ORIGINAL RESEARCH ARTICLE

Association of serum levels of zinc and copper with pathophysiological features of polycystic ovary syndrome

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Abstract

Trace elements may play a role as initiating environmental factors in polycystic ovary syndrome (PCOS). This study was designed to assess the relationship between circulating levels of zinc (Zn) and copper (Cu) and the pathophysiological features of PCOS**.** A cross-sectional study was conducted. Clinical data were recorded and blood samples were taken to analyze biochemical and hormonal parameters. The subgroup of participants defined with abnormal background had body mass excess, insulin resistance, or subclinical inflammation. In PCOS patients with insulin resistance, elevated Cu, Zn, and Cu/Zn ratio levels were observed. However, the Zn/Cu ratio was lower in those with body mass excess and subclinical inflammation. Significant correlations within PCOS patient subgroups, linking trace elements to anthropometric, metabolic, inflammatory, and hormonal variables, were revealed. Interactions of trace elements in PCOS pathophysiology are likely complex. Hence, supplementation, if considered, should be approached cautiously. *(Afr J Reprod Health 2026; 30 [9]: 14-25).*

Keywords: polycystic ovary syndrome, zinc, copper, insulin resistance, body mass, subclinical inflammation

Résumé

Les éléments traces peuvent figurer parmis les facteurs environnementaux initiateurs du syndrome des ovaries polykystiques (SOPK). L'objectif de ce travail était d'évaluer la relation entre les caractéristiques physiopathologiques du SOPK et les taux circulants de zinc (Zn) et de cuivre (Cu). Une étude cas-témoin a été menée. Des données cliniques ont été receuillies et des prélèvements sanguins ont été realisés. Les sous-groupes de participantes definis avec un background anormal avaient un excès-pondéral, une insulinorésistance, ou une inflammation-infraclinique. Chez les patientes porteuses de SOPK avec insulinoresistance, des taux élevés de Cu, Zn, et du rapport Zn/Cu ont été observés. Cependant, le rapport Zn/Cu était plus bas chez celles avec excès-pondéral et inflammation-infraclinique. Dans le SOPK, des correlations ont été révélées entre les elements traces et certaines caractéristiques anthropométriques, métaboliques, inflammatoires, et hormonales. L'intervention des elements traces dans la physiopathologie du SOPK semblerait être complexe. Une supplementation, si considérée, devrait être prudente. *(Afr J Reprod Health 2026; 30 [9]: 14-25).*

Mots-clés: syndrome des ovaires polykystiques, zinc, cuivre, résistance à l'insuline, indice de masse corporelle, inflammation-infraclinique

Introduction

Polycystic ovary syndrome (PCOS) is a prevalent endocrinopathy among reproductive-age women, characterized by various symptoms such as hirsutism, menstrual irregularities, and infertility.[1,2] Diagnosis typically involves identifying the association of oligo-anovulation, hyperandrogenism, and/or polycystic ovaries via ultrasound. Despite numerous hypotheses, the exact pathophysiology of PCOS remains elusive, encompassing factors beyond ovarian androgen hypersecretion and gonadotropin dysfunction, including body mass excess (BME), insulin resistance (IR), and subclinical inflammation (SCI), which collectively contribute to a self-reinforcing

cycle.[3–5] Additionally, environmental conditions are suggested to influence genetic traits by modulating their expression.[6,7]

Among environmental factors, trace elements, particularly zinc (Zn) and copper (Cu), have been implicated[8,9] These elements, ranked as the second and third most abundant essential trace elements in the human body, play pivotal roles in enzymatic reactions, oxidative responses, cytokine production, and hormonal signaling pathways (gonadotropin-releasing hormone, androgens, insulin). They also influence oxidative stress, apoptosis, and cardiovascular disease.[10,11] Some authors have postulated that disturbances in trace element metabolism could contribute to the onset of PCOS and related comorbidities.[8,11,12]

Numerous studies have explored the circulating levels of Zn and Cu in PCOS, yielding conflicting results regarding the direction of variation.[13,14] These disparities may stem from differences in the metabolic and inflammatory statuses of the participants. Additionally, some authors have examined the effects of Zn supplementation in PCOS, regardless of inflammatory and metabolic conditions.[15,16] Conflicting outcomes have been reported regarding hormonal and metabolic profiles, underscoring the contentious nature of the precise intervention mechanism of trace elements.

This study aimed to evaluate the relationship between serum levels of trace elements (Zn, Cu) and the pathophysiological features of PCOS namely body mass excess (BME), insulin resistance (IR), and subclinical inflammation (SCI).

Patients and methods

A cross-sectional study was conducted in hospitals in southern Tunisia, including Habib Bourguiba Hospital and Hedi Chaker Hospital. PCOS patients were enrolled from dermatology, gynecology, and endocrinology outpatient services. They were diagnosed based on at least two of the three Rotterdam criteria: oligo/anovulation, hyperandrogenism, and/or polycystic ovaries on ultrasound.[17] Controls were selected from the patients' acquaintances and individuals accompanying them to the hospital. They were age-paired to patients with PCOS. The exclusion criteria were medications acting on glucose or lipid metabolisms, and Zn or Cu supplements. Anthropometric measurements were conducted on women dressed in lightweight attire without shoes and Body mass index (BMI) was calculated using the formula: BMI = (weight (kg)) /(height (m))^2. BME was defined as BMI> 25 kg/m^2.[18] Waist circumference (WC) was measured from the midpoint between the last rib and hip, while hip circumference was measured at the level of the greater trochanter. All measurements and physical evaluations were performed by the same physician.

