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Association of metformin treatment with changes in metal dynamics in individuals with type 2 diabetes

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ABSTRACT

Introduction The metal-chelating activity of metformin, which has long been known but of unclear clinical relevance, has recently been implicated in the pleiotropic effects, including antitumorigenic and anti-inflammatory actions, of the drug. However, whether metformin actually influences metal dynamics in humans has remained unknown. We here investigate whether metformin influences serum metal levels in individuals with type 2 diabetes.

Research design and methods In this cross-sectional study, individuals with type 2 diabetes treated or not treated with metformin for at least 6 months were recruited. The primary outcome was the difference in serum copper concentration between metformin users and non-users. Secondary outcomes included differences in serum levels of iron, zinc, and vitamin B_{12} as well as in copper-related and iron-related parameters between the two groups.

Results A total of 189 individuals (93 metformin users and 96 non-users) were analyzed. Metformin users showed significantly lower serum copper (16.0 vs 17.8 µmol/L, p<0.001) and iron levels (16.3 vs 17.3 µmol/L, p=0.02) and higher zinc levels (13.3 vs 12.5 µmol/L, p=0.01) compared with non-users. Copper-related and iron-related parameters for metformin users were consistent with latent deficiencies of these metals. Serum homocysteine levels (12.2 vs 11.2 µmol/L, p=0.03) were significantly higher, whereas vitamin B_{12} levels (338.7 vs 412.8 pmol/L, p<0.001) were significantly lower, in metformin users. Multiple regression analysis including variables that potentially influence metal dynamics identified metformin use as an independent predictor of serum copper (B = −1.54 µmol/L, p<0.001) and iron levels (B = −2.49 µmol/L, p=0.004).

Conclusions Metformin use was associated with reduced serum levels of copper and iron, as well as with increased serum zinc levels. These changes in metal dynamics may be related to the pharmacological effects of this widely administered drug.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Metformin has long been recognized for its metal-chelating activity. However, the implication of this activity for metal homeostasis in humans and its clinical relevance remains incompletely elucidated.

WHAT THIS STUDY ADDS

⇒ In a cross-sectional analysis involving 189 individuals with type 2 diabetes (93 metformin users and 96 non-users), we found that metformin use was associated with lower serum levels of copper and iron and higher serum zinc levels.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The observed effects of metformin on metal dynamics may stem from its metal-chelating activity and contribute to its glucose-lowering and pleiotropic actions.

INTRODUCTION

Metformin, launched more than 60 years ago, is the most widely administered antidiabetes drug worldwide. Despite its long-term and widespread use, however, the mechanism of its antidiabetic effect remains incompletely understood. The suppression of hepatic gluconeogenesis appears to play a primary role in the glucose-lowering action of the drug. Inhibition of mitochondrial respiratory chain complex I and the consequent increase in the cellular AMP/ATP ratio is thought to contribute to such suppression.[1 2] However, various other mechanisms for this action have been uncovered over time.[1 2] Moreover, in addition to its glucose-lowering action, metformin manifests other pleiotropic actions that include anti-inflammatory,[3 4] antitumorigenic,[5 6] antiatherosclerotic,[7 8] and antiobesity effects.[9 10] The mechanisms underlying these effects also remain to be fully elucidated.

The chelation of various transition metals—including copper, nickel, and cobalt—has long been recognized as a biochemical action of metformin.[1] The chemical properties of complexes formed between metformin and metals, especially copper, have been well characterized.[11] However, the role of

metal chelation in the clinical actions of metformin is unknown. Although such chelation has been implicated in antimicrobial activity of metformin[12] and in its effect on AMP-activated protein kinase activity in cells,[13] these links remain ill-defined. Recent studies, however, have revealed that metformin-induced changes in copper dynamics affect mitochondrial function and related biological activities.[14 15] The binding of metformin to copper thus sequesters the metal in mitochondria, which contributes to the anticancer activity of the drug.[14] Furthermore, mitochondrial copper plays a pivotal role in macrophage activation, and a designed dimeric compound derived from metformin but with an ~1000-fold greater copper-chelating activity was found to manifest potent anti-inflammatory effects both in vitro and in vivo.[15] In addition, blood copper levels have been found to be higher in individuals with diabetes than in non-diabetic individuals[16 17] and to be associated with inadequate glycemic management[18] or with diabetic complications.[16] However, whether metformin treatment influences copper dynamics in human patients has remained unclear, as only one study with a small sample size reported no difference in serum copper levels between before and 3 months after the onset of metformin treatment.[19]

We therefore designed the current study primarily to examine whether metformin treatment is associated with changes in serum copper levels in individuals with type 2 diabetes. We also examined serum concentrations of iron and zinc, as well as serum parameters related to copper and iron dynamics, as secondary outcomes. Our findings provide evidence that metformin treatment is associated with changes in the serum levels of certain metals, including copper.

