

Association of whole blood copper, zinc and magnesium levels with risk of metabolic syndrome components in Chinese rural 6-12 years old children: the 2010-2012 China National Nutrition and Health Survey

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Research

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Abstract

Background

Copper, zinc and magnesium are essential elements and participate in a series of oxidative stress and inflammation related to metabolic reactions. There are many studies analyzed the relationship between Cu, Zn, Mg and metabolic syndrome(MetS), which involved in adults and adolescents, but there are still few in children.

Objective

We aim to estimate the association of whole blood copper, zinc and magnesium with metabolic syndrome components in Chinese rural 6–12 years old children.

Method:

A total of 911 children aged 6–12 were enrolled from the 2010–2012 China National Nutrition and Health Survey in this study. The basic characteristics, metals and MetS components parameters were collected. Multivariate logistic regression analysis was performed to confirm the independent relationship between metals and the incident of MetS components.

Results

Elevated waist was positively associated with higher whole blood Cu concentration(OR = 2.00) and higher ratio of Cu/Zn(OR = 2.08), whereas elevated TG was negatively associated with higher Cu (OR = 0.33) and higher Cu/Zn(OR = 0.35) level. And higher level of Zn (OR = 2.12) and Mg (OR = 2.34) were both positively associated with elevated TG. In the combination of low Cu and high Zn level, it increased the risk of elevated TG(OR = 2.21), which consistent with the single analysis of these two metals. Mg has no significant effect on elevated waist when analysis alone. However, when there was high Mg and Cu at the same time, the risk of elevated waist increased significantly (OR = 2.03). In addition, different from single analysis of Zn and Mg, when high Zn and low Mg combined, it can significantly decreased the risk of reduced HDL-C (OR = 0.47).

Conclusion

The level of whole blood Cu, Zn, Cu/Zn and Mg within the normal range in children is associated with the risk of MetS components, especially in elevated TG and elevated waist. And the combination of each two metals has also shown to be correlated to MetS components. The future research will conduct a cohort to further confirm these findings. And the results will also need to be verified in other populations.

1. Introduction

Metabolic syndrome (MetS) is a cluster of related risk factors of metabolic origin, promoting the development of cardiovascular disease (CVD) and increasing the risk for development of type 2 diabetes[1]. It may be the result of the interaction of genes, environment and lifestyle. Nowadays, the MetS is widespread in the world and estimates indicated that 34% of the population meet the criteria for the MetS and the number in China is 33.9%[2]. The prevalence of MetS is increasing rapidly and showing a trend of younger age. In Chinese children, the prevalence of MetS increased from 2.3% in 2004–2010 to 3.2% in 2011–2014[3].

Copper, zinc and magnesium are all important trace elements which are involved in various metabolic characteristics[4,5]. Copper and zinc play an important role in oxidative stress and inflammation[6,7], as they are the co-factors of antioxidant enzymes[8]. Magnesium is the second most abundant intracellular ion after potassium, and deficiency in magnesium is associated with several noncommunicable disease, such as type 2 diabetes, coronary heart disease[9]. A hypothesis of MetS indicates that oxidative stress presents as the main mechanism because of redox imbalance[10]. Thus, the metals may be related to the development of MetS. Several studies had already detect the relationship between single metal level such as Zn[11], Cu[12] and Mg[13] and the incident of MetS. The evaluation of the relationship between multiple elements and metabolic syndrome is still worth assessing especially in children. And also whole blood, which is a comprehensive indicator to reflect the nutritional status of elements in vivo, is rarely used as a biological specimen than serum and plasma. But as some studies show that the concentration of elements in whole blood is higher than that in serum or plasma, and can comprehensively reflect the exposure in the body[14,15].

Hence, in this cross-section study, we aimed to investigate the correlation between whole blood Cu, Zn and Mg and the risk of MetS components of Chinese children from 2010–2012 China National Nutrition and Health Survey (CNNS).