Blood samples were taken on the 3rd to the 5th day of the menstrual cycle, between 8 and 10 am. Participants were instructed to observe a 12-hour fast while maintaining an unrestricted diet. The measurement of glucose and high sensitive C-reactive protein (hsCRP) were measured using COBAS-e501 (Roche Diagnostics, Penzberg, Germany). Insulin, gonadotrophins (follicle stimulating hormone FSH, luteinizing hormone LH), antimullerian hormone (AMH), estradiol (E2), and testosterone (Testo) were performed on COBAS-e601 (Roche Diagnostics, Penzberg, Germany). Androstenedione (A4), dehydroepiandrosterone sulfate (DHEA-S), and 17-hydroxyprogesterone (17-OH-prog) were assessed using an ELISA kit (DRG, Germany). All assays were performed at the Department of Biochemistry of Sfax Hospital-Tunisia.

Homeostasis HOMA-IR (Homeostatic Model Assessment for Insulin Resistance) was calculated as fasting glucose (mmol/L) × fasting insulin (mU/L)/22.5. IR was likely at HOMA-IR > 2.5.[19] SCI was defined by hsCRP levels above 9.52 nmol/L (1 mg/L).[20] Hence, participants were categorized into subgroups based on BMI status lean/BME, insulin sensitivity/resistance profile, and the absence/presence of SCI. Individuals with BME, IR, or SCI were designated as having an "abnormal background".

Plasma trace elements were determined by an atomic absorption spectrophotometer (AAS) (Thermo scientific iCE 3000 series®, USA) at LARSEN—National Engineering School, Sfax, Tunisia. Specific wavelengths were used for copper: 324.7 nm, and for zinc: 213.8 nm. Zn and Cu Standards employed were Single element solutions for AAS with references J/8070/05 and J/8025/05, respectively (Fisher Scientific Co.,

Pittsburgh, Pa.15219). Samples underwent a dilution process (1+9 dilution of 500 µL of serum with analytical grade butan-1-ol 6%).[21, 22]

Concentrations of both metals were expressed in µmol/L. The molar ratio of Zn/Cu was then calculated. For trace elements and all other parameters, Internal and external scheme Biorad quality controls were used: Lyphocheck Assayed Chemistry Ref C310-5 and C315-5, Lyphocheck ImmunoAssay Control Ref: 370, External Quality Assurance Services (EQAS) Clinical Chemistry Monthly Program Ref BC50, and EQAS ImmunoAssay Monthly Program Ref: BC75.

Statistical analysis

The data were processed using the statistical package SPSS 25. Variables were expressed as mean +/-standard deviation or median (interquartile range). Normality was assessed by the Shapiro-Wilk test. Means were compared by student test or Mann-Whitney U Test depending on the variable distribution. Frequencies were checked by the X_2test. Pearson or Spearman correlation was applied in terms of distribution. The level of significance for rejecting the null hypothesis was $P < 0.05$.

Ethical considerations

This study was approved by the Southern Tunisian Ethics Committee «CPP SUD N°0197/2019». All participants gave informed written consent before being included ensuring confidentiality and full right protection.

Results

The basal characteristics of both PCOS patients and controls are summarized in Table 1. PCOS patients had significantly higher levels of BMI, WC, hsCRP, LH, LH/FSH, AMH, and steroids. However, there were no notable differences in glucose-insulin homeostasis and trace element profiles between the two groups (Table 1).

Table 1: Basal characteristics of PCOS patients and controls

	Unit	Controls 111	PCOS 117	p
Age	years	26.0 (22-31)	26.7 (22-29)	0.081
BMI	kg/m²	23.9 (21.3-27.3)	25.2 (221.1-29.8)	0.027
WC	cm	87.2 +/- 13.5	91.1 +/- 13.1	0.034
FSH	mUI/mL	6.3 (5.4-8.0)	6.0 (5.0-7.0)	0.081
LH	mUI/mL	5.7 (4.4-6.9)	7.4 (5.7-9.8)	<0.0001
LH/FSH	-	0.91 (0.63-1.14)	1.25 (0.97-1.61)	<0.0001
AMH	pmol/L	18.5 (11.5-29.8)	33.8 (20.6-45.5)	<0.0001
E2	pmol/L	123.3 (40.4-171.4)	148.6 (98.0-183.9)	0.009
Testo	nmol/L	0.85 (0.58-1.04)	1.11 (0.76-1.49)	<0.0001
17OHprog	nmol/L	0.2 (0.1-0.3)	0.3 (0.2-0.4)	0.016
A4	nmol/L	6.6 (3.6-7.5)	7.3 (5.6-10.5)	<0.0001
DHEA-S	µmol/L	5.2 +/- 2.2	6.1 +/- 2.8	0.014
Insulin	pmol/L	66.3 (48.2-102.1)	71.9 (55.5-111.4)	0.103
Glucose	mmol/L	5.00 (4.6-5.4)	5.02 (4.6-5.4)	0.612
HOMA-IR		2.09 (1.47-3.27)	2.19 (1.73-3.67)	0.104
hsCRP	nmol/L	9.4 (4.9-21.2)	13.7 (5.9-36.3)	0.022
Cu	µmol/L	14.3 (12.5-16.9)	14.1 (14.1-17.5)	0.749
Zn	µmol/L	11.6 +/- 1.7	11.7 +/- 1.9	0.785
Zn/Cu		0.80 (0.67-0.99)	0.84 (0.66-0.97)	0.665

17OHprog: 17-hydroxyprogesterone, A4: androstenedione, BMI: body mass index, Cu: copper, DHEA-S: dehydroepiandrosterone-sulfate,E2: estradiol, FSH: follicle stimulating hormone, HOMA-IR: Homeostatic Model Assessment for Insulin Resistance, hsCRP: high sensitive C-reactive protein, LH: luteinizing hormone, PCOS: polycystic ovary syndrome, Testo: testosterone, WC: waist circumference, Zn: zinc, Zn/Cu: Zinc to copper ratio.

