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The association between plasma zinc concentrations and markers of glucose metabolism in adults in Cameroon

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Abstract

An abnormal Zn status has been suggested to play a role in the pathogenesis of type 2 diabetes. However, epidemiological studies of the relationship between plasma Zn concentrations and diabetes are sparse and inconclusive. We aimed to investigate the association between plasma Zn concentrations and glycaemic markers (fasting glucose, 2-h glucose and homeostatic model assessment of insulin resistance) in rural and urban Cameroon. We studied 596 healthy adults (63·3 % women) aged 25–55 years in a population-based cross-sectional study. The mean plasma Zn concentration was 13·7 ± 2·7 μmol/L overall, with higher levels in men (14·4 ± 2·9 μmol/l) than in women (13·2 ± 2·6 μmol/l), *P*-value < 0·0001. There was an inverse relationship between tertiles of plasma Zn and 2-h glucose concentrations (*P*-value for linear trend = 0·002). The difference in 2-h glucose between those in the highest tertile of plasma Zn compared to the lowest was −0·63 (95 % CI − 1·02, −0·23) mmol/l. This remained significant after adjusting for age, sex, smoking status, alcohol intake, education level, area of residence, adiposity and objectively measured physical activity −0·43(−0·82, −0·04). Similar inverse associations were observed between plasma Zn concentrations and fasting glucose and homeostatic model assessment of insulin resistance when adjusted for socio-demographic and health-related behavioural characteristics. The current findings of an inverse association between plasma Zn concentrations and several markers of glucose homeostasis, together with growing evidence from intervention studies, suggest a role for Zn in glucose metabolism. If supported by further evidence, strategies to improve Zn status in populations may provide a cheap public health prevention approach for diabetes.

Key words: **Plasma Zn: Glycaemia: Insulin resistance: Africa**

The burden of diabetes has risen globally over the past three decades but at a faster rate in low- and middle-income countries where 80 % of people in the world live with diabetes[1]. In Africa, an estimated 24 million adults had diabetes in 2021, and this has been projected to be 55 million by 2045. Over 70 % of deaths of people with diabetes in Africa occur in those who are in an economically productive age group, which has substantial implications at the individual, household and societal levels[2]. Therefore, identifying the determinants of this growing diabetes burden is a major public health concern. In sub-Saharan Africa, the rise in diabetes prevalence has been attributed to a shift in dietary patterns along with physical inactivity, driven in part by urbanisation[3]. This dietary transition towards the consumption of processed foods is associated with diets that do not often meet recommended dietary intakes of some micronutrients[4].

Zn is an essential trace element naturally found mainly in meat, poultry, dairy products and seafood[5,6]. Fortified foods and plant foods like legumes and grains are also good dietary sources of Zn[7]. Although there is an evidence to suggest that Zn deficiency is a public health issue globally[8,9], the limited set of studies from Africa, which are mainly in children and women of reproductive age, suggest that the magnitude of Zn deficiency in Africa may be greater than in other parts of the world[8–10]. Based on estimates of dietary intake of Zn using national food balance sheets, 26 % of people in Africa have inadequate Zn intake (compared with 16 % globally)[9].

Abbreviations: GPAQ, global physical activity questionnaire; HOMA-IR, homeostatic model assessment of insulin resistance; PAEE, physical activity energy expenditure.

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There is evidence that Zn plays a role in glucose metabolism[11]. Findings from large prospective observational studies suggest an inverse association between dietary Zn intake and type 2 diabetes risk[12–14]. However, these studies relied on self-reports to assess dietary Zn intake which is subject to measurement error and recall bias. Measurement of blood Zn concentration to assess Zn status provides an objective measure that complements data on dietary Zn intake[15]. There are few studies on the association between blood Zn concentration and type 2 diabetes, and the results of these are inconsistent. In a previous cross-sectional study in the USA and a prospective study in Finland, plasma Zn was positively associated with diabetes prevalence or risk[16,17]. However, these studies were conducted in a context where the prevalence of Zn deficiency is low[9]. In other studies in China, where low plasma Zn concentration is more common, plasma Zn was either inversely associated with diabetes prevalence[18] or not markedly associated with diabetes[19]. Thus, it is possible that observations of the association between plasma Zn concentrations and diabetes could be affected by the frequency of low plasma Zn concentrations in the population studied.