2. Material And Method

2.1 Subjects

The study was based on the data obtained from 2010–2012 China National Nutrition and Health Survey (CNNS), which is a cross-sectional, nationally representative survey. According to Green's rule of numbers of participants used to examine relationships, the reasonable sample size is greater than $50 + 8m$

(where m is the number of independent variables) for testing the multiple correlation[16]. The independent variables were 15 in this study. As a consequence, the minimum sample size was 170. We randomly selected 1008 participants in 2010–2012 CNNS aged 6 to 12 years old in 72 rural areas according to the principles of gender equal, which all had a complete physical detections. Subjects were sampled by multi-stage stratified random sampling method. All the samples were re-examined after physical and biological data collection with the following exclusion criteria: 1) missing data for variables; 2) data does not meet test standards; 3) outlier for statistic analysis. Due to the exclusion of some samples, 911 samples were final recruited in this study. All subjects gave their informed consent for inclusion before they participated in the study.

2.2 Data collection

Physical examinations were performed by trained medical staff following standardized procedures. Height was measured by a Seca 213 Portable Stadiometer Height-Rod with a precision of 0.1 cm; body weight was measured by a Seca 877 electronic flat scale with a precision of 0.1 kg. Body mass index (BMI) was calculated as $\text{weight(kg)}/\text{square of height(m}^2\text{)}$. Waist circumference was measured by a tape with a precision of 0.1 cm. Systolic blood pressure (SBP, mmHg) and diastolic blood pressure (DBP, mmHg) were assessed twice in the right upper arm using Omron HBP-1300 professional BP monitor. The second of the two measures was recorded if the difference between the two was less than 10 mmHg. A third measure was performed if the difference between the first two measures was more than 10 mmHg, and any two measures with a difference less than 10 mmHg were recorded. The venous blood was collected and divided into the anticoagulation tube and serum separator tube, respectively. The blood samples in the serum separator tube were promptly centrifuged at 3000 g for 15 min after blood collection, divided into aliquots of serum and frozen at -80°C for subsequent assays. Serum fasting glucose, high-density lipoprotein cholesterol (HDL-C) and triglyceride (TG), were measured by an enzymatic method using Hitachi 7600 automatic biochemical analyzer (Japan). The whole blood Cu, Zn and Mg concentration was measured by inductively coupled plasma mass spectrometry (NexION 350D, PerkinElmer) from the anticoagulation tube in KED model.

2.3 Definitions of MetS and its components

As the subjects in this study were aged 6 to 12 and no consistent definition of metabolic syndrome (MetS) could be used here. We adopt the modified criteria for MetS from American Academy of Pediatrics (AAP) and International Diabetes Federation (IDF)[17], which indicate MetS as the presence of ≥ 3 of the risk factors. The MetS components were followed as: 1) obesity: waist ≥ 95 th percentile of children of the same age and gender or BMI ≥ 95 th percentile of children of the same age and gender; 2) hypertension: blood pressure ≥ 95 th percentile of children of the same age and gender (fast identify: systolic BP ≥ 120 mmHg or diastolic BP ≥ 80 mmHg); 3) dyslipidemia: a.Reduced HDL-C(< 1.03 mmol/L); or b.Elevated TG(≥ 1.47 mmol/L); 4) hyperglycemia: fasting glucose ≥ 5.6 mmol/L.

2.4 Statistic analysis

Statistical analyses were performed using SPSS version 19.0. The results of descriptive characteristics were expressed as average \pm standard deviation. The relationships among clinical indexes and the number of MetS components were analyzed using Kruskal-Wallis test. Spearman correlation coefficients were applied to assess the relationship between metals and MetS components. The odds ratios (ORs) and 95% confidence intervals (95%CI) were determined by multivariate logistic regression to investigate the associations between MetS components and tertiles of whole blood metals. We categorized the level of whole blood Cu, Zn, the ratio of Cu to Zn, and Mg into tertiles. Using the lowest tertile as the reference. P-trend analysis was done by treating the tertiles as a continuous variable in the regression analyses. The combination of metals defined low as the concentration was lower than the 50 percentage of whole value and high as the concentration was equal or greater to the 50 percentage value. All statistical test were two side and statistical significance was determined at a $P < 0.05$.