Table 2: clinical and biochemical studied characteristics of controls according to normal/abnormal background

Unit		BMI<25kg/m2	BMI>25kg/m2	HOMA-IR<2.5	HOMA-IR>2.5	HsCRP<9.5nmol/L	HsCRP>9.5nmol/L
Number (%)		69 (62.2)	42 (37.8)	70 (63.1)	41 (36.9)	58 (52.2)	53 (47.7)
Age	years	25.0 (22.0-28.0)[a]	30.5 (22.0-36.2)[a,c]	24.5 (22.0-30.2)	26.0 (23.0-30.7)	24.5 (22.0-34.0)	25.0 (22.0-34.0)
BMI	Kg/m²	22.8 (20.2-23.4)[a]	24.4 (26.9-31.6)[a]	22.8 (20.6-27.6)	23.9 (21.5-27.0)[c]	22.8 (20.6-24.8)[a]	25.7 (22.5-30.7)[a]
WC	cm	83.6 +/- 11.4[a]	91.0 +/- 15.0[a,c]	84.4 +/- 11.7[a]	91.2 +/- 15.7[a,c]	83.6 +/- 11.4[a,b]	91.0 +/- 15.0[a,c]
FSH	mUI/mL	6.6 (5.4-8.1)[b]	6.3 (5.6-7.8)	6.1 (5.3-8.0)	6.7 (5.6-7.8)	6.3 (5.6-7.7)[b]	6.6 (5.3-7.8)
LH	mUI/mL	6.4 (4.7-7.2)[a,b]	4.7 (4.1-6.5)[a,c]	5.0 (4.2-6.6)[b]	6.5 (4.7-7.3)[c]	6.1 (4.5-7.5)[b]	5.3 (4.2-6.8)[c]
LH/FSH		0.94 (0.70-1.21)[b]	0.78 (0.58-1.01)[c]	0.83 (0.61-1.14)[b]	0.96 (0.70-1.21)[c]	0.95 (0.67-1.21)[b]	0.89 (0.61-1.13)[c]
AMH	pmol/L	20.3 (12.6-20.3) [b]	17.6 (9.4-23.8) [c]	19.3 (8.5-29.0) [b]	18.3 (12.2-31.3) [c]	16.5 (9.8-27.9) [b]	21.5 (12.3-30.3) [c]
E2	poml/L	137.6 (43.7-193.0)[a]	99.8 (37.8-154.7)[a,c]	77.2 (33.9-142.5)[b]	146.1 (94.0-195.1)	142.7 (34.8-187.6)	107.5 (40.4-143.9)[c]
Testo	nmol/L	0.87 (0.62-1.06)[b]	0.73 (0.52-0.92)[c]	0.71 (0.51-1.04)[b]	0.87 (0.66-1.07)[c]	0.80 (0.58-1.07)[b]	0.73 (0.59-0.97)[c]
17OHP	nmol/L	0.3 (0.2-0.3)[b]	0.2(0.1-0.3)	0.2 (0.1-0.3)[a,b]	0.3 (0.2-0.3)[a]	0.2 (0.1-0.3)	0.2 (0.1-0.3)[c]
A4	nmol/L	6.8 (4.6-7.8)[b]	6.3 (3.5-7.0)[c]	6.9 (3.5-7.2)[b]	6.4 (4.6-7.5)[c]	6.9 (4.4-7.8)	6.4 (3.5-6.9)[c]
DHEA-S	µmol/L	5.5 +/-2.1	4.8 +/-2.4[c]	4.8 +/-2.2[b]	5.5 +/-2.1	5.4 +/- 2.3	5.1 +/- 2.1
HOMA-IR		1.99 (1.38-2.96)	2.04 (1.60-3.67)	1.40 (1.07-1.67)[a]	3.00 (2.36-4.29)[a]	2.25 (1.49-3.18)	1.92 (1.48-4.07)
Glucose	mmol/L	5.0 (4.6-5.4)	5.0 (4.5-5.4)	4.7 (4.36-5.2)[a]	5.3 (5.0-5.7)[a]	5.2 (4.6-5.5)	5.1 (4.8-5.3)
hsCRP	nmol/L	6.7 (4.5-14.4)[a]	14.6 (8.5-24.5)[a, c]	9.12 (5.4-19.3)	7.0 (4.8-19.7)[c]	5.3 (4.1-7.2)[a]	21.7 (14.3-37.3)[a]
Cu	µmol/L	13.5 (11.0-15.3) [a]	15.7 (12.9-18.6) [a]	15.0 (12.9-16.8)	13.4 (11.2-16.4)	13.8 (11.0-16.2)[a]	15.3 (13.0-17.1)[a]
Zn	µmol/L	11.5 +/- 1.4	11.6 +/-2.1	11.2 +/- 1.6[a]	11.9 +/- 1.7[a]	11.5 +/-1.6	11.6 +/-1.8
Zn/Cu		0.85 (0.72-1.04)[a]	0.74 (0.59-0.93)[a]	0.74 (0.67-0.87) [a]	0.86 (0.70-1.10) [a]	0.86 (0.71-1.15)[a]	0.75 (0.64-0.89)[a]

17OHprog: 17-hydroxyprogesterone, A4: androstenedione, BMI: body mass index, Cu: copper, DHEA-S: dehydroepiandrosterone-sulfate, E2: estradiol, FSH: follicle stimulating hormone, HOMA-IR: Homeostatic Model Assessment for Insulin Resistance, hsCRP: high sensitive C-reactive protein,LH: luteinizing hormone, PCOS: polycystic ovary syndrome, Testo: testosterone, WC: waist circumference, Zn: zinc, Zn/Cu: Zinc to copper ratio

[a]: significant difference between controls with normal/abnormal background

[b]: significant difference between controls and PCOS patients with normal background

[c]: significant difference between controls and PCOS patients with abnormal background

