Serum Micronutrient and Micromineral Concentrations and Ratios in Healthy Omani Subjects

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Key Words

Omani · α -Tocopherol · Caeruloplasmin · Cholesterol · Copper · Retinol · Zinc

Abstract

Objective: This study aimed to establish reference ranges of serum concentrations of copper, zinc, retinol, α -tocopherol, copper:caeruloplasmin and copper:zinc ratios in a group of healthy Omani men and women. **Materials and Methods:** Assay techniques employed were atomic absorption spectrophotometry (copper and zinc), reverse-phase high-pressure liquid chromatography with isocratic elution (retinol and α -tocopherol), immunonephelometry (caeruloplasmin) and spectrophotometry (albumin and cholesterol). **Results:** The mean $\pm$ SD (μ M) obtained for copper, zinc, retinol, and α -tocopherol were 15.9 ± 3.0 , 14.2 ± 2.0 , 1.45 ± 0.39 and 16.9 ± 4.4 , respectively. The mean $\pm$ SD for copper:zinc and copper:caeruloplasmin ratios were $1.15 \pm 0.30 \mu\text{mol}/\text{mmol}$ and $6.99 \pm 0.84 \mu\text{mol}/\text{g}$, respectively. Significantly higher ($p < 0.0001$) copper and caeruloplasmin concentrations, copper:zinc and copper:caeruloplasmin ratios and lower zinc, retinol, α -tocopherol, cholesterol concentrations and α -tocopherol:cholesterol ratio were present in women compared to men. Age appeared to be associated with copper and retinol concentrations, and copper:caeruloplasmin ratios in women; in men, the association was mostly moderate with caeruloplasmin, α -tocopherol, cholesterol concentra-

tions and α -tocopherol:cholesterol ratios. Smokers had decreased albumin ($p = 0.002$), zinc ($p = 0.023$) concentrations, and copper:caeruloplasmin ratios ($p = 0.002$), increased α -tocopherol concentrations ($p = 0.016$) and α -tocopherol:cholesterol ratios ($p = 0.021$) compared with non-smokers. Deficiency incidence was $\leq 5\%$ for all investigated parameters. **Conclusions:** Reference ranges of micronutrient concentrations and micromineral ratios were established for Omani subjects. The mean values of several micronutrients were lower than those reported for other populations and some showed gender effects.

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Introduction

In the Sultanate of Oman, estimated occurrences of nutritional disorders per 10,000 population are malnutrition (protein energy malnutrition, vitamin A mineral deficiencies) 19, obesity and other metabolic disorders (excluding gout) 9, other nutritional deficiencies and symptoms related to nutrition and development 9, thus making a total of 37 (0.37%) [1]. In this context, information about micronutrient status of the people of this region is lacking. The present study embarked on the investigation of levels of copper, zinc, retinol, and α -tocopherol and micromineral ratios in healthy Omani men and women.

The microminerals copper and zinc are essential enzyme cofactors and antioxidants [2]. Copper is an important mineral in the normal growth and development of the skeletal system [2]. Zinc status regulates vitamin A metabolism, including absorption, transport and its utilization [3] and its deficiency is associated with decrease in bone density. Zinc is bound mainly to plasma albumin [2], which is considered a nutrition marker [4]. It also inhibits the intestinal absorption of copper. Hence, the risk of copper deficiency is increased when the molar ratio of zinc to copper (Zn:Cu) is high [5]. Copper:zinc ratio is thought to be clinically more useful than the concentration of either metal [6]. Caeruloplasmin, an endogenous antioxidant and a major copper binder, is important in the interpretation of serum copper results [7]. Copper:caeruloplasmin ratio has recently been suggested as an alternative index of copper status [8, 9] that enables adjustment for relatively high caeruloplasmin levels without recourse to producing gender- and age-derived reference intervals [9].

Retinol and α -tocopherol are essential micronutrients [10, 11]; α -tocopherol is also a powerful antioxidant [11], but the benefits to humans of the potential antioxidant activity of retinol are not clear [12]. Serum α -tocopherol:cholesterol ratio is considered a more reliable index of vitamin E nutrition than α -tocopherol concentration alone [13].

This study aimed at establishing reference ranges for serum copper, zinc, retinol, α -tocopherol levels, copper:caeruloplasmin and copper:zinc ratios in healthy Omani men and women, the effect of gender and age on them and to determine the prevalence of serum deficiency of these parameters.