RESEARCH DESIGN AND METHODS

Study participants

This single-center, cross-sectional study was conducted in accordance with the Declaration of Helsinki. The study was registered in the University Hospital Medical Information Network (UMIN registration number 000054359). Participants were individuals with type 2 diabetes who were aged 18 years or older and who had either been using metformin for at least 6 months or not used it for at least 6 months. Written informed consent was obtained from all participants, who did not receive any financial compensation for their participation. Exclusion criteria included the use of iron, zinc, vitamin B_{12}, or folic acid supplements; hemoglobin levels below the reference range (<135.0 g/L for men or <115.0 g/L for women under 60 years of age; <120.0 g/L for men or <105.0 g/L for women aged 60 to 70 years; or <110.0 g/L for men or <105.0 g/L for women aged 70 years or older); pregnancy or breastfeeding; a history of gastrectomy, duodenectomy, or small bowel resection; malabsorption disorders; an estimated glomerular filtration rate of <30 mL min^{-1} 1.73 m^{-2}; liver cirrhosis; and malignancy.

The primary endpoint of this study was the difference in serum copper concentrations between metformin users and non-users. Although we hypothesized that metformin use would lower serum copper levels, there was no definitive scientific basis to predict the extent of this reduction. We therefore assumed that, even if metformin reduced serum copper levels, the values would still remain within the normal range. At Kobe University Hospital, the reference range for serum copper is 10.7–20.1 µmol/L, with both the mean and median around 15.7 µmol/L. Based on this, we estimated the intergroup difference to be approximately 2.4–3.9 µmol/L. Assuming a SD of 3.1–4.7 µmol/L—approximately one-third to one-half of the reference range—we calculated that a total of 200 participants (100 per group) would provide ≥80% power to detect such a difference using a two-sided t-test at a significance level of 5%. This sample size was also considered feasible based on the expected number of eligible participants over an approximately 2-year recruitment period.

Beginning in August 2022, all outpatients with diabetes seen by participating physicians and all inpatients with diabetes admitted to our department were screened for eligibility. Those who met the inclusion criteria and did not meet any exclusion criteria were invited to participate, and written informed consent was obtained from those who agreed. By December 2023, 2,322 patients had been screened, and 204 individuals had provided informed consent. Because multiple physicians conducted outpatient clinics on the same days, the number of participants who consented slightly exceeded the predefined target of 200. Although eligibility was assessed prior to obtaining consent, five participants were subsequently found to meet exclusion criteria after consent had been obtained. As a result, 199 participants were ultimately enrolled in the study (online supplemental figure S1).

Demographic and clinical characteristics of the study participants

Age, sex, duration of illness, and prescribed medications were extracted from medical records. The primary outcome was the difference in serum copper levels between metformin-treated and metformin-untreated individuals. Secondary outcomes included differences in serum ceruloplasmin, iron, ferritin, unsaturated iron-binding capacity (UIBC), transferrin, zinc, vitamin B_{12}, homocysteine, and cobalt levels. Serum copper, iron, and zinc concentrations were determined using colorimetric methods: the 3,5-DiBr-PAESA (3,5-Dibromo-2-pyridylazoethylsulfanylbenzoic acid) method for copper, the Nitroso-PSAP (2-Nitroso-5-(N-propyl-N-(3-sulfopropyl)amino)phenol) method for iron, and the Nitro-PAPS (2-(5-Nitro-2-pyridylazo)−5-(N-n-propyl-N-(3-sulfopropyl)amino)phenol) method for zinc. Serum ceruloplasmin was analyzed by nephelometric immunoassay, whereas ferritin and transferrin were measured using a chemiluminescent immunoassay. Serum vitamin B_{12} was determined by electrochemiluminescence immunoassay,