Table 3: clinical and biochemical studied characteristics of PCOS patients according to normal/abnormal background

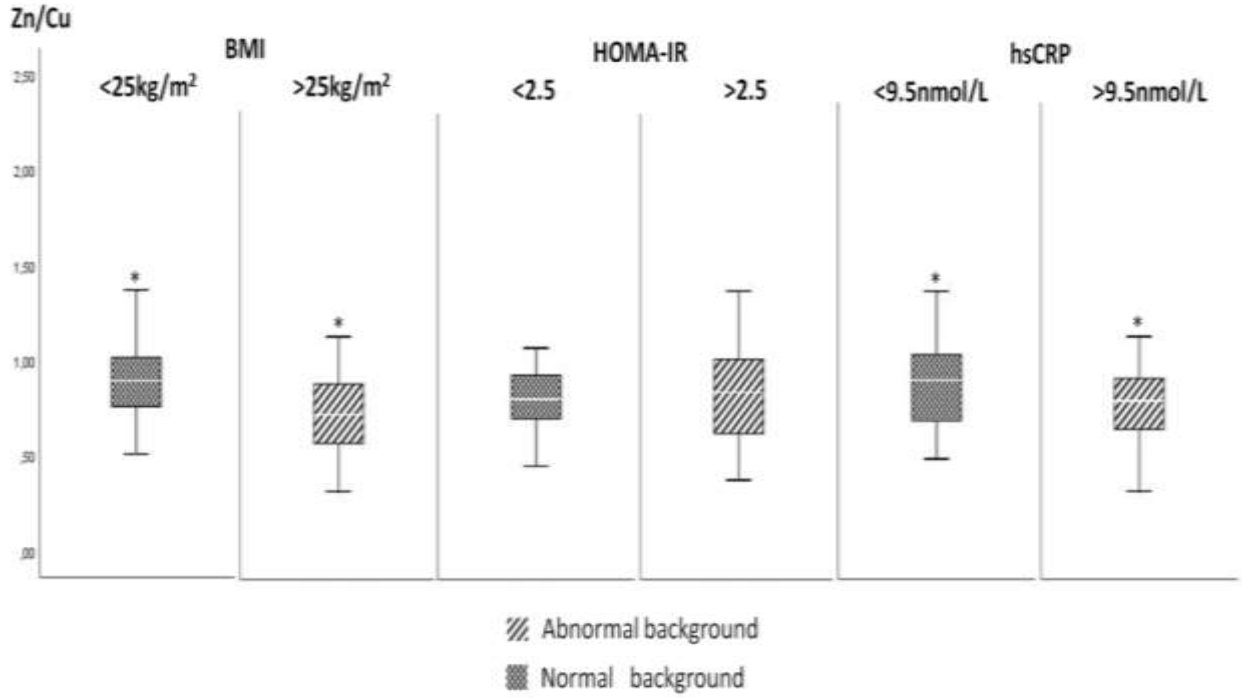
Unit		BMI<25kg/m2	BMI>25kg/m2	HOMA-IR<2.5	HOMA-IR>2.5	HsCRP<9.5nmol/L	HsCRP>9.5nmol/L
Number (%)		58 (49.6)	59 (50.4)	69 (59.0)	48 (41.0)	43 (36.7)	74 (63.2)
Age	years	25.0 (22.0-29.0)	25.0 (21.5-28.0)[c]	25.0 (21.5-29.5)	25.0 (22.0-28.0)	23.0 (21.0-25.0)	25.0 (22.0-29.2)
BMI	Kg/m²	22.1 (20.8-23.8)[d]	29.7 (27.3-32.1)[d]	24.2 (21.0-28.6)[d]	26.8 (23.6-30.1)[c,d]	23.7 (21.0-26.8)[d]	27.5 (24.3-31.4)[d]
WC	cm	85.2 +/- 12.0[d]	94.0 +/- 12.3[d,c]	86.3 +/- 11.7[d]	96.5 +/- 12.0[c,d]	85.2 +/- 12.0[b,d]	94.0 +/- 12.3[c,d]
FSH	mUI/mL	5.8 (4.9-6.5)[b]	6.2 (5.2-7.2)	6.0 (5.0-8.1)	5.7 (4.8-6.6)	5.6 (4.5-6.4)[b]	5.9 (5.1-7.4)
LH	mUI/mL	7.8 (6.3-9.8)[b,d]	6.5 (5.0-9.9)[c,d]	6.7 (5.3-8.2)[b]	7.3 (5.8-11.0)[c]	7.8 (5.1-9.9)[b]	7.2 (5.9-10.8)[c]
LH/FSH		1.37 (1.06-1.82)[b,d]	1.21 (0.73-1.51)[c,d]	1.07 (0.78-1.48)[b]	1.35 (1.06-1.96)[c]	1.51 (1.06-1.96)[b]	1.24 (0.91-1.60)[c]
AMH	pmol/L	38.1 (23.9-47.2)[b,d]	28.6 (15.0-38.8) [c,d]	29.8 (18.6-41.7) [b]	36.9 (23.3-47.2) [c]	39.9 (25.5-47.9) [b,d]	29.6 (18.7-40.7) [c,d]
E2	poml/L	148.3 (100.7-184.4)[d]	143.9 (89.0-183.1)[c,d]	112.3 (87.3-162.0)[b]	152.8 (112.2-194.6)	116.3 (85.1-164.0)	149.2 (109.6-184.2)[c]
Testo	nmol/L	0.97 (0.75-1.43)[b]	1.02 (1.02-1.61)[c]	1.28 (0.80-1.58)[b]	1.09 (0.73-1.66)[c]	1.07 (0.78-1.42)[b]	1.25 (0.75-1.71)[c]
17OHP	nmol/L	0.3 (0.3-0.4)[b]	0.3 (0.2-0.4)	0.4 (0.3-0.4)[b]	0.3 (0.2-0.4)	0.3 (0.2-0.4)	0.3 (0.2-0.4)[c]
A4	nmol/L	7.4 (5.7-10.1)[b]	7.2 (5.6-10.5)[c]	10.1 (6.3-10.5)[b]	7.2 (5.4-8.8)[c]	7.6 (6.7-10.1)	7.1 (5.1-9.9)[c]
DHEA-S	µmol/L	6.1 +/-2.8	6.1 +/-3.0[c]	6.2 +/ - 3.0[b]	5.9 +/- 2.7	5.7 +/- 2.8	6.2 +/- 2.9
HOMA-IR		2.17 (1.70-2.65)	2.80 (1.82-4.42)	1.70 (0.99-1.88)[d]	3.16 (2.31-4.24)[d]	2.46 (1.99-3.71)	2.45 (1.72-4.11)
Glucose	mmol/L	5.0 (4.6-5.4)	5.0 (4.7-5.5)	4.8 (4.4-5.2)[d]	5.1 (4.8-5.6)[d]	4.9 (4.7-5.5)	5.1 (4.7-5.4)
hsCRP	nmol/L	8.0 (4.7-14.8)[d]	27.6 (12.0-44.3)[d, c]	14.5 (5.9-32.6)	13.4 (5.9-37.1)[c]	5.7 (3.9-6.9) [d]	30.0 (14.0-56.8) [d]
Cu	µmol/L	12.9 (11.3-15.3)[d]	16.0 (13.3-19.8)[d]	13.7 (11.5-15.8)[d]	14.7 (12.5-18.0)[d]	12.8 (11.2-15.3)[d]	14.0 (12.4-17.8)[d]
Zn	µmol/L	11.8 +/-1.9	11.7 +/-1.9	11.4 +/- 1.8[d]	12.3 +/- 1.9[d]	11.8 +/- 1.7	11.7 +/- 2.0
Zn/Cu		0.89 (0.76-1.02)[d]	0.72 (0.57-0.89)[d]	0.80 (0.70-0.93) [d]	0.84 (0.62-1.02) [d]	0.91 (0.69-1.04)[d]	0.79 (0.64-0.91)[d]