Materials and Methods

The institutional Medical Research and Ethics Committees approved the study and informed consent was obtained from the participating subjects before the study. Exclusion criteria were pregnancy, females on oral contraceptives, subjects with clinical conditions such as malabsorption, hyperlipidaemia, hepatic disorders and persons on medication (cholestyramine, neomycin, mineral oil, antacid, phenobarbitone, cortisone). Two-fifty Omani indigenes comprising men ($n = 130$, aged 18–62 years, mean 30.3) and women ($n = 120$, aged 18–60 years, mean 32.3), who were apparently healthy, with no infection or chronic inflammatory disease, were enrolled. None of the subjects ingested micronutrient supplements. Menopausal women (aged >40 years) numbered 21. Thirty-four men and 1 woman were smokers.

Fasting blood samples were drawn from each subject into plastic tubes (W. Sarstedt, Inc., Princeton, N.J., USA) to avoid contamination of copper and zinc. They were kept in the dark to clot

Table 1. Electrothermal furnace programme for copper analysis

Temperature	Ramp, s	Hold, s
110°C	3	30 (drying)
130°C	15	30 (drying)
1,200°C	10	20 (ashing)
2,300°C	0	5 (atomization)
2,400°C	1	3 (cleaning)

followed by centrifugation at 2,000 rpm for 10 min. Using disposable plastic pipettes, haemolysis-free serum was separated from the cells into Beckman microfuge tubes (Beckman Instruments), wrapped in foil to prevent photodecomposition of retinol and α -tocopherol and frozen at -80°C pending analyses within a fortnight.

The electrothermal AAS copper method of Evenson and Warren [14] was modified for copper analysis on Model 600 atomic absorption spectrophotometer (PerkinElmer Corp., PTE Ltd., Singapore). Essentially, after calibration with copper working standards, the controls and samples were diluted 20-fold with 0.1% HNO_3 , followed by auto-injection of 20- μl aliquot into the graphite cuvette for analysis. Analysis sequence and monitoring at 324.7 nm (copper emission line) using the furnace program are shown in table 1.

Zinc measurement by flame AAS on a Shimadzu 6800 (Shimadzu, Kyoto, Japan) interfaced to a PC integrator and a printout system was based on a previously described method [15]. Trace Element Control Serum Levels I and II (Utak Laboratories, Inc., 25020 Avenue Tibbits, Valencia, Calif., USA) were used to monitor serum copper and zinc levels.

Analysis of serum concentrations of retinol and α -tocopherol by reverse-phase HPLC followed a previous method [16], with modification. Essentially, the modification was the use of photodiode array that continuously monitored the column effluents over a selected wavelength range of 210–400 nm that covered the wavelength maxima of retinol (325 nm), α -tocopherol (292 nm) and α -tocopherol acetate as internal standard (285 nm). Quality control was monitored by using control levels I and II (Chromsystems Instruments and Chemicals GmbH, München, Germany) (table 2).

IMMAGE Immunochemistry system was used to measure caeruloplasmin by rate immunonephelometry using Vigil Protein QC levels I, II and III as reference material (Beckman Coulter, USA). Albumin and cholesterol levels were measured by routine spectrophotometric methods on the Beckman Synchron CX7 clinical chemistry system with Multiquant levels I, II and III (Biorad Laboratories, Irvine, Calif., USA) as reference material.

Statistical analyses were performed using SPSS 11.0 for Windows software (SPSS, Chicago, Ill., USA). Because histogram plots to assess the relative frequency of concentrations showed normal curves for all the tests, reference ranges were established using parametric test. The reference range data were reported as mean $\pm$ SD with 95% confidence interval (CI) in the result tables. Differences between two groups were evaluated by independent sample t tests. Correlation analyses were performed. Probability (p) values were two-tailed; $p < 0.05$ was considered significant.

Table 2. Imprecision studies of internal quality control sera

Analyte levels:	Intra-batch CV, % (n = 11)			Inter-batch CV, %		
	I	II	III	I	II	III
Copper	2.6	2.7	2.4	4.3	4.7	4.8
Zinc	1.7	2.4	1.7	3.5	3.8	3.7
Caeruloplasmin	7.2	4.2	6.5	8.4	6.1	6.6
Albumin	0.9	1.3	1.2	2.0	2.8	2.3
Cholesterol	1.4	1.1	1.4	2.5	2.4	2.9
Retinol	3.3	3.1	n.a.	5.0	6.1	n.a.
α -Tocopherol	4.8	4.5	n.a.	6.6	7.2	n.a.