Despite the high prevalence of inadequate Zn intake in Africa compared with other regions of the world, we did not find any previous population-based study in an African population linking dietary Zn intake or Zn biomarkers to glycaemic markers. Dietary patterns in many low- and middle-income countries are rich in intake of phytates, which bind to Zn and inhibit its absorption[7,15]. In such settings, blood Zn may be a better indicator of Zn exposure than dietary Zn intake. In a previous study in Cameroon, the prevalence of low plasma Zn concentrations (< 70 μg/dl) in women aged 15–49 years was 82 %[20].

In this population-based study, including participants from rural and urban settings of Cameroon, a country with a high prevalence of low Zn status[8,9,20], we aimed to examine the independent associations between plasma Zn concentration and glycaemic markers. We hypothesised that plasma Zn concentrations would be inversely associated with glycaemic markers given the high prevalence of low Zn concentration in Cameroon.

Methods

Study population and design

The methods used for this study have been described in detail elsewhere[21]. In brief, this was a population-based cross-sectional study conducted in 2005–2006 in two urban sites and two rural sites in Cameroon. The urban sites were Yaoundé, the capital city of Cameroon (Centre region) and Bamenda, the capital city of the North-west region and the rural sites were Mbankomo in the Centre region and Bafut in the North-west region. All adults aged 25–55 years without a history of diabetes or CVD were approached through door-to-door recruitment in the four sites. A total of 651 participants (rural: *n* 303, mean age 38·5 ± 8·3 years; urban: *n* 348, mean age 37·9 ± 9·1 years) agreed to take part in this study. The mean age and sex ratio of volunteers was similar to all 3854 eligible participants identified in the delimited areas. We excluded 55 participants who did not have blood samples available for plasma Zn analysis. Ethical approval was obtained from the Cameroon National Ethics Committee, and all participants provided written informed consent.

Data collection

Fasting and 2-h glucose post 75 g oral glucose tolerance test were measured on fresh capillary whole blood using a Hemocue B-Glucose Analyzer (HemoCue AB) onsite. Fasting blood samples collected from all the participants were centrifuged at ~ 1400 *g*, and plasma aliquots were stored at −80°C. Plasma samples collected were transported on dry ice by air to Cambridge and stored at −80°C until analysis.

Measurement of plasma zinc. Plasma Zn concentrations were determined by inductively coupled plasma mass spectrometry (ICP-MS) using a Perkin Elmer NEXION 300D at Southampton University Hospitals in 2022. An internal standard (rhodium) was added to the plasma diluted in one in fifty in distilled water and to the quality control to normalise for sample preparation and instrument variability. Samples were run against matrix-matched calibration solutions prepared with bovine serum (Sigma-Aldrich). The Zn isotope signals (^{66}Zn) were compared against the internal standard to determine the concentration of plasma Zn. Quality control materials were run with each analytical batch and consisted of a certified reference material (Sero, Norway) and an in-house material. The inter-batch coefficient of variation was less than 10·1 %, and values for the CRM were within the acceptable range. External quality assurance was performed as part of the Trace Element Quality Assurance Scheme (TEQAS) (UK NEQAS).

Measurement of metabolic markers. Fasting plasma insulin was measured by fluorometric assay on a 1235 AutoDELFIA automatic immunoassay system (kit by Perkin Elmer Life Sciences). C-reactive protein, plasma cholesterol and TAG were measured using automated assays on the Dade Behring Dimension RxL analyser. C-reactive protein was measured using a particle-enhanced turbidimetric technique, and total cholesterol, HDL-cholesterol and TAG were measured by enzymatic method. LDL-cholesterol concentrations were derived by the Friedewald formula (LDL = total cholesterol − (TAG/2·2) − HDL), when TAG levels were < 4·5 mmol/l. These analyses were conducted at the National Institute for Health Research (NIHR) Cambridge Biomedical Research Centre (BRC), Core Biochemical Assay Laboratory.