3. Results

3.1 Characteristics of the study population

This study was carried out with 911 individuals (included 467 male and 444 female) with complete data on MetS components and whole blood Cu, Zn and Mg concentrations. The basic characteristics of subjects were shown in Table 1. There were gender-based differences in the comparison of clinical and biological indexes: waist, HDL-C, Cu concentration and Cu/Zn ratio were higher in male, but TG concentration was higher in female. We also analyzed these variables by different numbers of MetS components. We observed that the value of age, height, weight, waist, BMI, the rate of obesity and the levels of fasting serum glucose, SBP, Cu and Cu/Zn had an ascending trend, while the HDL level had a descending trend with increasing number of MetS components. We also test the difference between each two groups defined with different MetS components numbers. Participants in the group with one or two MetS components were more likely to have a greater age, weight, height, waist, BMI, rate of obesity, TG, FG level, and DBP level and a lower HDL-C level when compared with non-MetS components group. The concentration of waist, FG, Cu, Cu/Zn and the obesity rate were significantly higher among the group with 3 MetS components when compared with the other 3 group respectively.

Table 1
Basic characteristics according to gender and the number of MetS components

Indexes	Total (N = 911)	Male (N = 467)	Female (N = 444)	Numbers of MetS components			
				0(N = 497)	1(N = 336)	2(N = 71)	3(N = 7)
Age(years)	9.12 ± 1.7	9.01 ± 1.7	9.23 ± 1.7	8.93 ± 1.69 [#]	9.31 ± 1.68 ^a	9.55 ± 1.67 ^a	9.17 ± 1.84
Height(m)	131.52 ± 11.84	131.06 ± 11.41	132.01 ± 12.27	130.08 ± 10.71 [#]	132.71 ± 12.58 ^a	135.46 ± 13.74 ^a	136.99 ± 16.29
Weight(kg)	28.95 ± 8.74	29.17 ± 8.96	28.71 ± 8.49	26.95 ± 6.81 [#]	30.33 ± 9.27 ^a	35.43 ± 11.72 ^{a,b}	38.84 ± 17.24 ^{a,b}
Waist(cm)	55.96 ± 7.91	56.76 ± 7.93	55.11 ± 7.82 [*]	53.4 ± 5.01 [#]	57.58 ± 8.69 ^a	64.65 ± 10.28 ^{a,b}	71.33 ± 10.5 ^{a,b,c}
BMI(kg/m ²)	16.41 ± 2.78	16.66 ± 2.98	16.15 ± 2.53	15.72 ± 2.27 [#]	16.86 ± 2.82 ^a	18.84 ± 3.62 ^{a,b}	19.65 ± 4.17 ^{a,b}
Obesity(%)	16.02%	14.56%	17.56%	0% [#]	27.08% ^a	67.6% ^{a,b}	100% ^{a,b,c}
TG(mmol/L)	0.79 ± 0.48	0.74 ± 0.46	0.85 ± 0.5 [*]	0.67 ± 0.29 [#]	0.93 ± 0.62 ^a	1.03 ± 0.57 ^a	0.68 ± 0.25 ^c
HDL-C (mmol/L)	1.27 ± 0.3	1.31 ± 0.3	1.23 ± 0.29 [*]	1.40 ± 0.26 [#]	1.15 ± 0.28 ^a	1.01 ± 0.2 ^{a,b}	1.01 ± 0.24 ^a
FG(mmol/L)	4.85 ± 0.71	4.9 ± 0.73	4.8 ± 0.68	4.71 ± 0.53 [#]	4.94 ± 0.77 ^a	5.25 ± 1.02 ^{a,b}	6.41 ± 1.21 ^{a,b,c}
SBP(mmHg)	92.44 ± 11.42	92.68 ± 10.88	92.19 ± 11.96	91.44 ± 10.16 [#]	92.92 ± 12.31	96.41 ± 13.43 ^{a,b}	100.38 ± 17.31 ^a
DBP(mmHg)	60.23 ± 8.65	60.23 ± 8.07	60.23 ± 9.24	59.41 ± 7.72 [#]	60.77 ± 9.47 ^a	63.34 ± 9.58 ^{a,b}	61.52 ± 12.32
Cu(mg/L)	1.01 ± 0.13	1.03 ± 0.14	0.99 ± 0.13 [*]	1.00 ± 0.13 [#]	1.01 ± 0.13	1.05 ± 0.14 ^{a,b}	1.19 ± 0.10 ^{a,b,c}
Zn(mg/L)	5.21 ± 1.07	5.19 ± 1.06	5.24 ± 1.08	5.21 ± 1.05	5.22 ± 1.09	5.23 ± 1.11	4.87 ± 1.07
Cu/Zn	0.2 ± 0.05	0.20 ± 0.05	0.20 ± 0.04 [*]	0.20 ± 0.04 [#]	0.20 ± 0.05	0.21 ± 0.04	0.25 ± 0.05 ^{a,b,c}
Mg(mg/L)	41.44 ± 5.28	41.29 ± 5.24	41.60 ± 5.32	41.16 ± 5.26	41.74 ± 5.34	41.94 ± 5.13	41.77 ± 4.39
MetS, metabolic syndrome; BMI, body mass index; TG, triglycerides; HDL-C,high density lipoprotein cholesterol;FG, fasting glucose; SBP,systolic blood pressure; DBP, diastolic blood pressure;*,p < 0.05 for gender; #, p < 0.05 for trend;a, compare with non-MetS component group; b, compare with one MetS component group ; c, compare with two MetS component group							