Table 1 Principal clinical parameters for study participants at baseline

Parameter	MET group (n=93)	Non-MET group (n=96)	P* value
Demographics			
Age (years), median (IQR)	67.0 (56.0–74.0)	68.5 (60.0–74.6)	0.34
Male, no. (%)	57 (61)	57 (59)	0.79
Disease duration (years), median (IQR)	16.5 (8.5–23.0)	9.0 (3.0–19.5)	<0.001
BMI (kg/m^2), median (IQR)	25.7 (22.4–29.8)	24.2 (21.7–28.6)	0.21
Laboratory parameters			
HbA_{1c} level (%), median (IQR)	7.3 (6.8–7.9)	7.0 (6.6–7.6)	0.05
Plasma glucose (mmol/L), median (IQR)	7.9 (6.9–9.0)	7.2 (6.3–9.1)	0.14
RBC count (10^{12}/L), mean (SD)	4.75 (0.61)	4.68 (0.51)	0.37
Hemoglobin (g/L), mean (SD)	142.0 (17.0)	143.0 (14.0)	0.66
MCV (fL), mean (SD)	91.2 (5.3)	92.7 (4.2)	0.03
MCH (pg/cell), median (IQR)	30.2 (29.0–31.6)	30.7 (29.6–32.1)	0.05
MCH concentration (g/L), mean (SD)	330.0 (11.0)	332.0 (11.0)	0.33
SD of RBC distribution width (fL), median (IQR)	43.8 (41.6–45.9)	44.0 (42.0–46.9)	0.36
CV of RBC distribution width (%), median (IQR)	13.0 (12.5–13.6)	13.0 (12.5–13.5)	0.86
Hematocrit (proportion of 1.0), mean (SD)	0.43 (0.05)	0.43 (0.04)	0.84
WBC count (10^9/L), median (IQR)	6.7 (5.5–7.7)	6.0 (5.1–7.4)	0.07
Platelet count (10^9/L), median (IQR)	220.0 (187.5–258.0)	198.5 (171.8–241.0)	0.02
Creatinine (µmol/L), median (IQR)	69.0 (56.6–88.4)	71.6 (56.6–87.5)	0.63
eGFR (mL/min^{-1}/1.73 m^{-2}), median (IQR)	70.9 (53.8–84.8)	65.0 (53.4–78.9)	0.34

*P values for comparisons between MET and Non-MET groups were determined with the t test or the Mann-Whitney U test, depending on normality of the data for continuous variables, and the χ^2 test for categorical variables, including sex distribution.
BMI, body mass index; CV, coefficient of variation; eGFR, estimated glomerular filtration rate; HbA_{1c}, glycosylated hemoglobin; MCH, mean corpuscular hemoglobin; MCV, mean corpuscular volume; MET, metformin; RBC, red blood cell; WBC, white blood cell.

and serum homocysteine levels were quantified by liquid chromatography-mass spectrometry (MS). Serum cobalt concentrations were measured using inductively coupled plasma MS (ICP-MS). Transferrin saturation was calculated by dividing the serum iron concentration by the total iron-binding capacity, which is the sum of the serum iron level and UIBC, and then multiplying the result by 100 for expression as a percentage. If vitamin B_{12} levels exceeded the upper limit of quantitation (1,106.7 pmol/L), a value of 1,106.7 pmol/L was assigned.

Statistical analysis

All statistical analysis was conducted with SPSS V.29.0 software (IBM, Armonk, New York, USA). A two-sided p value of<0.05 was considered statistically significant for all analyses. Data are presented as number (%) for categorical variables, and as either mean (SD) or median (IQR) for continuous variables depending on the normality of the distribution, which was assessed with the Shapiro-Wilk test. Continuous variables were compared between metformin users and non-users with the t test for normally distributed data or the Mann-Whitney U test for non-normally distributed data. Categorical variables were compared with the χ^2 test.

Multiple linear regression analysis was performed to identify factors influencing serum copper, iron, and zinc concentrations. Independent variables incorporated in the analysis included metformin use, age, sex, body mass index (BMI), hemoglobin A_{1c} (HbA_{1c}) level, estimated glomerular filtration rate, and use of proton pump inhibitors or histamine type 2 receptor antagonists (model 1). The use of anticoagulants or antiplatelet agents was also included for the analysis of iron, and that of diuretics or ACE inhibitors for the analysis of zinc. These variables were selected on the basis of previous studies indicating their potential impact on serum copper, iron, and zinc levels.[20–26] Iron, copper, and zinc levels were added as complementary factors to the existing factors for a model 2. Multicollinearity among the independent variables was checked by calculation of the variance inflation factor, with values of >5 indicating significant multicollinearity.