Covariate measurement. Data on socio-demographic characteristics (age, sex, education level, rural or urban residence) and health-related behaviours (alcohol intake, smoking, physical activity, self-reported fruit and vegetable intake) were collected by interviewers using an adapted version of the WHO STEPS questionnaire[22]. Based on responses to the questions 'have you ever smoked any tobacco product/consumed a drink that contains alcohol?' and 'do you currently smoke any tobacco product/did you consume a drink that contains alcohol within the past 12 months?', smoking status and alcohol intake were categorised as never, past or current.

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Self-reported and objectively measured physical activity data were collected from all participants. Data on self-reported physical activity were collected using the global physical activity questionnaire (GPAQ) and estimates of energy expenditure in different domains (work, leisure and travel) and overall physical activity energy expenditure (GPAQ PAEE) were derived in metabolic equivalents of task-min/week[22]. PAEE was measured objectively over seven continuous days using a combined heart rate and movement sensor (Actiheart; Cambridge Neurotechnology). The validity of this method was assessed in this population against PAEE measured with doubly labeled water ($r = 0{\cdot}40$)[23]. PAEE scaled for body weight was expressed as kJ/kg per d after calibration using individual heart rate. Three categories based on time spent in minutes per day at different intensities of physical activity were created: < 1·5 metabolic equivalents of tasks, sedentary behaviour; 1·5–3 metabolic equivalents of tasks, light physical activity > 3 metabolic equivalents of tasks, moderate to vigorous physical activity[24]. Throughout this manuscript, we use the term PAEE to refer to objectively measured PAEE.

Three measurements of blood pressure were taken using an automated blood pressure measuring device (OMRON M4–1) on the dominant arm of the participants after at least 5 min of rest and at 1-minute intervals. The blood pressure value was computed as the average of the three recordings. Waist circumference was measured to the nearest 0·1 cm in participants wearing light clothing using a non-stretch fiberglass tape at the level of the midpoint between the lower costal margin and the anterior superior iliac crests, and height was measured using a standard rigid stadiometer. Body weight was measured using electronic scales, and body composition was assessed using bioelectrical impedance analysis (Tanita TBF-531 scales; Tanita UK). BMI (kg/m^2) was computed as the body weight (kg) divided by the square of height (m^2).

Outcomes. Outcomes were markers of glucose homeostasis, including fasting glucose, 2-h glucose and homeostatic model assessment of insulin resistance (HOMA-IR)). HOMA-IR was computed using the formula = ((FPI × FBG)/22·5)), where FPI is fasting plasma insulin (mU/l) and FBG is fasting blood glucose (mmol/l)[25].

Statistical analysis

All the statistical analyses were performed using Stata 15 (StataCorp). Descriptive statistics are presented as means ± SD (or median and (25th–75th percentile) for non-normally distributed data) or numbers and percentages. We tested differences in means using the *t* test (or differences in medians using the Mann–Whitney test) and differences in proportions using the chi-squared test. Using the sex-specific cut-offs for defining Zn deficiency recommended by the International Zinc Nutrition Consultative Group, we reported the proportion of participants with plasma Zn deficiency (< 10·7 μmol/l in women and < 11·3 μmol/l in men)[26]. Linear trends across tertiles of plasma Zn were obtained from a linear regression model for continuous variables including tertiles of plasma Zn as a continuous exposure and chi-squared test for trend (Cochran–Armitage test or Cochran–Mantel–Haenszel test for categorical variables with 2 or ≥ 3 levels, respectively). We fitted linear regression models adjusted for age and sex to identify potential correlates of plasma Zn.

To examine the independent associations between plasma Zn and glycaemic markers, we categorised plasma Zn into tertiles and fitted three statistical models incrementally adjusted for potential confounding variables. After fitting crude regression models, we further adjusted for age (continuous) and sex, and then for smoking (never, past or current), alcohol intake (never, past or current), level of education (less than primary school, completed primary school, secondary school and university), residential site (4 sites), PAEE (continuous) and BMI (continuous). *P*-values for trend were obtained from linear regression models including plasma Zn as an ordinal variable across tertile categories. HOMA-IR was log-transformed to account for its skewed distribution. Complete case analysis was performed.