3.2 The association between tertiles of Cu, Zn, Cu/Zn and Mg and MetS components indicators

The relationships between Cu, Zn, Cu/Zn and Mg and MetS components indicators were shown in Table 2. The value of BMI, waist and DBP tended to be higher in the upper tertiles of Cu, whereas TG value was lowest in the third tertile. In Zn group, both SBP and DBP had an ascending trend with increasing tertile of Zn level. But in Cu/Zn group, the trend was opposite, the value of TG, SBP and DBP decreased as the increasing of Cu/Zn tertiles. There was a significantly ascending trend of TG, FG and DBP in Mg group as the tertile raised. We also assessed the correlation between metals and MetS components indicators. Whole blood copper was positively associated with waist and DBP and negatively associated with TG. There were positive correlations between Zn and TG, FG, SBP and DBP. Opposite to Cu group, the ratio of Cu to Zn was negatively correlated to TG, DBP and SBP. But waist was also positive associated with Cu/Zn. The correlation in Mg was only found in DBP.

Table 2
Distribution of MetS components indicators by tertiles of Cu, Zn, Cu/Zn and Mg

Indexes	Cu (mg/L)				Zn (mg/L)				Cu/Zn			Mg (mg/L)			
	T1 < 0.94	T2 0.94-1.06	T3 > 1.06	P value	T1 < 4.64	T2 4.64-5.70	T3 > 5.70	P value	T1 < 0.17	T2 0.17-0.21	T3 > 0.21	P value	T1 < 38.89	T2 38.89-43.66	T3 > 43.66
N = 911	294	315	302		300	310	301		295	311	305		300	310	301
BMI(kg/m2)	16.16	16.23	16.85	0.004	16.21	16.45	16.58	0.250	16.45	16.48	16.31	0.733	16.25	16.56	16.42
Waist(cm)	55.91	55.21	56.78*	0.046	55.52	55.98	56.36	0.430	55.96	56.36	55.54*	0.444	55.13	56.10	56.64
TG(mmol/L)	0.86	0.78	0.74#	0.011	0.74	0.82	0.82*	0.063	0.91	0.75	0.72#	0.001	0.73	0.85	0.79
HDL-C (mmol/L)	1.28	1.27	1.26	0.085	1.25	1.28	1.28	0.325	1.28	1.28	1.26	0.695	1.26	1.28	1.27
FG(mmol/L)	4.79	4.89	4.88	0.201	4.82	4.81	4.93*	0.062	4.87	4.87	4.82	0.601	4.81	4.80	4.95
SBP(mmHg)	92.27	91.94	93.13	0.412	90.54	92.94	93.83*	0.001	93.86	92.84	90.67#	0.002	91.22	92.84	93.25
DBP(mmHg)	59.28	60.21	61.19*	0.026	58.58	60.95	61.15*	0.001	61.17	60.35	59.21#	0.02	58.98	60.56	61.15*

BMI, body mass index; TG, triglycerides; HDL-C, high density lipoprotein cholesterol; FG, fasting glucose; SBP, systolic blood pressure; DBP, diastolic blood pressure; *, positively correlation; #, negatively correlation.