The mean % recoveries for retinol and α -tocopherol obtained by spiking sera with known concentrations (μM) of 2.0 and 12.0 of the respective analytes were 98 (n = 5) and 96 (n = 5), respectively. Inter-batch CV values were obtained from analyses on 20 consequent days.

Table 3. Mean $\pm$ SD and 95% CI of serum parameters in a representative sample of Omani men and women

Parameter	Total	Men	Women	p value ¹
Copper, μM	15.9 $\pm$ 3.0	14.2 $\pm$ 2.7	17.8 $\pm$ 2.1	<0.0001
95% CI	10.2 – 21.3	9.8 – 21.0	13.5 – 22.3	
Zinc, μM	14.2 $\pm$ 2.0	15.0 $\pm$ 2.2	13.4 $\pm$ 1.5	<0.0001
95% CI	11.0 – 19.8	10.5 – 20.6	11.1 – 17.4	
Cu:Zn, $\mu\text{M}/\mu\text{M}$	1.15 $\pm$ 0.30	0.97 $\pm$ 0.24	1.34 $\pm$ 0.24	<0.0001
95% CI	0.69 – 1.60	0.59 – 1.40	0.95 – 1.71	
Caeruloplasmin, g/l	0.301 $\pm$ 0.044	0.291 $\pm$ 0.053	0.312 $\pm$ 0.028	<0.0001
95% CI	0.215 – 0.427	0.207 – 0.439	0.257 – 0.354	
Cu:caeruloplasmin, $\mu\text{mol/g}$	6.99 $\pm$ 0.84	6.49 $\pm$ 0.81	7.53 $\pm$ 0.45	<0.0001
95% CI	5.46 – 8.14	5.03 – 7.70	6.86 – 8.21	
Retinol, μM	1.45 $\pm$ 0.39	1.69 $\pm$ 0.39	1.20 $\pm$ 0.20	<0.0001
95% CI	0.62 – 1.65	0.66 – 1.80	0.55 – 1.15	
α -Tocopherol, μM	16.9 $\pm$ 4.4	18.1 $\pm$ 5.8	15.6 $\pm$ 1.4	<0.0001
95% CI	10.3 – 30.3	9.1 – 33.8	13.1 – 20.0	
Cholesterol, mmol/l	4.5 $\pm$ 0.7	4.7 $\pm$ 0.9	4.4 $\pm$ 0.4	<0.0001
95% CI	3.3 – 6.5	3.1 – 7.1	3.7 – 5.3	
α -Tocopherol:cholesterol, $\mu\text{M}/\text{mmol}$	3.71 $\pm$ 0.64	3.81 $\pm$ 0.85	3.60 $\pm$ 0.22	<0.0001
95% CI	2.19 – 5.27	2.14 – 5.82	3.0 – 4.2	
Albumin, g/l	45.1 $\pm$ 2.3	45.3 $\pm$ 2.4	44.9 $\pm$ 2.2	0.166
95% CI	40.0 – 50.0	40.3 – 50.0	39.0 – 49.0	

Statistical significance was considered at $p < 0.05$. ¹ Men versus women.

Results

The mean $\pm$ SD and 95% CI values for all the parameters of the study group are presented in table 3. The serum copper and caeruloplasmin levels were much higher ($p < 0.0001$) in women compared to men. The same also applied to copper:zinc and copper:caeruloplasmin ratios.

Higher Zn, retinol, α -tocopherol, cholesterol concentrations ($p < 0.0001$) and α -tocopherol:cholesterol ratio ($p = 0.006$) were observed in men. Incidences of deficiency of the micronutrients and their ratios, considered at below reference range values, were between 1 and 4%.

Correlation analyses of the relationship between age and various biochemical parameters for men and women

Table 4. Relationship between age and the parameters in Omani men and women

Parameter	Men (n = 130)		Women (n = 120)	
	r	p value	r	p value
Copper	0.249	0.004	0.439	<0.0001
Zinc	-0.007	0.938	0.163	0.076
Copper:zinc	0.191	0.029	0.158	0.085
Caeruloplasmin	0.417	<0.0001	0.302	0.001
Cu:caeruloplasmin	-0.234	0.007	0.386	<0.0001
Retinol	0.150	0.088	0.508	<0.0001
α-Tocopherol	0.625	<0.0001	0.113	0.221
Cholesterol	0.562	<0.0001	0.267	0.003
α-Tocopherol:cholesterol	0.368	<0.0001	-0.223	0.015
Albumin	-0.351	<0.0001	0.011	0.905

Statistical significance was considered at p < 0.05.