In sensitivity analysis, (a) with missing data (PAEE, *n* 53; 2-h glycaemia, *n* 9; HOMA-IR, *n* 5) assumed to be missing at random, we imputed missing data by using multiple imputations by chained equations to create ten imputed datasets and using Rubin's rules to combine estimates[27]; (b) we further adjusted for self-reported fruit and vegetable intake as a proxy for overall dietary quality in model 3 and (c) replaced BMI by body fat in model 3. We investigated non-linear associations of plasma Zn with glycaemic markers by fitting restricted cubic splines with three knots corresponding to the 25th, 50th and 75th percentile of continuously distributed plasma Zn using model 3 and assessed the shape of the association with glycaemic markers. We tested for effect modification by sex, rural–urban residence and BMI categories on the association between plasma Zn and glycaemic markers and subgroup analysis was performed if the p–value for interaction was < 0·05.

Results

The characteristics of the study participants are presented in Supplementary Tables S1 and S2. Of the 596 participants with measurements for plasma Zn, 63·3 % were women. The mean (±SD) age of the participants was 38·3 ± 8·6 years. There was no difference in the mean plasma Zn concentration between rural (13·5 ± 2·9 μmol/l) and urban (13·8 ± 2·6 μmol/l) participants, *P*-value = 0·35. The mean concentration of plasma Zn was 13·7 ± 2·7 μmol/l with higher levels in men (14·4 ± 2·9 μmol/l) than in women (13·2 ± 2·6 μmol/l), *P*-value < 0·0001. Using pre-established cut-offs for plasma Zn deficiency, 13·8 % of women had plasma Zn concentrations below 10·7 μmol/l and 11·9 % of men below 11·3 μmol/l.

Level of education and smoking status were positively associated with plasma Zn while female sex, physical activity, self-reported vegetable intake, 2-h glucose and adiponectin were all inversely associated (Tables 1 and 2). There was no evidence of a linear trend in fasting glucose and HOMA-IR across increasing tertiles of plasma Zn.

After adjusting for age, and sex, positive correlates of plasma Zn were male sex (adjusted for age only) and measures of

Table 1. Socio-demographic and behavioural characteristics by tertiles of plasma Zn concentrations (Cameroon study, *n* 596)

	Plasma Zn categorised by tertiles						
	1st		2nd		3rd		
Characteristics	*n*	%	*n*	%	*n*	%	*P* for linear trend
Zn (μmol/l), range	6·2–12·4		12·5–14·5		14·6–25·8		
Age (years)							
Mean	38·7		38·9		37·3		0·09
SD	8·1		8·9		8·8		
Sex							
Women	146	72·6	127	64·1	104	52·8	< 0·001
Education							0·04
Completed							
< Primary school	36	18·0	37	18·7	30	15·2	
Primary school	100	50·0	83	41·9	81	41·1	
Secondary School	41	20·5	53	26·8	56	28·4	
University	23	11·5	25	12·6	30	15·2	
Smoking status							0·02
Never	165	82·1	155	78·3	144	73·1	
Past	26	12·9	26	13·1	27	13·7	
Current smoker	10	5·0	17	8·6	26	13·2	
Alcohol intake							0·07
Never	21	10·4	17	8·6	27	13·7	
Past	14	7	22	11·1	22	11·2	
Current	166	82·6	159	80·3	148	75·1	
Residence							
Rural	100	49·7	77	38·9	98	49·7	0·99
	Mean	SD	Mean	SD	Mean	SD	
PAEE (kJ/kg per d)	51·5	23·6	51·3	22·2	48·7	23·7	0·26
Sedentary time (min/d)	960·9	154·1	937·0	148·8	968·9	154·7	0·63
LPA time (min/d)	356·2	103·7	381·2	105·2	362·7	108·8	0·57
MVPA time (min/d)	122·9	90·3	121·7	84·3	108·4	85·3	0·13
	Median	25th–75th percentile	Median	25th–75th percentile	Median	25th–75th percentile	
GPAQ PAEE (kJ/kg per d)	48·6	6·7–146·9	20·3	3·3–113·7	7·6	3·2–71·2	< 0·001
GPAQ work (MET-min/week)	3840	0–12 480	0	0–10 080	0	0–5760	< 0·001
GPAQ leisure (MET-min/week)	0	0–0	0	0–0	0	0–0	
GPAQ travel (MET-min/week)	1440	560–3360	840	280–3360	840	280–1680	0·002
Fruit intake (times/week)	2	1–6	3	1–5	2	1–3	0·09
Vegetable intake (times/week)	6	1–8	4	2–8	3	2–5	< 0·001