3.3 Odds ratios for MetS components in tertiles of Cu, Zn, Cu/Zn and Mg

Table 3 summarized the odds ratios (ORs and 95%CI) for MetS components associated with the concentrations of whole blood copper, zinc, the ratio of copper to zinc and magnesium, which were categorized into tertiles. In elevated waist, significant associations were found for copper and Cu/Zn in crude model. After adjusting age, gender and BMI, the multivariable adjusted ORs was 2.00 (1.56–3.89) for Cu and 2.08 (1.22–3.55) for Cu/Zn. No significant correlation were found between zinc and magnesium and the incident of elevated waist. All of the metals were associated with the risk of incident for elevated TG either in crude or adjusted model. There were significant decreases in the risk for elevated TG in T3 group compared with T1 group in Cu and Cu/Zn. The OR and 95% CI for Cu and Cu/Zn was 0.34 (0.17–0.66) and 0.31 (0.15–0.62) respectively in crude model. The trends persisted after adjustment, the OR turned to be 0.33 (0.16–0.65) and 0.35 (0.17–0.71), respectively. However, TG level were inversely associated with zinc and magnesium. The ORs (95%CI) for elevated TG of individuals categorized in Zn and Mg tertile 2 were 2.27 (1.18–4.35) and 2.36 (1.20–4.62), respectively, when no adjusted. The ORs (95%CI) were attenuated but remained statistically significant after adjustment [T2 vs. T1: 2.12 (1.10–4.10) for Zn, 2.34 (1.19–4.61) for Mg]. There was no significant association between metals and hypertension, reduced HDL-C and hyperglycemia.

Table 3
Odds ratios for MetS components according to whole blood metals

Index		Cu				Zn				Cu/Zn				Mg	
		T1	T2	T3	P	T1	T2	T3	P	T1	T2	T3	P	T1	T2
N = 911		294	315	302		300	310	301		295	311	305		300	310
Elevated	n	32	44	70		51	50	45		37	50	59		40	49
Waist	Crude	1	1.32 (0.82–2.16)	2.47 (1.56–3.89)	0.001	1	0.93 (0.61–1.43)	0.85 (0.55–1.32)	0.789	1	1.33 (0.84–2.11)	1.67 (1.07–2.61)	0.053	1	1.22 (0.77–1.91)
	Adjusted	1	1.23 (0.71–2.12)	2.00 (1.18–3.28)	0.001	1	0.78 (0.48–1.28)	0.62 (0.37–1.04)	0.194	1	1.32 (0.77–2.28)	2.08 (1.22–3.55)	0.019	1	1.21 (0.71–2.04)
Hypertension	n	9	7	13		7	8	14		14	10	5		9	9
	Crude	1	0.72 (0.26–1.95)	1.42 (0.59–3.38)	0.334	1	1.10 (0.39–3.09)	2.04 (0.81–5.13)	0.205	1	0.66 (0.29–1.52)	0.33 (0.12–0.94)	0.096	1	0.96 (0.37–2.47)
	Adjusted	1	0.94 (0.33–2.64)	1.95 (0.79–4.82)	0.175	1	0.91 (0.32–2.60)	1.48 (0.70–2.85)	0.513	1	0.83 (0.35–1.93)	0.58 (0.20–1.69)	0.610	1	0.85 (0.32–2.24)
Elevated TG	n	34	20	13		14	31	22		34	21	12		13	30
	Crude	1	0.51 (0.29–0.92)	0.34 (0.17–0.66)	0.002	1	2.27 (1.18–4.35)	1.61 (0.80–3.21)	0.041	1	0.55 (0.31–0.93)	0.31 (0.15–0.62)	0.002	1	2.36 (1.20–4.62)
	Adjusted	1	0.52 (0.28–0.93)	0.33 (0.16–0.65)	0.002	1	2.12 (1.10–4.10)	1.41 (0.70–2.85)	0.066	1	0.57 (0.32–1.02)	0.35 (0.17–0.71)	0.010	1	2.34 (1.19–4.61)
Reduced HDL-C	n	62	62	75		74	66	59		53	73	73		68	64
	Crude	1	0.91 (0.61–1.36)	1.23 (0.84–1.81)	0.281	1	0.82 (0.56–1.20)	0.74 (0.51–1.09)	0.310	1	1.40 (0.94–2.08)	1.43 (0.96–2.13)	0.145	1	0.88 (0.60–1.30)
	Adjusted	1	0.90 (0.61–1.35)	1.23 (0.83–1.82)	0.282	1	0.81 (0.55–1.18)	0.72 (0.49–1.07)	0.266	1	1.40 (0.93–2.09)	1.48 (0.98–2.23)	0.132	1	0.88 (0.60–1.31)
Hyperglycemia	n	25	37	32		37	26	31		25	33	36		31	25
	Crude	1	1.43 (0.83–2.44)	1.27 (0.73–2.21)	0.414	1	0.65 (0.38–1.10)	0.81 (0.49–1.35)	0.277	1	1.28 (0.74–2.13)	1.44 (0.84–2.47)	0.399	1	0.76 (0.43–1.32)
	Adjusted	1	1.57 (0.91–2.70)	1.36 (0.77–2.39)	0.429	1	0.62 (0.36–1.06)	0.75 (0.45–1.26)	0.212	1	1.40 (0.80–2.44)	1.69 (0.97–2.95)	0.177	1	0.73 (0.41–1.27)