Table 5. Effect of cigarette smoking on plasma levels of some biochemical parameters in Omani men

Parameter	Smokers (n = 34)	Non-smokers (n = 96)	p value
Copper, μM	13.9 ± 2.5	14.3 ± 2.8	0.477
Zinc, μM	14.2 ± 1.4	15.1 ± 2.4	0.023
Copper:zinc, μM/μM	0.99 ± 0.24	0.97 ± 0.28	0.727
Caeruloplasmin, g/l	0.306 ± 0.054	0.289 ± 0.055	0.112
Cu:caeruloplasmin, μmol/g	6.06 ± 0.81	6.58 ± 0.81	0.002
Retinol, μM	1.14 ± 0.23	1.11 ± 0.27	0.576
α-Tocopherol, μM	20.2 ± 5.2	17.6 ± 5.9	0.016
Cholesterol, mM	4.9 ± 1.0	4.6 ± 0.9	0.211
α-Tocopherol:cholesterol, μM/mM	4.11 ± 0.70	3.76 ± 0.89	0.021
Albumin, g/l	44 ± 1	46 ± 3	0.002

Statistical significance was considered at p < 0.05.

are shown in table 4. There was a moderate association between copper and age in women (r = 0.439), but not in men. A similar effect of age was observed for caeruloplasmin, α-tocopherol, cholesterol and α-tocopherol:cholesterol ratio (with significant r values being 0.417, 0.625, 0.562, and 0.368, respectively) in men, and for retinol in women (r = 0.508). A weak but negative relationship was seen between age and albumin (r = -0.351) in men. The

Table 6. Mean plasma micronutrient concentrations and copper:caeruloplasmin ratios reported for various countries

Country	Cu μM	Zn μM	Retinol μM	α-To-coph- erol μM	Cu:caerulo- plasmin ratio μmol/g	Ref.
Oman	15.9	14.2	1.45	16.9	6.99	this study
UK					6.81	6
Kuwait	23.2	16.1	1.76	20.0		13, 14
Japan			2.60	24.4		16
Italy			2.09	26.2		17
Spain			1.70	28.2		18
USA			1.91	25.7		19
Switzerland			2.09	29.3		20
Thailand	18.0	12.7				22
Norway	17.5	15.5				23
N. Ireland	18.1	12.4				24
Canary	17.3	17.7				25

effect of smoking on the parameters in men is shown in table 5. Smokers had significantly lower zinc and albumin concentrations and copper:caeruloplasmin ratio than non-smokers. Higher α-tocopherol concentration and α-tocopherol:cholesterol ratio were observed in smokers compared to non-smokers.

The mean values for the micronutrients and copper:caeruloplasmin ratio published for different countries are presented in table 6. Omani mean values for retinol, α-tocopherol, and copper were lower relative to reported mean values for Americans, Europeans, the Japanese, Kuwaitis and Thais. Their mean zinc value was lower than the values reported for the Norwegians and Canarians, but higher compared to the mean for Greeks, the Irish and Thais. The Omani mean for the copper:caeruloplasmin ratio was similar to the value reported for the UK population.

Discussion

The mean values of serum copper, zinc, retinol, α-tocopherol, caeruloplasmin, albumin and cholesterol for Omanis were within the established reference ranges [2]. However, compared with other populations, like Kuwaiti, Japanese, Italian, Spanish, American and Swiss populations [16–20] (table 6), the mean values of retinol and α-tocopherol obtained for Omanis were lower. Similarly,

the Omani mean for copper was lower compared to those of Kuwaitis, Thais, Greeks, Norwegians, the Irish and Canarians [15, 21–25]. Omani mean for zinc levels varied and was higher than the values reported for Thais [21], Greeks [22] and Norwegians [23], but lower than those of the Irish and Canarians [24, 25]. These findings may have two explanations. Firstly, the method specificity may be higher here than in earlier studies, which may be attributable to improvement of the relevant analytical techniques over the years. Secondly, for Omanis, the dietary habits reflecting their affluence and purchasing power may be different from those of Kuwaitis, the Japanese and Westerners. The Japanese and the Westerners would also be expected to be nutritionally more aware and are likely to ingest nutrient supplements.