PAEE, physical activity energy expenditure; LPA, light physical activity; MVPA, moderate to vigorous physical activity; GPAQ, Global Physical Activity Questionnaire; MET, metabolic equivalents of task.
n 596 except for PAEE where *n* 543.
Results are presented as arithmetic mean ± SD (or median (25th–75th percentile) for non-normally distributed variables) or *n* (%). *P*-values for trend are from a chi-squared test for trend for categorical variables or from a linear regression model for continuous variables including tertile of plasma Zn as a continuous exposure.

adiposity (online Supplementary Table S3). Measures of physical activity and self-reported intake of fruit and vegetables were negatively correlated with plasma Zn. Rural/urban residential site was not associated with plasma Zn concentrations.

Table 3 shows the results of multiple linear regression analyses between plasma Zn concentration and glycaemic markers. 2-hour glucose was lower by −0·63 (95 % CI − 1·02, −0·23) mmol/l among those in the highest tertile of plasma Zn compared with those in the lowest tertile (*P*-value for linear trend 0·002) in unadjusted analysis. This remained significant in model 3 adjusted for age, sex, smoking status, alcohol intake, education level, area of residence, adiposity and objectively measured physical activity (β: −0·43(95 % CI − 0·82, −0·04) mmol/l, *P*-value for linear trend = 0·03.

Similar inverse associations were observed between plasma Zn and fasting glucose and HOMA-IR after adjusting for potential confounders. Compared with participants in the lowest tertile of plasma Zn, being in the highest tertile was associated with lower fasting glucose (–0·25 (–0·48, −0·01) mmol/l) and lower HOMA-IR (–0·23(–0·44, −0·03)), both *P*-value for linear trend < 0·05 in a multivariable model adjusted for socio-demographic characteristics and health-related behaviours (model 3). Results were unchanged in sensitivity analyses further adjusting for self-reported fruit and vegetable intake or BMI replaced by body fat in model 3.

There was no evidence of a non-linear association between plasma Zn and any of the outcomes using restricted cubic splines. The test for interaction between sex, rural/urban area of residence or BMI categories and plasma Zn concentrations on any of the glycaemic markers was not significant.

Discussion

In this population-based cross-sectional study of 596 participants in Cameroon, we observed that plasma Zn concentration was inversely associated with glycaemic markers (2-h glucose, fasting glucose and HOMA-IR). The inverse associations between

Table 2. Metabolic characteristics of the population by tertiles of plasma Zn concentrations (Cameroon study, *n* 596)