TG, triglycerides; HDL-C, high density lipoprotein cholesterol; *P*, *P* for trend; Crude, non-adjust; Adjusted, adjust for age, gender, BMI.

3.4 Odds ratios for MetS components in tertiles of the combination of metals

As the metals were all associated with MetS components, we combined each two of them to detect the joint correlations. In the joint analysis of Cu and Zn, the combination of low Cu and high Zn increased the risk of incident for elevated TG [OR(95%CI) 2.21 (1.18–4.13)], when compared to the joint of low Cu and low Zn. There was no significantly association between MetS components and other Cu and Zn combinations. The correlations were also observed when we considered the interaction of Cu and Mg. High Cu and high Mg were associated with increased risk of elevated waist [OR(95%CI) 2.03 (1.26–3.27)]. But when Mg level was low, the risk of elevated TG was attenuated to 0.40 (0.16–0.95) combined with high Cu. In the joint of Zn and Mg, the significantly correlation could only exist in reduced HDL-C. The trend of ORs were decreased, we observed a negatively association between reduced HDL-C and high Zn with low Mg. And there was also no association between the combination of Zn and Mg and other MetS components.

Table 4
Odds ratios for MetS components according to the combination of metals

Metal	Elevated Waist			Hypertension			Elevated TG			Reduced HDL-C			Hyperglyc
	N	OR	P value	N	OR	P value	N	OR	P value	N	OR	P value	N
Cu + Zn													
Low Cu + Low Zn	38/257	1	0.008	6/257	1	0.224	18/257	1	0.001	58/257	1	0.134	29/257
Low Cu + High Zn	18/196	0.58(0.32–1.05)		7/196	1.54(0.51–4.68)		28/196	2.21(1.18–4.13)		38/196	0.82(0.52–1.31)		15/196
High Cu + Low Zn	34/185	1.29(0.78–2.15)		3/185	0.69(0.17–2.79)		8/185	0.60(0.25–1.41)		51/185	1.30(0.84–2.01)		20/185
High Cu + High Zn	56/273	1.48(0.94–2.33)		13/273	2.09(0.78–5.58)		13/273	0.66(0.31–1.38)		52/273	0.80(0.53–1.22)		30/273
Cu + Mg													
Low Cu + Low Mg	32/275	1	0.016	8/275	1	0.890	23/275	1	0.003	59/275	1	0.730	24/275
Low Cu + High Mg	24/178	1.18(0.67–2.08)		5/178	0.96(0.31–2.99)		23/178	1.62(0.88–2.99)		37/178	0.96(0.66–1.52)		20/178
High Cu + Low Mg	35/198	1.63(0.97–2.73)		8/195	1.40(0.51–3.81)		7/195	0.40(0.16–0.95)		49/195	1.20(0.78–1.85)		18/195
High Cu + High Mg	55/260	2.03(1.26–3.27)		8/260	1.06(0.39–2.86)		14/260	0.62(0.31–1.24)		54/260	0.96(0.63–1.45)		32/260
Zn + Mg													
Low Zn + Low Mg	44/306	1	0.319	6/306	1	0.103	18/306	1	0.301	83/306	1	0.017	32/306
Low Zn + High Mg	28/136	1.12(0.27–4.57)		3/136	1.12(0.27–4.57)		8/136	1.00(0.42–2.36)		26/136	0.63(0.38–1.04)		17/136
High Zn + Low Mg	23/167	3.18(1.13–8.92)		10/167	3.18(1.13–8.92)		12/167	1.23(0.58–2.63)		25/167	0.47(0.28–0.77)		10/167
High Zn + High Mg	51/302	1.71(0.61–4.77)		10/302	1.71(0.61–4.77)		29/302	1.70(0.92–3.13)		65/302	0.73(0.50–1.07)		35/302