Sensitivity analysis—including subgroup analysis by sex and analysis excluding participants using specific medications potentially affecting copper, iron, and zinc metabolism—was also conducted to evaluate the robustness of the findings.

Table 2 Comparison of serum metal dynamics between metformin (MET) and Non-MET groups

Parameter	MET group (n=93)	Non-MET group (n=96)	P* value
Copper (µmol/L)	16.0 (14.8–18.1)	17.8 (15.7–19.5)	<0.001
Ceruloplasmin (mg/L)	224.0 (207.0–256.0)	254.0 (219.0–275.0)	<0.001
Iron (µmol/L)	16.3 (11.6–19.6)	17.3 (13.6–22.0)	0.02
Ferritin (µg/L)	66.1 (26.4–126.5)	107.5 (72.6–206.8)	<0.001
UIBC (µmol/L)	44.6 (39.5–51.8)	38.7 (32.7–47.9)	<0.001
Transferrin (µmol/L)	31.9 (29.2–36.1)	30.0 (26.5–34.0)	0.008
Transferrin saturation† (%)	26.7 (19.5–32.3)	31.0 (23.7–38.0)	<0.001
Zinc (µmol/L)	13.3 (12.1–14.6)	12.5 (10.9–14.1)	0.01
Vitamin B_{12} (pmol/L)	338.7 (244.9–449.7)	412.8 (323.5–565.7)	<0.001
Homocysteine (µmol/L)	12.2 (10.2–16.8)	11.2 (9.0–15.2)	0.03

Data are expressed as median (IQR).
*P values for comparisons between the two groups were determined with the t test or Mann-Whitney U test, depending on normality of the data.
†Transferrin saturation is calculated by dividing the serum iron level by the total iron-binding capacity (TIBC), which is the sum of serum iron and UIBC, and then multiplying the result by 100 for expression as a percentage.
UIBC, unsaturated iron-binding capacity.

RESULTS

Participants and background

The patient flow diagram is shown in online supplemental figure S1, with a total of 93 individuals in the metformin (MET) group and 96 individuals in the Non-MET group being included in the analysis. Table 1 presents the baseline characteristics of the study participants.

The median age was 67.0 years in the MET group and 68.5 years in the Non-MET group, with this difference not being statistically significant. Although there was a higher proportion of men overall, the sex distribution also did not differ significantly between the groups (p=0.79). The median dosage of metformin in the MET group was 1,500 mg (IQR of 1,000–2,000 mg). Whereas hemoglobin levels did not differ significantly between the groups, the mean corpuscular volume was significantly lower in the MET group than in the Non-MET group (91.2±5.3% vs 92.7±4.2%, p=0.03).

Outcomes

Data on serum metal dynamics are presented in table 2.

Serum copper (16.0 vs 17.8 µmol/L, p<0.001) and ceruloplasmin concentrations were significantly lower in the MET group than in the Non-MET group. In addition, serum iron (16.3 vs 17.3 µmol/L, p=0.02) and ferritin (66.1 vs 107.5 µg/L, p<0.001) levels were significantly lower, whereas transferrin (31.9 vs 30.0 µmol/L, p=0.008) and UIBC (44.6 vs 38.7 µmol/L, p<0.001) levels were significantly higher, in the MET group. The level of transferrin saturation was significantly lower in the MET group (26.7% vs 31.0%, p<0.001). In contrast, serum zinc levels were significantly higher in the MET group than in the Non-MET group (13.3 vs 12.5 µmol/L, p=0.01). Vitamin B_{12} levels were also significantly lower in the MET group (338.7 vs 412.8 pmol/L, p<0.001), whereas homocysteine levels were significantly higher (12.2 vs 11.2 µmol/L, p=0.03). Vitamin B_{12} levels exceeded the upper limit of measurement in three individuals (3%) of the MET group and in six individuals (6%) of the Non-MET group, which might have been attributable to supplementation of this vitamin. After exclusion of these nine participants, analysis of the remaining 90 individuals in each group revealed a significantly lower vitamin B_{12} concentration in the MET group (332.8 (242.7–430.1) pmol/L) compared with the Non-MET group (392.5 (318.0–536.4) pmol/L, p<0.001). Serum cobalt levels were below the detection limit of the assay (1.7 nmol/L) in many samples; measurable levels were observed in only 53 individuals in the MET group and 63 individuals in the non-MET group. When comparing only the samples with detectable levels, no significant difference was observed between the MET and non-MET groups (2.2 (1.9–3.7) vs 2.4 (2.0–3.3) nmol/L, p=0.722) (online supplemental table S1).