	Plasma Zn categorised by tertiles						
	1st		2nd		3rd		
Characteristics	Mean	SD	Mean	SD	Mean	SD	*P* for linear trend
Waist circumference (cm)	88·0	12·2	89·2	12·2	88·6	12·0	0·63
BMI (kg/m^2)	25·9	5·3	26·4	5·2	25·9	5·2	0·97
Body fat (%)	28·8	11·1	28·9	10·7	26·7	11·4	0·05
Systolic blood pressure (mmHg)	120·7	20·5	122·6	22·1	124·6	19·6	0·07
Diastolic blood pressure (mmHg)	75·2	12·8	76·8	13·9	77·3	13·4	0·11
Fasting glucose (mmol/l)	4·82	1·06	4·79	1·31	4·71	1·61	0·40
2-hour glucose (mmol/l)	6·68	1·85	6·20	1·78	5·99	1·99	0·0003
Fasting insulin (pmol/l)							
Median	21·7		18·8		22·7		0·63
25th–75th percentile	11·5–37·5		11·0–34·8		12·4–33·4		
HOMA-IR index							
Median	0·75		0·66		0·76		0·48
25th–75th percentile	0·37–1·32		0·34–1·23		0·39–1·21		
Total cholesterol (mmol/l)	3·78	0·97	3·85	0·99	3·91	0·97	0·18
HDL cholesterol (mmol/l)	1·20	0·35	1·23	0·34	1·24	0·30	0·29
LDL cholesterol (mmol/l)	2·18	0·84	2·25	0·84	2·30	0·84	0·17
TAG (mmol/l)							
Median	0·75		0·73		0·74		0·59
25th–75th percentile	0·61–0·93		0·58–0·92		0·57–0·98		
CRP (mg/l)							
Median	3·97		5·28		5·43		0·44
25th–75th percentile	2·44–7·37		2·69–8·39		2·47–10·12		

HOMA-IR, Homeostatic model assessment of insulin resistance; CRP, C-reactive protein.
(*n* 596, except for 2-h glycaemia where, *n* 587 and HOMA-IR, *n* 591).
Results are presented as an arithmetic mean ± SD (or median (25th–75th percentile) for non-normally distributed variables) or *n* (%). *P*-values for trend are from a chi-squared test for trend for categorical variables or from a linear regression model for continuous variables, including tertile of plasma Zn as a continuous exposure.

Table 3. Associations between plasma Zn concentrations and glycaemic markers (Cameroon study)

		Tertile 2		Tertile 3		
	Tertile 1	*β*-coefficient	95 % CI	*β*-coefficient	95 % CI	*P*-value for linear trend
Fasting glucose (mmol/l) (*n* 543)						
Model 1	1·0 (ref)	−0·04	−0·29, 0·21	−0·20	−0·45, 0·04	0·10
Model 2	1·0 (ref)	−0·03	−0·27, 0·20	−0·18	−0·41, 0·06	0·14
Model 3	1·0 (ref)	−0·06	−0·29, 0·17	−0·25	−0·48, −0·01	0·04
2-h glucose (mmol/l) (*n* 536)						
Model 1	1·0 (ref)	−0·49	−0·86, −0·12	−0·63	−1·02, −0·23	0·002
Model 2	1·0 (ref)	−0·47	−0·83, −0·11	−0·54	−0·94, −0·15	0·007
Model 3	1·0 (ref)	−0·42	−0·77, −0·07	−0·43	−0·82, −0·04	0·03
HOMA-IR (*n* 540)						
Model 1	1·0 (ref)	−0·07	−0·27, 0·14	−0·02	−0·24, 0·19	0·81
Model 2	1·0 (ref)	−0·02	−0·22, 0·18	0·07	−0·14, 0·28	0·53
Model 3	1·0 (ref)	−0·13	−0·30, 0·05	−0·23	−0·44, −0·03	0·02

HOMA-IR, Homeostatic model assessment of insulin resistance.
Model 1: Unadjusted.
Model 2: Adjusted for age and sex.
Model 3: model 2 + smoking status, alcohol intake, education level, residential site (4 sites), BMI (continuous) and objectively measured physical activity (continuous).

plasma Zn and fasting glucose and HOMA-IR became significant only after adjusting for socio-demographic characteristics and health-related behaviours. To our knowledge, this is the first population-based study in a sub-Saharan African population to examine the relationship between plasma Zn concentrations and markers of glucose homeostasis.

There are limited data from representative surveys on plasma Zn distribution from Africa in part because of the financial and technical resources required to analyze plasma Zn compared with the assessment of dietary Zn intake. The mean plasma Zn concentration in our study was comparable to those reported in studies in the USA[28] and Europe[29,30], but higher than in previous studies in Africa[20,31–33]. This could be because the previous studies in Africa were conducted mostly in children (< 5 years) or women of reproductive age. These population sub-groups are known to have higher Zn turnover[29,30]. In addition, blood samples in some of the studies were collected in non-fasting participants and in the afternoons. Plasma Zn concentrations follow a diurnal variation and are higher in the mornings and in fasting participants[15].