17OHprog: 17-hydroxyprogesterone, A4: androstenedione, BMI: body mass index, Cu: copper, DHEA-S: dehydroepiandrosterone-sulfate, E2: estradiol, FSH: follicle stimulating hormone, HOMA-IR: Homeostatic Model Assessment for Insulin Resistance, hsCRP: high sensitive C-reactive protein,LH: luteinizing hormone, PCOS: polycystic ovary syndrome, Testo: testosterone, WC: waist circumference, Zn: zinc, Zn/Cu: Zinc to copper ratio

[b]: significant difference between controls and PCOS patients with normal background

[c]: significant difference between controls and PCOS patients with abnormal background

[d]: significant difference between PCOS patients with normal and abnormal background



BMI: body mass index, HOMA-IR: Homeostatic Model Assessment for Insulin Resistance, hsCRP: high sensitive C-reactive protein, Zn: zinc, Zn/Cu: Zinc to copper ratio
*: significant difference between PCOS patients with normal and abnormal background

Figure 1: Zn/Cu ratio levels in PCOS patients according to normal/abnormal background

Upon subdividing participants based on background status (normal/abnormal), a significant elevation in WC was identified in abnormal backgrounds. In PCOS patients with BME and SCI, AMH was significantly lower and hsCRP significantly higher than those with normal background (Table 2 and 3)

Table 2 and 3 also highlights elevated Cu levels in PCOS patients with IR and all participants with BME and SCI. Zn levels were elevated exclusively in those with IR. Furthermore, the Zn/Cu ratio was higher in those with IR and lower in those with BME and SCI (Table 2 and 3).

Significant correlations within PCOS patients, linking trace elements to anthropometric, metabolic, inflammatory, and hormonal variables, are detailed in Table 4. There were significant correlations of Cu and Zn/Cu ratio levels with age, BMI, and WC. Despite observed positive correlations of insulin with Cu and Zn in PCOS patients, no correlation was noted with Zn/Cu ratio. The relationship observed for Zn/Cu ratio levels with LH/FSH, Testo, and DHEA-S was stronger than those of Cu or Zn taken alone (Table 4). Further exploration of correlations in PCOS patients with an abnormal background revealed positive associations between Zn/Cu ratio with LH/FSH and androgens (Testo, A4, DHEA-S). In this context, Testo and DHEA-S were positively correlated with Zn. In BME and SCI, Zn levels were positively associated with insulin and HOMA-IR (Table 5). Moreover, AMH exhibited a negative correlation with Cu and a positive one with Zn/Cu in PCOS patient subgroups with IR and those without BME and SCI (Table 5). Significant correlations were observed between Zn/Cu ratio levels and the LH/FSH ratio in PCOS patients, regardless there background (Figure 2). In contrast, AMH and testo levels showed significant correlations with Zn/Cu ratio levels only in specific categories (Figures 3 and 4).