Multiple regression analysis of serum copper, iron, and zinc levels

We next performed multiple regression analysis for serum copper, iron, and zinc levels to elucidate the relations and interactions between variables. We examined multicollinearity and conducted residual analysis to assess model performance. Variance inflation factor values for all variables were <2.4, indicating no multicollinearity issues. To help visualize the pairwise relationships among serum metal levels, scatter plots with Spearman's correlation coefficients and trend lines are shown in online supplemental figure S2. Residual analysis revealed that serum copper, iron, and zinc residuals were normally distributed.

Table 3 Multiple linear regression analysis of serum copper concentration

	Model 1			Model 2		
Variable	**B (95% CI)**	**β**	**P* value**	**B (95% CI)**	**β**	**P* value**
Age	0.02 (–0.02 to 0.06)	0.08	0.39	0.02 (–0.02 to 0.07)	0.09	0.35
Sex (male=0, female=1)	1.70 (0.91 to 2.50)	0.28	<0.001	1.65 (0.86 to 2.44)	0.27	<0.001
Body mass index	0.12 (0.05 to 0.18)	0.28	<0.001	0.12 (0.05 to 0.19)	0.29	<0.001
Hemoglobin A_{1c}	0.20 (–0.04 to 0.44)	0.11	0.10	0.22 (–0.02 to 0.46)	0.12	0.08
Estimated glomerular filtration rate	0.01 (–0.01 to 0.03)	0.08	0.31	0.01 (–0.01 to 0.03)	0.08	0.31
Metformin (no=0, yes=1)	–1.54 (–2.30 to –0.78)	–0.26	<0.001	–1.71 (–2.51 to –0.92)	–0.29	<0.001
Proton pump inhibitor (no=0, yes=1)	–0.29 (–1.12 to 0.60)	–0.04	0.52	–0.38 (–1.28 to 0.51)	–0.06	0.40
H_2 receptor antagonist (no=0, yes=1)	0.38 (–1.47 to 2.23)	0.03	0.69	0.31 (–1.54 to 2.16)	0.02	0.74
Iron	Not included			–0.05 (–0.12 to 0.02)	–0.10	0.16
Zinc	Not included			0.08 (–0.13 to 0.30)	0.05	0.45

*P values indicate the statistical significance of each independent variable in the model. A p value of <0.05 suggests a significant contribution to the outcome variable.
H_2, histamine type 2.

With regard to serum copper levels, we first constructed model 1 for copper concentration as the outcome variable and with explanatory variables based on previous studies but with the addition of metformin use. Metformin use was identified as a statistically significant independent factor influencing serum copper concentration (table 3).

Model 2 was then constructed by the addition of serum iron and zinc levels as explanatory variables to model 1. Neither iron nor zinc emerged as significant factors, whereas metformin remained a significant predictor.

Similar multiple linear regression analysis identified zinc, in addition to metformin use, as an independent factor influencing serum iron concentration (table 4).

Model 1 identified metformin use as an independent predictor of serum iron concentration, whereas model 2 identified both metformin use and serum zinc as significant independent factors influencing iron levels.

Subgroup analysis based on medications and sex

The medications used by the participants are listed in online supplemental table S1. We therefore conducted an analysis excluding participants using these six medication types (online supplemental table S3). Even after the exclusion of these individuals, serum copper, iron, and ferritin levels remained significantly lower in the MET