Previous epidemiological studies on the association between dietary Zn intake and diabetes have been limited by measurement error of dietary Zn assessment and show inconclusive results[12–14,34]. The quantification of plasma Zn offers the advantage of being an objective marker of both dietary Zn intake and body stores but has not been widely applied to test diet–disease association, probably owing to the high cost of the plasma Zn analysis compared with the assessment of dietary Zn intake using subjective methods. As a result, evidence from previous observational studies using plasma Zn concentration is limited, with the majority of the studies coming from China[16,35–38].

Our findings of an inverse association between plasma Zn concentrations and glycaemic markers are consistent with previous case–control studies showing that higher plasma Zn concentration was associated with lower odds of type 2 diabetes[18,36,37]. In contrast, In contrast, a cross-sectional study in the USA in 5153 adults reported that higher serum Zn concentration was associated with higher odds of pre-diabetes and diabetes[16]. A similar positive association between serum Zn and risk of type 2 diabetes was reported in a 20-year prospective study of middle-aged and older men in Finland[17]. In these studies showing a positive association between serum Zn and type 2 diabetes, the prevalence of low blood Zn concentration was low and it is has been suggested that excessive bioavailability of Zn may lead to overactive β-cells and eventually β-cell failure due to prolonged overactivity of the β-cell[39].

Some of these previous studies investigating the association between plasma Zn concentration and diabetes prevalence or risk did not account for health-related behaviours such as physical activity that confound the relationship between Zn and glycaemia. In this study, self-reported fruit and vegetable intake and physical activity were inversely associated with plasma Zn concentrations. Fruit and vegetable intake may be a proxy for a diet low in meat or high in plant-rich diets in this study, which are high in phytates that bind to Zn to form an insoluble complex, thereby inhibiting Zn absorption in the intestines[7]. The inverse association between plasma Zn concentrations and physical activity is consistent with previous studies suggesting that physical activity promotes higher Zn excretion in sweat and urine[34].

Plasma Zn concentrations respond to Zn supplementation[31,40]. To date, published studies of supplementation trials of Zn for diabetes prevention and management are mostly of small sample sizes and short duration. Previous meta-analysis of intervention studies reported a reduction in fasting glucose, postprandial glucose, glycated haemoglobin, fasting insulin and HOMA-IR with Zn supplementation compared with controls[41,42]. Notably, over 80 % of the studies included in this meta-analysis were of short duration (< 1 year) and from Asia, where inadequate Zn exposure from low dietary Zn and high phytate intakes are prevalent[9]. A Mendelian randomisation study reported no causal association between blood Zn and risk of type 2 diabetes[43]. However, uncertainty remains due to the small sample size and only two SNP included in the analyses.

Mechanistic evidence of the potential role of Zn in the pathogenesis of type 2 diabetes comes from animal and human studies[11,44,45]. Zn has a positive effect on insulin signaling in the skeletal muscles by stimulating the tyrosine phosphorylation of insulin receptors, thus promoting glucose uptake[44]. Moreover, Zn is found in abundance in the pancreatic β-cells and is essential for the synthesis of Zn-insulin crystals (insulin crystallisation), the form in which insulin is stored in the pancreas. It has been suggested that the type, size and morphology of the Zn-insulin crystals regulate the conversion of pro-insulin to insulin[45]. Zn also appears to be an insulin-mimetic with the potential to modulate insulin storage, secretion and receptor signal transduction[11,44]. Some of the anti-inflammatory effects of Zn could explain its beneficial role in diabetes. Finally, Zn also acts as a co-factor for superoxide dismutase and other enzymes against oxidative stress[46].