Table 4: correlations in PCOS patients with trace elements

		Cu	Zn	Zn/Cu
Age	r	0.244	-	-0.256
	p	0.008	-	0.006
BMI	r	0.459	-	-0.389
	p	<0.0001	-	<0.0001
WC	r	0.393	-	-0.389
	p	<0.0001	-	<0.0001
Glucose	r	0.209	-	-
	p	0.023	-	-
Insulin	r	0.205	0.294	-
	p	0.005	0.001	-
HOMA-IR	r	0.269	0.312	-
	p	0.003	0.001	-
hsCRP	r	0.340	-	-0.281
	p	<0.0001	-	0.002
FSH	r	0.268	-	0.221
	p	0.003	-	0.016
LH	r	-	0.253	0.236
	p	-	0.006	0.01
LH/FSH	r	0.206	0.210	0.317
	p	0.026	0.022	0.001
AMH	r	-0.223	-	0.187
	p	0.005	-	0.02
E2	r	-	0.275	-
	p	-	0.003	-
Testo	r	-0.207	0.222	0.307
	p	0.025	0.016	0.001
17OHprog	r	-0.283	-	0.276
	p	0.003	-	0.004
A4	r	-0.331	-	0.230
	p	0.001	-	0.020
DHEA-S	r	-0.248	0.253	0.327
	p	0.012	0.01	0.001

17OHprog: 17-hydroxyprogesterone, A4: androstenedione, BMI: body mass index, Cu: copper, DHEA-S: dehydroepiandrosterone-sulfate, E2: estradiol, FSH: follicle stimulating hormone, HOMA-IR: Homeostatic Model Assessment for Insulin Resistance, hsCRP: high sensitive C-reactive protein, LH: luteinizing hormone, PCOS: polycystic ovary syndrome, Testo: testosterone, WC: waist circumference, Zn: zinc, Zn/Cu: Zinc to copper ratio.

Discussion

Our results revealed various connections between trace elements and hormonal, metabolic, and inflammatory profiles in PCOS and these were expressed in the presence of coexisting abnormal backgrounds. Given the mutually antagonistic nature of Zn and Cu metabolisms, the Zn/Cu ratio emerges as more informative than the independent levels of Zn or Cu.[11,12] In our study, the positive correlations observed between the Zn/Cu ratio and LH/FSH ratio strongly suggest a central effect of trace elements on the gonadotropic axis. Cu has been reported to promote anovulatory menstruation by interacting with gonadotropic axis signaling pathways.[23]

However, among patient subgroups without BME or SCI, AMH levels were elevated and exhibited a significant positive correlation with Cu and Zn/Cu ratio. A similar relationship was observed in PCOS patients with IR. This brings to mind the median levels of the Zn/Cu ratio among normal and abnormal subgroups. A close interconnection between AMH effects and intrafollicular trace elements has been previously suggested.[24] We propose a tight interaction of trace elements with PCOS pathophysiology based on normal or abnormal background.

Zn has been reported to participate in oocyte maturation and inhibit follicular differentiation and recruitment in animal models.[25,27] Through intracellular signaling pathways, Zn induces apoptosis, one of the hypothetical models reported in PCOS.[28] Moreover, in animal models, elevated intrafollicular levels of zinc could be induced by testosterone.[29] Positive correlations between androgens and the Zn/Cu ratio were observed in PCOS subgroups with abnormal backgrounds. If favorable effects of Zn supplementation were reported on the metabolic profile in PCOS, the effect on steroid hormones would be worse.[30]

In BME and SCI, we observed significantly lower Zn/Cu ratios. The pro-inflammatory state, a commonly reported feature in BME and SCI, promotes cytokines and is involved in decreasing circulating levels of Zn and increasing levels of Cu.[11] Thus, ceruloplasmin synthesis would be promoted,[31] and circulating Zn would be redistributed to the liver.[32] Significant negative correlations between the Zn/Cu ratio and hsCRP, BMI, and WC bolster this observation. However, anthropometric parameters consistently demonstrated the strongest relationship with Cu. This suggests that disturbances in Cu metabolism may be a significant contributing factor to BME-related PCOS. Additionally, in all PCOS patients

with abnormal backgrounds, we noted the strongest correlations of hsCRP with Cu levels. Thus, Cu elevation would be associated with a worsened subclinical inflammatory state. Cu metabolism disturbances could potentially establish a connection between abnormal glucose metabolism and PCOS via oxidative stress.[33,34] In our study, both HOMA-IR and glucose in the PCOS subgroup with IR exhibited positive correlations with Cu levels. Furthermore, this subgroup demonstrated significantly higher Cu levels compared to the subgroup of PCOS patients without IR. Contrary to our expectations of a decreased Zn/Cu ratio in IR, the ratio was higher in both PCOS patients and

Table 5: Significant correlations of trace elements with clinical and biochemical studied characteristics in PCOS patients according to normal/abnormal background

		Excess body weight			Insulin-resistance			Subclinical inflammation		
		Cu	Zn	Zn/Cu	Cu	Zn	Zn/Cu	Cu	Zn	Zn/Cu
BMI	N	0.311	-	-	0.608	-	-0.543	0.411	-	-0.363
	AN	0.274	-	-	0.370	-	-0.358	0.441	-	-0.296
WC	N	0.286	-	-	0.686	-	-0.587	-	-	-
	AN	-	-	-	-	-	-	0.422	-	-0.250
Glucose	N	-	-	-	-	-	-	-	-	-
	AN	-	-	-	0.250	-	-	-	-	-
Insulin	N	-	0.350	-	0.318	-	-	-	-	-
	AN	-	0.274	-	-	-	-	0.331	0.388	-
HOMA-IR	N	-	0.407	-	-	-	-	-	-	-
	AN	-	0.271	-	0.237	-	-	0.325	0.374	-
hsCRP	N	-	-	-	0.310	-	-	-	-	-
	AN	0.311	-	-	0.326		-0.270	0.397	-	-0.254
FSH	N	-	-	-	-	-	-	-	-	-
	AN	-	-	-	0.300	-	-0.261	-	-	-
LH	N	-	0.380	-	-	0.336	0.419	-	0.311	0.322
	AN	-	-	-	-	-	-	-	-	-
LH/FSH	N	-	0.271	0.251	-	-	0.390	-0.347	-	0.347
	AN	-	-	0.253	-0.244	-	0.251	-	-	0.235
AMH	N	-0.284	-	0.344	-	-	-	-0.463	-	0.496
	AN	-	-	-	-0.478		0.451	-	-	-
E2	N	-	-	-	-	0.322	-	-	0.362	-
	AN	-	0.392	0.291	-	-	-	-	-	-
Testo	N	-0.251	-	-	-	-	-	-0.334	-	-
	AN	-0.303	0.379	0.455	-0.336	0.265	0.383	-	0.354	0.387
17OHprog	N	-	-	0.329	-	-	-	-	-	0.499
	AN	-	-	-	-	-	0.250	-	-	-
A4	N	-	-	-	-	-	-	-0.315	-	-
	AN	-0.500	-	0.328	-0.358	-	0.284	-0.396	-	0.330
DHEA-S	N	-	-	-	-	-	0.361	-	-	
	AN	-	0.388	0.483	-	0.301	0.340	-	0.304	0.434