TG, triglycerides; HDL-C, high density lipoprotein cholesterol; *P* value, *P* for trend; Low means less than 50% concentration; High means greater or equal to 50% concentration.

4. Discussion

In this study, we explored whether the level of metals within normal reference ranges would affect the risk of metabolic syndrome components. The present findings in this study provides evidence that whole blood Cu and the ratio of Cu to Zn were both positively associated with a higher odds ratio of elevated waist and negatively associated with a higher OR of elevated TG either in crude or adjusted model. We also observed that whole blood Zn and Mg were

positively associated with the risk of elevated TG. We have not only analyzed the function of single element, but also made a preliminary attempt of multi-element joint analysis. With regard to the interaction of metals, high Cu with high Mg increased the risk of elevated waist. Exposure to low level of Cu and high level of Zn, the risk of elevated TG was much higher than the other combination of this two metals, whereas low level of Cu and high level of Mg was inversely negative associated with elevated TG. The joint of high Zn and low Mg was also decreased the risk of reduced HDL-C.

In our study population, we assessed both the single and combined effects of copper and zinc on MetS components. When it was first recognized by Hart et al[18] in 1928, Cu is a essential metal for normal growth and development. With the further research, people find the two most important functions of copper. One function is as an electron transfer intermediate in redox reactions[19]. It has a high level of oxidation and could lead to excessive damaging reactive oxygen species (ROS) by redox reaction[20]. And ROS is a main factor to induce insulin resistance. A number of copper-containing proteins display oxidative reductase activity. Thus Cu is an essential cofactor in oxidative and reductase enzymes[21], such as superoxide dismutase and ceruloplasmin. And also Zn is a an integral component of a large number of proteins and enzymes. It could take part in a variety of metabolic processes. And some researches have indicated that zinc overload would lead to Cu deficiency and reduced HDL-C[22]. As a consequence, the interaction of copper and zinc should not be separated, they would be residual confounding factor for each other. This suggests that we need to break the previous analysis of single elements and consider the interactions among these metals.

The concentrations of copper and zinc in the whole blood of our study were all in the normal range(Cu 0.61-1.9mg/L, Zn 3.1-9.8mg/L), referring to the study of Swedish adolescents[23]. We observed a higher level within the normal range of whole blood copper lead to increase risk of elevated waist, which was a representative of obesity. In agreement with our result, Catherine et al[24] also observed positive relationship of serum copper with abdominal obesity. We also observed that the risk of elevated TG was decreased with the tertiles of Cu. The effects of copper on blood lipid metabolism is certain, but the effect on each index of lipid metabolism is still inconsistent. Some studies suggested that serum copper level was positively associated with total cholesterol, TG or low density lipoprotein, such as the study in Kuwaitis 15-80 years people[25] and in American young adults[26]. But in the study of 2011-2014 NHANES, there was no association between serum copper and TG in children and adolescent[27]. By contrast, in the study of Leslie et al[28], the increased concentration of total cholesterol and a reduction of HDL cholesterol were observed in subjects with a low copper, which may be consistent with our results.