We did not find evidence of a non-linear association between plasma Zn and any of the glycaemic markers. However, a recent large cohort study in China showed a U-shaped relationship between dietary Zn intake and diabetes risk, with an inflection point at 9·1 mg/d[34]. Future prospective studies are needed to confirm our findings of an inverse association between plasma Zn and diabetes. If evidence of a beneficial effect of Zn in diabetes is shown in intervention studies, public health strategies to increase dietary Zn intake may offer a cheap and complementary primary prevention approach for diabetes.

Strengths and limitations

The major strength of this study lies in the use of an objective indicator of Zn status since the measurement of plasma Zn concentration does not rely on memory. Inadequate Zn status may result from insufficient dietary Zn intake, but also poor dietary Zn absorption (e.g. high dietary phytate intake that inhibits Zn absorption). Thus, in low- and middle-income settings where dietary phytate intake is high, plasma Zn may be a better indicator of Zn exposure than the estimated dietary intake of Zn[10,47]. In addition, variability in the bioavailability of Zn from foods limits its dietary Zn assessment using subjective methods[15]. Food composition tables are sometimes unavailable for local food items to calculate Zn and phytate intakes accurately, which is a drawback as the Zn content of plant-based foods may be influenced by soil Zn concentrations and phytates inhibit dietary Zn absorption[15]. Plasma Zn concentration has also been shown to respond to dietary Zn intake and Zn supplementation and is a useful indicator of Zn status at the population level[48]. However, many factors independent of Zn statuses such as infections, inflammation, duration of fasting, pregnancy and hormonal contraceptives affect plasma Zn concentrations[26]. We conducted these analyses in a population-based study with the inclusion of participants from rural and urban areas of Cameroon and adjusted for socio-demographic characteristics and health-related behaviours in the analysis.

This study has several limitations. The blood samples were not collected in trace element-free tubes, and the resulting plasma Zn concentrations may have been affected by contamination from environmental Zn, including Zn from the tubes in which the samples were stored or even the long-term storage. However, plasma Zn appears to be relatively stable after long-term storage, and the contamination from tubes has been shown to be minimal[49]. Even if plasma Zn concentrations were affected by contamination or long-term storage, this is unlikely to affect our observed associations as the effect of storage or

contamination was likely to be random. In this cross-sectional study, we used fasting glucose, 2-hour glucose and HOMA-IR as intermediate markers of key pathways to diabetes rather than simply the prevalence of diabetes. Fasting and 2-hour glucose are also the means by which diabetes diagnosis is made, thus we are able to study the links between Zn status and diabetes risk, plus study its relationship with insulin resistance as a major pathophysiological pathway to diabetes. The estimates of insulin resistance derived from HOMA-IR are strongly correlated with estimates from the hyperinsulinaemic clamp ($r = 0{\cdot}88$), widely considered the gold standard for assessing insulin sensitivity[25]. The absence of a measure of insulin secretion meant we were unable to study how Zn status might on diabetes risk via an effect on early-phase insulin secretion. Another limitation is the cross-sectional study design of this study, which means we cannot rule out the possibility of reverse causation as an explanation of the associations observed. For instance, it is possible that the chronic hyperglycaemia present in patients with diabetes increases oxidative stress by producing free radicals, which leads to lower Zn concentrations since Zn has anti-oxidative properties. Diabetes may also lead to low levels of Zn due to the higher levels of Zn excretion from the kidneys[50]. Despite our attempt to control for a wide range of potential confounders, our observed associations may be affected by residual confounding. Our study was conducted in healthy adults aged 25–55 years, and our findings may not be generalisable outside of this age range and beyond the geographical location in which the study was conducted.

Conclusion

Our population-based study in rural and urban Cameroon shows that plasma Zn concentration was independently inversely associated with fasting glucose, 2-h glucose and HOMA-IR. This suggests a role of Zn in glucose metabolism, possibly involving both insulin secretion and insulin resistance. Given that Zn is a biomarker that is elevated by intake of protein-rich foods, further work is required to disentangle the specific effects of Zn on diabetes from the effects of the food groups that influence Zn status. Additionally, the current cross-sectional findings should be investigated in prospective study designs.

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Supplementary material

For supplementary material/s referred to in this article, please visit https://doi.org/10.1017/S0007114523000223

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