Table 4 Multiple linear regression analysis of serum iron concentration

	Model 1			Model 2		
Variable	**B (95% CI)**	**β**	**P* value**	**B (95% CI)**	**β**	**P* value**
Age	–0.03 (–0.13 to 0.06)	–0.07	0.52	0.01 (–0.09 to 0.10)	0.01	0.92
Sex (male=0, female=1)	–0.79 (–2.59 to 1.02)	–0.07	0.39	–0.35 (–2.22 to 1.52)	–0.03	0.71
Body mass index	0.01 (–0.14 to 0.15)	0.01	0.95	0.06 (–0.09 to 0.21)	0.08	0.40
Hemoglobin A_{1c}	0.09 (–0.46 to 0.64)	0.02	0.76	0.24 (–0.30 to 0.78)	0.07	0.39
Estimated glomerular filtration rate	–0.003 (–0.05 to 0.04)	–0.01	0.91	0.001 (–0.04 to 0.05)	0.003	0.97
Metformin (no=0, yes=1)	–2.49 (–4.19 to –0.78)	–0.21	0.004	–3.33 (–5.09 to –1.56)	–0.28	<0.001
Proton pump inhibitor (no=0, yes=1)	–1.69 (–3.90 to 0.51)	–0.13	0.13	–1.92 (–4.08 to 0.24)	–0.15	0.08
H_2 receptor antagonist (no=0, yes=1)	–0.26 (–4.56 to 4.03)	–0.01	0.90	–0.77 (–4.97 to 3.42)	–0.03	0.72
Antiplatelet (no=0, yes=1)	–0.30 (–2.94 to 2.34)	–0.02	0.82	–0.18 (–2.76 to 2.40)	–0.01	0.89
Anticoagulant (no=0, yes=1)	0.31 (–2.78 to 3.40)	0.02	0.84	0.89 (–2.16 to 3.95)	0.05	0.57
Copper	Not included			–0.24 (–0.56 to 0.08)	–0.12	0.14
Zinc	Not included			0.71 (0.26 to 1.16)	0.23	0.002

*P values indicate the statistical significance of each independent variable in the model. A p value of <0.05 suggests a significant contribution to the outcome variable.
H_2, histamine type 2.

group, whereas zinc levels did not differ significantly between the two groups.

Sex-specific subgroup analysis revealed that both men and women showed lower serum copper and ceruloplasmin levels and lower serum ferritin, and transferrin saturation levels in the MET group than in the non-MET group (online supplemental table S4). Vitamin B_{12} levels were significantly lower in women and tended to be lower in men of the MET group. Zinc levels were significantly higher only for men in the MET group.

DISCUSSION

This study is the first to show that individuals with type 2 diabetes treated with metformin have lower serum copper levels than do those not receiving this drug. Serum ceruloplasmin levels were also lower in metformin users, which is consistent with copper deficiency. Multiple regression analysis accounting for variables that could influence metal dynamics identified metformin use as an independent determinant of serum copper concentration. In addition, analysis excluding individuals taking other medications that potentially affect metal dynamics revealed that metformin use remained associated with reduced copper levels. Given that orally administered metformin remains in the intestinal tract for an extended period,[27] chelation by metformin in the intestine might reduce copper absorption. Effects of metformin on the gut have recently attracted considerable attention.[28 29] However, we cannot rule out the possibility that the reduced serum copper levels of individuals taking metformin are the result of a mechanism other than chelation.

We also found that metformin users had lower serum levels of iron and ferritin as well as higher levels of transferrin and UIBC, all of which are indicative of iron deficiency. Reduced iron levels were associated with metformin use even after exclusion of individuals taking other medications that potentially affect metal dynamics. A small-scale study previously showed that individuals taking metformin for >5 years had lower serum iron levels than did those using the drug for shorter times,[30] consistent with our findings. However, in this earlier study, both short- and long-term users manifested anemia, with hemoglobin levels of 107.8 and 106.2 g/L, respectively. Longer-term users are also showing higher ferritin levels,[30] which typically do not indicate iron deficiency. In addition, individuals with polycystic ovary syndrome treated with metformin for 12 weeks were found to have lower ferritin levels compared with those not treated with the drug,[31] further suggesting a link between metformin and reduced iron levels. Of note, our study is the first to comprehensively evaluate iron-related parameters, with all results indicating latent iron deficiency, in metformin users.

The interaction between iron and metformin is less well understood compared with that between copper and the drug. Metformin forms a complex with Fe(III) in aqueous solution, including ethanol-based solvents.[32 33] However, it remains unclear whether such complex formation occurs under physiological conditions. Unlike in the case of copper, the structure of the iron-metformin complex is intricate and largely depends on the reaction conditions.[32 34] Further investigation into the chemical properties of iron-metformin interaction is warranted to understand the mechanism underlying the effects of the drug on iron dynamics.