17OHprog: 17-hydroxyprogesterone, A4: androstenedione, AN: abnormal background with insulin resistance and/or subclinical inflammation, BMI: body mass index, Cu: copper, DHEA-S: dehydroepiandrosterone-sulfate, E2: estradiol, FSH: follicle stimulating hormone, HOMA-IR: Homeostatic Model Assessment for Insulin Resistance, LH: luteinizing hormone, N: normal background with no insulin resistance and no subclinical inflammation, PCOS: polycystic ovary syndrome, Testo: testosterone, WC: waist circumference, Zn: zinc, Zn/Cu: Zinc to copper ratio

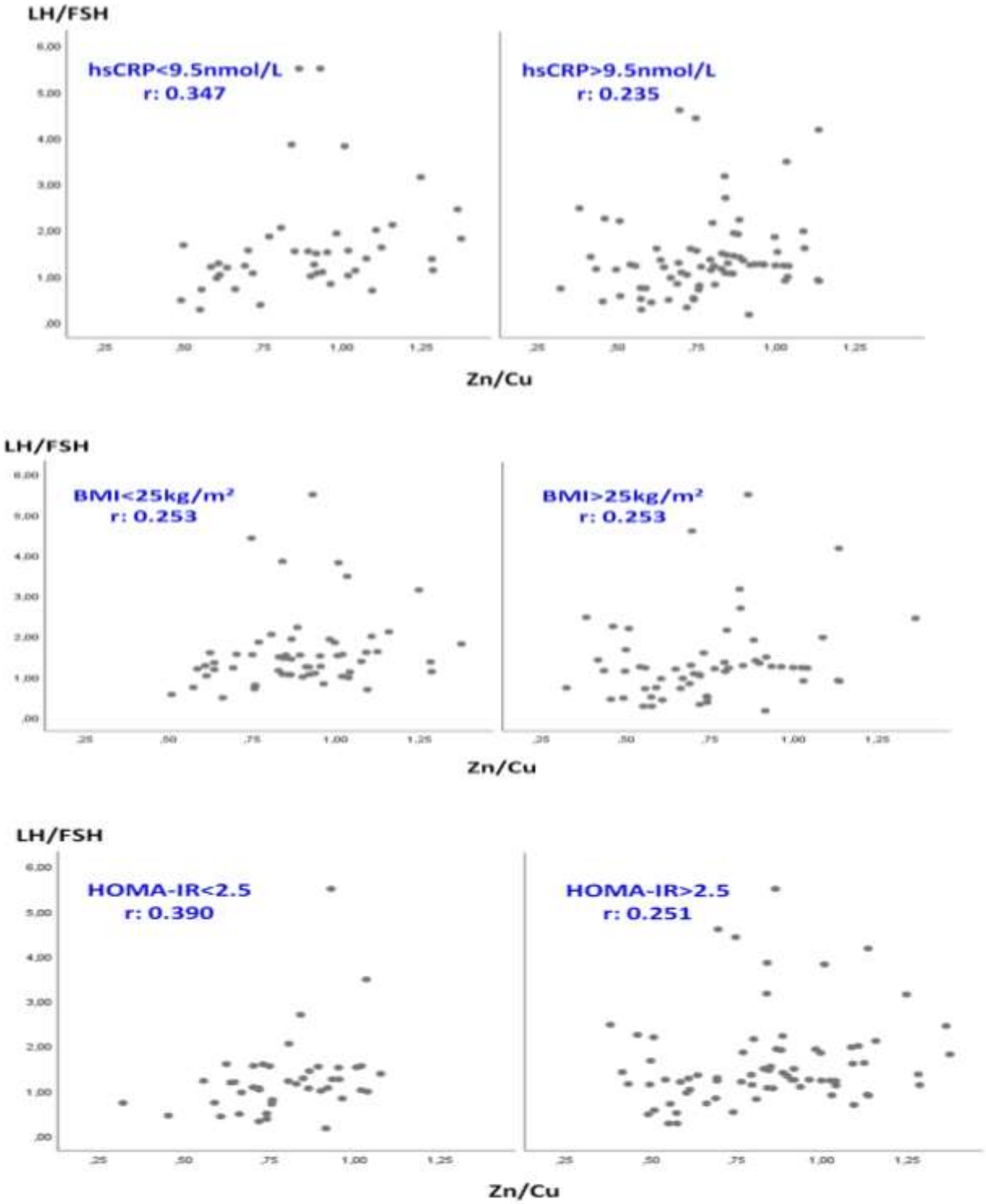


Figure 2: Significant correlations between LH/FSH ratio and Zn/Cu ratio levels in PCOS patients according to normal/abnormal background