Gender was an important determinant as serum copper values being higher in women than men (table 3) even though the exclusion criteria included pregnancy and intake of oral contraceptives. High serum copper values seen in Omani women corroborated reports for women of Kuwaiti, Thai, and Western populations. Gender influence was also noticeable for zinc and retinol levels, which were higher in men than in women (table 3); the high retinol levels contrasted with the report by Winkelhofer-Roob et al. [20], but confirmed other studies [16–19]. The finding here of lower α -tocopherol level in Omani women than men is at discord with the study by Ito et al. [17] on the Japanese.

The present study's copper:caeruloplasmin ratio (mean $\pm$ SD, $\mu\text{mol/g}$) of 6.99 ± 0.84 (table 3) is similar to 6.81 ± 0.87 reported by Twomey et al. [9]. Also, the Omani reference range of 5.46–8.14 $\mu\text{mol/g}$ for copper:caeruloplasmin ratio (table 3) is close to 5.2–7.3 and 5.08–8.55 $\mu\text{mol/g}$ Beetham [8] and Twomey et al. [9] reported, respectively. Furthermore, Omani reference ranges of 10.2–21.3 μM (copper) and 0.215–0.427 g/l (caeruloplasmin) are similar to the 10–25 μM (copper) and 0.200–0.500 g/l (caeruloplasmin) reported by Twomey et al. [9]. However, the observation that the copper:caeruloplasmin ratio was markedly higher in women compared to men (table 3) contrasted with the previous reports [8, 9]. The persistent gender effect found here may be related to the types of subjects in the present study and that of Twomey et al. [9]. A sizeable number of patients in the study by Twomey et al. [9] had liver disorders that impinge on the capacity to synthesize caeruloplasmin compared to the hepatic synthetic capacity of healthy Omani women. Twomey et al. [9] did not indicate the ratio of women to men in their study.

Depressed copper and elevated zinc is a common trace-metal imbalance; copper and zinc compete for ligands and impair each other's function [2]. Absorbed dietary zinc acts directly with a nuclear metalloregulatory binding protein that controls the synthesis of metallothionein, an avid copper binder in the intestinal mucosal cells [2]. Thus, when mucosal cells are rich in metallothionein, little copper traverses the cells into the blood, because most of the copper is returned to the intestinal lumen when intestinal cells turn over [2]. The reference range for copper:zinc ratios of 0.69–1.60 (mean = 1.15) reported here is comparable to the stated optimum of 0.70–1.00 [5]. A deficiency of 4% obtained for the Omanis may be insignificant.

The effect of age on the parameters varied and was dependent on gender (table 4). Copper concentration increased with age, and more so in women than in men contrasting with caeruloplasmin. The copper:caeruloplasmin ratio complemented copper in that it increased with age in women, but unlike copper it negatively correlated with age in men. There is no previous study to compare these findings with. The increased caeruloplasmin in the aging males may be an acute-phase response to rising inflammation consistent with ageing. Serum albumin levels correlate with nutritional parameters including anthropometric measurements [4]. Albumin measured in this study primarily as a marker of nutrition adequacy was negatively associated with age in men, which may portend diminishing hepatic synthesis in ageing men than women [26].

α -Tocopherol concentrations are frequently adjusted for plasma cholesterol concentrations to avoid misclassification of vitamin E status [13]. A stronger association of α -tocopherol:cholesterol ratio with age in men compared to women (table 4) suggests greater antioxidant protection for the former group against lipid peroxidation and atherosclerotic lesion [11]. The 1–3% incidence of deficiency of α -tocopherol and of α -tocopherol:cholesterol ratio in Omani men and women is unlikely to be of any real significance. The relationship of serum cholesterol concentration with age was less striking in women than in men. That retinol level related with age in Omani women may mean improved aging-related immunity [10]. The benefits to humans of the potential antioxidant activity of retinol are not clear [12].

Cigarette smoke is implicated in free radical-related cytotoxicity [27]. Albumin, an antioxidant and a major zinc binder and transporter [2], complemented zinc in depletion in smokers relative to non-smokers (table 5), possibly implying a connection between cigarette smoke

cytotoxicity and the hepatic synthesis of albumin. Conversely, increased α -tocopherol level and α -tocopherol:cholesterol ratio in smokers compared with non-smokers may confer antioxidant protection against the deleterious effect of smoking [28]. Previous studies have also reported varied effects of smoking on biological antioxidant status [28, 29].

Conclusion

This study has shown that in the Omani population, the serum concentrations of copper, zinc, retinol, α -tocopherol and copper:caeruloplasmin ratio are influenced by gender. The mean values of several micronutrients were lower than the corresponding values reported for other populations.

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