In general, zinc is not considered a typical chelation partner for metformin.[11] We did not observe reduced levels of zinc in metformin users; indeed, metformin treatment was associated with higher serum zinc levels, although the mechanism underlying this association remains unclear. Excessive zinc intake reduces copper absorption through upregulation of the metal-binding protein metallothionein in the intestine.[35] However, case reports have revealed that individuals with copper deficiency due to malabsorption associated with celiac disease may present with elevated serum zinc levels,[36 37] suggesting that impaired copper absorption may not only result from excess zinc but also lead to increased zinc levels. Furthermore, our multivariate analysis indicated that iron and zinc serve as mutual explanatory factors, independent of metformin. The finding that increased zinc levels were significant only in men among metformin users suggests a role for sex-specific physiological factors. Regardless of the mechanism, the increase in zinc levels with metformin use may not be due to a direct interaction of the metal with metformin but may rather be a secondary effect.

We found that metformin use was associated with reduced levels of vitamin B_{12}, as has been found in previous studies.[38 39] Whereas the mechanism by which metformin induces vitamin B_{12} deficiency remains uncertain, this vitamin contains cobalt within its molecular structure, and metformin is known to chelate cobalt.[11 34] Given that cobalt shortage is related to vitamin B_{12} deficiency,[40] we attempted to measure serum cobalt levels by ICP-MS, which allows the analysis of trace elements with high sensitivity and precision.[41] Unfortunately, the serum cobalt levels of 73 of the 189 study participants (38.6%) were below the measurable limit of our system. Further analysis with a refined system may shed light on metformin-induced vitamin B_{12} deficiency and its relation to the cobalt-chelating activity of the drug.

The observed changes in serum metal levels in this study may not necessarily reflect latent adverse effects of metformin, unlike the decrease in vitamin B_{12} levels. Recent preclinical studies suggest that the chelation and depletion of copper in mitochondria may contribute to the anti-inflammatory and anticancer effects of metformin.[14 15] Higher blood copper levels have previously been observed in individuals with diabetes[16 17] and found to be positively correlated with HbA_{1c} levels[18] and diabetic complications.[16] Of note, copper-chelating agents have been shown to ameliorate glucose intolerance[42] and heart failure[43] in diabetic animal models.

These findings collectively suggest that the reduced copper levels of metformin users uncovered in the present study may be linked to the glucose-lowering and complication-preventive effects of the drug. In addition, higher iron and lower zinc levels in blood are generally associated with glucose intolerance.[44 45] The observed changes in the blood levels of these metals may therefore also be related to the antidiabetes action of metformin.

Our study has several limitations. Given that it is an observational study, causality cannot be definitively established, and unmeasured confounding variables may have influenced the results. We did not collect detailed dietary information or menstrual histories, both of which might have affected the findings. Although we excluded individuals who reported taking iron, zinc, vitamin B_{12}, or folic acid supplements, three metformin users and six non-users showed vitamin B_{12} levels exceeding the upper limit of quantitation. This finding suggests that they may have taken vitamin B_{12}–containing supplements and were not properly excluded. However, no participants showed extremely high levels of copper, iron, or zinc (exceeding the mean by more than 3.5×SD). We also excluded individuals with anemia from this study, although deficiencies of copper, iron, or vitamin B_{12} can cause anemia. Individuals with severe deficiencies of these nutrients may therefore have been excluded, potentially affecting the results. In addition, metformin users tended to have higher BMI, which may have influenced serum metal concentrations. However, obesity and weight gain are generally associated with increased serum copper levels and decreased serum zinc levels,[46] findings that contrast with our current results. Serum iron levels, on the other hand, are generally lower in individuals with obesity.[46] While we cannot entirely rule out the possibility that the tendency toward higher BMI influenced the results, it is unlikely that the observed alterations in metal concentrations can be explained by this factor alone.

In summary, our study has revealed that metformin use in the clinical setting is associated with lower serum copper levels as well as with lower iron and higher zinc levels, providing previously unrecognized insight into metformin action. The antidiabetes drug imeglimin, which is structurally similar to metformin but thought to lack metal-chelating properties as a result of its cyclic structure, was recently launched in Japan.[47] Prospective studies to examine the relation between metal levels and the glucose-lowering and complication-preventive effects of metformin, as well as to compare the effects of metformin and those of imeglimin, may further clarify the clinical relevance of metal chelation by metformin. Still, our findings provide novel and important perspectives on the mechanisms of action of metformin, a widely used antidiabetic drug whose effects remain incompletely understood, and might eventually contribute to future therapeutic choices.

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Data availability statement Data are available upon reasonable request. The data from this study are not publicly available due to restrictions on data management and analysis, which were permitted only within Kobe University Hospital to ensure data privacy and security. However, the datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

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