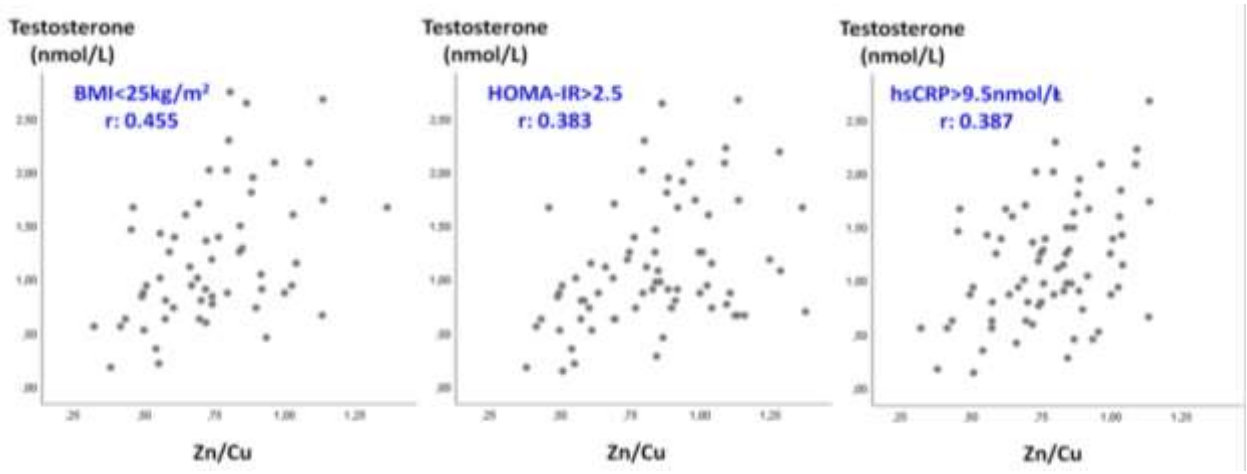


Figure 3: Significant correlations between testosterone and Zn/Cu ratio levels in PCOS patients

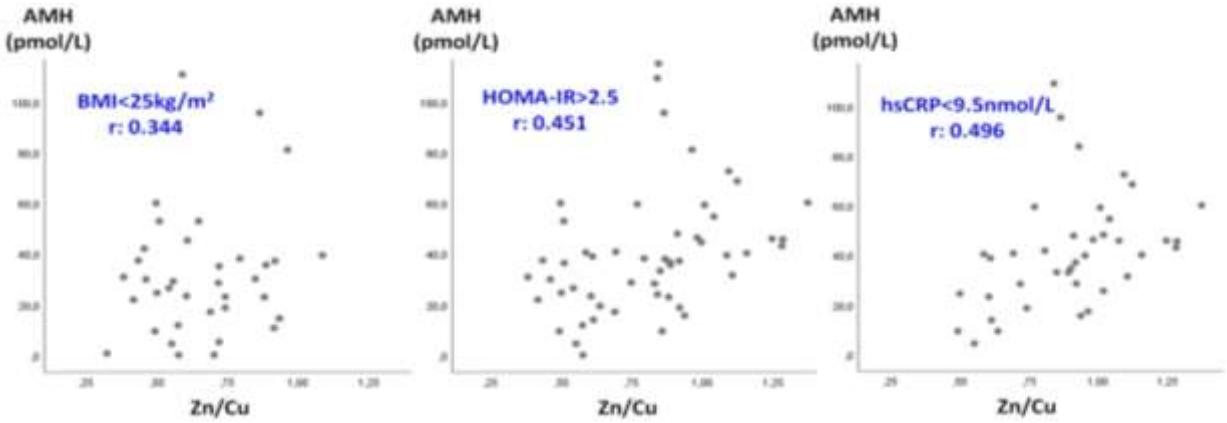


Figure 4: Significant correlations between AMH and Zn/Cu ratio levels in PCOS patients

controls with IR compared to normal background subgroups. Despite the lack of a significant correlation between glucose, insulin, or HOMA-IR, there was a noticeable increase in Zn levels in individuals with IR. Moreover, Zn contributes to insulin metabolism and action.[35] This finding suggests that Cu and Zn may exhibit complex interactions independently in IR PCOS patients.Furthermore, alterations in Zn/Cu ratio levels may indicate the body's ability to counter oxidative stress, which is markedly elevated in BME and SCI associate with reduced Zn/Cu ratio levels. The observed correlations between the Zn/Cu ratio and LH/FSH ratio, testosterone, and AMH may reflect a compensatory response to the pronounced oxidative stress typical of PCOS.[36-38]

Strength and limitation

Our study is the first investigation of Zn and Cu levels in PCOS within the South Mediterranean region, with a particular focus on southern Tunisia. We believe that evaluating the Zn/Cu ratio contributes valuable insight to our research, which integrates trace element status along with inflammatory and oxidative stress responses in PCOS. These findings underscore the importance of Zn and Cu status in managing PCOS and its associated comorbidities. Further investigations, including serum albumin and ceruloplasmin analysis, could provide clarity on the variations in trace elements. Increasing the size of the studied population would enhance the statistical

significance of the observed differences. While participants' eating habits were assumed to reflect a Mediterranean diet, some variation could have been introduced by utilizing a dietary recall diary.

Conclusion

An elevation of the Zn/Cu ratio could exacerbate hyperandrogenemia in PCOS when associated with an abnormal background. We observed a close interaction with AMH and gonadotrophins in both normal and abnormal background PCOS. The multifaceted implication of Zn/Cu equilibrium in PCOS pathophysiology was evident. Our findings underscore the complexity of the role of trace elements in PCOS, highlighting the need for further research into trace element supplementation in PCOS.

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Competing interests

The authors have no competing interests to declare.

Contribution of authors

Khansa Chaabouni, Dana Jallouli, and Fatma Khanfir conceived and designed the study. Wassim Guidara, Khansa BelHsan and Madiha Frikha collected and analysed the data. Khansa Chaabouni and Aida Elleuch prepared the manuscript. Fatma Ayadi approved the final version.All authors mentioned in the article approved the manuscript.

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