50 gms, of the total have been found to be combined with 106 μg of copper to synthesis the ceruloplasmin molecule.

In order to study the change in plasma copper following the intake of food, estimations of plasma copper were carried out on 10 subjects, 5 males and 5 females,

TABLE II

Statistical constants in normal healthy medical lactovegetarian students of plasma proteins and thier various fractions.

Statistical constants.	Total Plasma proteins g. per cent	Albumin.	Protein fractions as gm. per cent. Globulin Fractions. Alpha-1.	Alpha-2.	Beta	Gamma	Total Globuli
			65 males				
Range.	6.50-8.0	3.8-4.4	0.11-0.65	0.12-0.80	0.72-1.12	1.10-2.40	2.5-4.10
Mean.	7.46	4.10	0.33	0.50	0.78	1.68	3.28
Standard Deviation	∓0.72	∓0.39	∓0.11	∓0.47	∓0.18	∓0.30	∓0.47
Coefficient of variation per cent	1.82	10.09	34.0	94.4	23.0	17.6	14.20
			35 females				
Range.	6.5-7.7	3.6-4.40	0.11-0.91	0.21-0.91	0.61-1.46	1.26-2.24	2.89-4.18
Mean.	7.20	3.85	0.30	0.63	08.6	1.67	3.27
Standard Deviation.	∓0.37	∓0.36	∓0.13	∓0.22	∓0.22	∓0.24	∓0.45
Coefficient of variation per cent	5.10	9.47	43.3	44.0	22.4	14.10	13.00

TABLE III

The values of plasma copper and its fractions with the copper binding fractions of plasma proteins.

No and Sex.	Total Copper	Total protein	Total albumin	Albumin bound copper	Total alpha. two glob.	Alphatwo globulin bound copper.
Males (65)	110	7.46	4.10	6.4	0.50	106.
Females (35)	131	7.20	3.85	7.2	0.63	126.

Copper values are expressed in micrograms per 100 c.c. of plasma.
Protein values are expressed in grams per 100 c.c. of plasma.

before meal and again 2 hours after the meal. The results are presented in the Table IV. A rise in plasma copper was observed in all instances after the meal. The average increase in these subjects was 25 μg. in males and 28 μg. in females.

TABLE IV

Relationship between plasma copper level and injestion of vegetarian diet

No. of Subject.	Plasma copper per 100 c.c. in microgram		Increased in copper level in microgram
	Before meals	2 Hours after Meals	Y
1. (M)	107	130	23
2. (M)	91	107	16
3. (M)	104	137	33
4. (M)	95	125	30
5. (M)	110	135	25
6. (F)	130	150	20
7. (F)	120	147	27
8. (F)	101	145	44
9. (F)	110	140	30
10. (F)	115	135	20

M = Males. F = Females.

A comparison of the differt plasma copper values reported in literature is given in Table V.

TABLE V

Plasma copper level in normal individuals as reported by different authors.

Authors.	Plasma copper values in normal in microgram per cent.
Bearn (1)	90—120
Brendstrup (2)	113 (M). 122 (F)
Butler and Newman (3)	66.2—84.9
Cartwright et al (5)	116.
Chopra and Balsubrahmanyan. (7)	80—126.
Cummings et at (8)	90—180
Lahey et al (20)	105 ∓ 16 (40M) 116 ∓ 16 (23F)
Markowitz et al. (22)	108.
Rangam and Bhagwat (29)	132—146
Reift and Sehnieden (31)	96 ∓ 8.0
Wintrobe (35)	70—120
Present author. (1965)	110. (65M) 131. (35F)

DISCUSSION

The values of plasma copper obtained in this study in 65 males vary between 92 and 140 μg% with an average of 110 ± 13.5 μg% and in 35 females between 100 and 150μg% with an average of 131.0 ± 14.5 μg%. These figures are in good agreement with the values published by the other investigators. Thus, the normal average values reported by a number of workers on a large number of subjects of both the sexes ranged between 109 to 148μg per 100 c.c. of plasma with the individual values varying from 68 to 187μg% (2, 4, 6, 14, 21, 24, 26, 32, 33). The data presented here indicates that the plasma copper values are significantly higher in females as compared to the males. Similar observations have also been recorded by several workers (6,20,25,26).

Lahey *et al.* (21) have also shown that about 6.0% of the total copper present in plasma is in loose combination with the plasma albumin, the albumin fraction of the plasma proteins being the transporter of the copper in the blood. In the study persued 5.6% of the total copper in males and 5.5% of the total copper in females are the albumin bound fractions of the total plasma copper. Bearn and Munkel (1) have conformed the evidence that the direct reacting copper, *i e.* the copper fraction which directly reacts with the copper colorimeteric reagent, (sodium diethyl dithio carbamate,) without acid treatment is bound to plasma albumin.

About 94.4% of the copper present in the plasma samples of males and 94.5% of the copper present in the plasma samples of females do not directly react with the copper colorimetric reagent but will react after removal of protein by treatment with hydrochloric acid. This is called indirectly reacting copper which is present in the ceruloplasmin molecule. Gubler *et al.* (10) have found that about 94.% of the total copper is tightly bound to one of the plasma proteins to form ceruloplasmin. This protein is *alpha*-2 globulin fraction of plasma proteins as pointed out by Holmberg and Laurell (15). They have again shown that ceruloplasmin is an enzyme capable of acting on sustrate, paraphenylene diamine. This enzyme activity is associated with the copper containing portion of ceruloplasmin. As observed here *beta*-2 globulin fraction of the plasma protein is more in females than in males and hence more of the plasma copper is in bound form in case of females forming excess of ceruloplssmin than in males.

The copper oxidase values are on an average 67.% in the terms of per cent transmission for male plasma samples and 66% for female plasma samples. Ravin (30) had observed these value from 50 to 80% transmission, corresponding to 100-200 microliter uptake of oxygen/ml/hour in the normal sample of plasma.

A very small but significant diurnal variation in plasma copper values have been reported by Lahey *et al.* (21) and Nielson (26). To obtain fairly uniform duplicate results the blood samples were collected approximately at the same time in the morning (at 9-00 A.M.) during the day.

The finding that the plasma copper is raised after meals is in contrast to that of other workers like Heilmeyer *et al.* (13), Lahey *et al.* (20), who observed that the rise in plasma copper after meal was insignificant. An average increase of 25μg in the five male subjects studied and of 28μg in the five female subjects studied has been observed in this study. The increase of plasma copper after meals may be attributed to absorption of

copper from the diet. The copper content of an average Indian balanced diet has been shown to be 2.48 mg by Chaudhary (6).

The electrophoretic patterns of plasma proteins in apparently healthy individuals show minor differences. The total proteins and albumin fractions of plasma proteins in males are higher than in females. This is in contrast to the observations of Kulkarni *et al.* (19) who observed no difference between the plasma protein contents of both the sexes. Lahey *et al.* (21) have found the correlationship between *alpha* 2 and *alpha*-3 fractions of plasma proteins and total plasma copper as well as *beta*-1 and *beta* 2 fractions of plasma proteins and the copper concentration in the plasma. In both the cases copper levels increase with the increase of these fractions. They however did not find any significant correlation between the plasma copper level and other protein fractions determined electro-phoretically. In the study persued herewith, an increase in the ceruloplasma bound copper with the increase in *alpha*-2 fraction of plasma proteins have been found in cases of males as well as in females which substentiates the observations reported by Lahey *et al.* (20).

The author is thankful to Dr. B. N. Mazumdar, Professor of Physiology B. J. Medical College, Ahmedabad, for helpful suggestions, encouragement and facilities provided.

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