In the current study, although the concentration of Zn was in the normal range, we still observed the tertiles of Zn was positively associated with risk of elevated TG, which was in agreement with the report of Ghasemi et al.[29] conducted in Tehran's urban population(OR=1.60) and Jin-A et al.[30] in Korean adults(OR=1.47). It has been confirmed by Sukalski et al.[31] that the reduced serum Cu concentration would lessens Cu/Zn superoxide dismutase activity. And the Cu/Zn ratio is used to determine the level of oxidative stress. In this study, all the value of Cu/Zn were less than 1.0, which indicated the antioxidant mechanism in this population was normal[32]. And the correlations of Cu/Zn with elevated waist and elevated TG were consisted with Cu, which also observed in the study of Monika Fedor et al.[33]. With regard to the combination of Cu and Zn, we found low level of Cu combined with high level of Zn would increased the risk of elevated TG, which would provide suggestions to regulate TG levels with the joint of this two metals.

Mg is also a coenzyme, which would affect the metabolism of glucose and lipid. Unlikely with some reports indicated that Mg supplement decrease the TG and blood pressure[34], we observed an increase risk of elevated TG in the second tertile of Mg. There are also some studies about that Mg supplement could significantly decreased the risk of MetS[35,36], which means low level of Mg may effect the incidence of some MetS components. In the combination of high level of Zn and low level of Mg, the incidence of reduced HDL-C was increased. And high value of Cu with low value of Mg could decreased the risk of elevated TG, which was consisted with the function of single metal. When the value of Cu and Mg were both in a high level, the risk of elevated waist was raised. This was in agreement with a cross-section study conducted by Beydoun et al.[37].

In this study, there have some strengths. It is the first one to assess the relationship between MetS components and Cu, Zn, Mg, both individually and jointly among Chinese rural children. And the study population also have a national representative. Secondly, this study evaluated the metals biological function in whole blood samples. Different from the commonly used serum and plasma samples, some studies show that the whole blood can reflect more comprehensively the long-term status of metals in the body and may not be affected by current dietary intake[38,39]. Thirdly, considering the interaction between elements, we break the previous analysis of single element, and try to analyze the joint action of multiple metals. This provides data support for the future research on the joint use of multiple elements. The fourth one is about the study population. We conducted the research among 6-12 years old children in Chinese rural. Although the relationship of metabolic syndrome in children and adolescents has attracted more and more attention in recent years, there are still few studies involving such a low age group.

In addition to the advantages, the limitations of our study need to be mentioned. Firstly, based on the physiological functions of copper and zinc, they could influence the process of inflammation. However, we could not adjust the inflammatory indicators, such as C-reaction protein, as a confounding factor in the analysis because it was not test in this study. The second one is the lack of dietary factors. Dietary factors have a great influence of these metals. Thus, we could only get the long-term association between these three metals but not generalized a comprehensive correlation between Cu, Zn, Mg and MetS components. Due to the relatively small age of the sample population, the prevalence of metabolic syndrome is also very low, so the relationship between the metals and the components of MetS was discussed instead.

Conclusions

In conclusion, higher level of whole blood Cu and Cu/Zn were positively associated with the risk of elevated waist and negatively associated with the risk of elevated TG. Additionally, higher Zn and Mg level increased the risk of elevated TG. The combination of each two metals could give suggestions to regulate the conditions of elevated waist, elevated TG and reduced HDL-C. In the future research, we will conduct a prospective cohort with more diet and genetic factors to better study the relationship between trace elements and metabolic syndrome.

Declarations

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of Institute for Nutrition and Food Safety, China CDC (now change to National Institute for Nutrition and Health, China CDC) (file number 2013-018).

Consent for publication

The authors consent to the publication of the data.

Availability of data and materials

The datasets used or analysed during the current study are available from the corresponding author Lichen Yang on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Author Contributions

H.Z. and Q.M. wrote the paper and analyzed data; P.S., S.L. and Y.Q. provided essential materials; X.L. conducted research; L.W. performed statistical analysis; L.Y. designed research and had primary responsibility for final content. All authors read and approved the final manuscript.

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List Of Abbreviations

MetS: metabolic syndrome; AAP: American Academy of Pediatrics; IDF: International Diabetes Federation; BMI, body mass index; TG, triglycerides; HDL-C, high density lipoprotein cholesterol; FG, fasting glucose; SBP, systolic blood pressure; DBP, diastolic blood pressure;

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