Clinical Nutrition 42 (2023) 972–986

Contents lists available at ScienceDirect

Clinical Nutrition

journal homepage: http://www.elsevier.com/locate/clnu

Original article

Association of plasma zinc levels with anti-SARS-CoV-2 IgG and IgA seropositivity in the general population: A case–control study

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ARTICLE INFO

Article history:
Received 10 February 2023
Accepted 4 April 2023

Keywords:
Micronutrients
Copper
Vitamin D
Deficiency
Public health
Immunity

SUMMARY

Introduction: Some micronutrients have key roles in immune defence, including mucosal defence mechanisms and immunoglobulin production. Altered micronutrient status has been linked with COVID-19 infection and disease severity. We assessed the associations of selected circulating micronutrients with anti-SARS-CoV-2 IgG and IgA seropositivity in the Swiss community using early pandemic data.
Methods: Case-control study comparing the first PCR-confirmed COVID-19 symptomatic cases in the Vaud Canton (May to June 2020, n = 199) and *controls* (random population sample, n = 447), seronegative for IgG and IgA. The replication analysis included seropositive (n = 134) and seronegative (n = 152) close contacts from confirmed COVID-19 cases. Anti-SARS-CoV-2 IgG and IgA levels against the native trimeric spike protein were measured using the Luminex immunoassay. We measured plasma Zn, Se and Cu concentrations by ICP-MS, and 25-hydroxy-vitamin D_3 ($25(OH)D_3$) with LC-MS/MS and explored associations using multiple logistic regression.
Results: The 932 participants (54.1% women) were aged 48.6 ± 20.2 years (±SD), BMI 25.0 ± 4.7 kg/m^2 with median C-Reactive Protein 1 mg/l. In logistic regressions, $\log_2$(Zn) plasma levels were negatively associated with IgG seropositivity (OR [95% CI]: 0.196 [0.0831; 0.465], P < 0.001; replication analyses: 0.294 [0.0893; 0.968], P < 0.05). Results were similar for IgA. We found no association of Cu, Se, and $25(OH)D_3$ with anti-SARS-CoV-2 IgG or IgA seropositivity.
Conclusion: Low plasma Zn levels were associated with higher anti-SARS-CoV-2 IgG and IgA seropositivity in a Swiss population when the initial viral variant was circulating, and no vaccination available. These results suggest that adequate Zn status may play an important role in protecting the general population against SARS-CoV-2 infection.
Registry: Corona Immunitas:: ISRCTN18181860.

1. Introduction

Early 2020, the world was confronted with one of the worst respiratory virus pandemics since the 1918 Spanish flu. Three years later, we remain confronted with multiple unanswered questions related to the host immunity against SARS-CoV-2. The COVID-19 pandemic has highlighted the higher risk of some groups such as obese [1], and old adults [2,3] to develop severe forms of the

Abbreviations: $25(OH)D_3$, vitamin D3; CRP, C-reactive protein; Cu, copper; IgA, immunoglobulin A; IgG, immunoglobulin G; ICP-MS, inductively coupled plasma mass spectrometry; UPLC-MS/MS, ultra-performance liquid chromatography tandem mass spectrometry; MN, micronutrient; Se, selenium; PCR, polymerase chain reaction; Zn, zinc; MFI, Mean Fluorescence Intensity.

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https://doi.org/10.1016/j.clnu.2023.04.007

disease. These two groups are frequently malnourished and exhibit dysregulated immune responses [4].

Some micronutrients (MNs) have key roles in immune defence [5,6], including in the mucosal defence mechanisms, critical for the prevention of infection or colonization of the respiratory tract by pathogens. Respiratory epithelial cells have been shown to mediate the active transport of polymeric immunoglobulin-A (IgA) from the lamina propria to the airway lumen [7]. IgA exists under a monomeric and polymeric form, often called "secretory IgA" present in mucous membranes. The role of IgA in the defence of mucosal surfaces has expanded from a limited scavenger role for exogenous material to a broader protective function [7]. Very recently, the role of IgA in the immunity against the omicron variant has been stressed [8]. Among the MNs, vitamins A, C, D and E, as well as copper (Cu), zinc (Zn) and selenium (Se) have been shown to modulate the humoral response and especially the synthesis of the immunoglobulins IgA and IgG [5,9,10].

Searching for SARS-CoV-2 infection risk factors, multiple reports have linked an altered MN status with COVID-19 infection and disease severity [11]. As early as 2020, Chinese authors reported positive correlations between COVID-19 disease and low Se status [12], and German authors with altered Zn status [13]. Similar observations regarding plasma Zn and Se levels were reported in Belgium [14]. In Switzerland, a high prevalence of MN deficiencies (vitamin D, vitamin A, Zn and Se) was observed at admission in 57 consecutively hospitalized COVID-19 patients [15]. A systematic review, including 49 studies [16], confirmed significantly lower Zn, iron (Fe), and Se blood levels in COVID-19 patients than healthy controls; levels being lower among severe COVID-19 cases [16]. Of note the levels of inflammation which causes micronutrient redistribution was not documented.

Vitamin D_3 ($25(OH)D_3$) has been widely investigated as it supports immune defences against viral infection in general [17–20]. The largest meta-analysis, including 38 studies published up to April 2021 including over 200′000 participants [21], seemed to show that vitamin D supplementation was associated with significantly lower risk of severe COVID-19 disease and mortality [22–24]. Calcifediol ($25(OH)D_3$) administration might have protective effects in the sickest critical care patients [23]. A large case–control study from Catalonia including 4′643′139 subjects [25], pointed towards a slight protection against SARS-CoV-2 infection. But uncertainty persists, as shown by another meta-analysis including publications until January 2022, which found no significant effect of vitamin D supplementation on the risk of COVID-19 infection, insufficient data to analyse the association with the risk of hospitalization, but a reduced risk of ICU admission with supplementation [26].

European studies indicate suboptimal and even deficient status in a range of MNs particularly in old adults [27,28] and in people with low dietary intakes [29]. Switzerland seems to have higher prevalence rates of suboptimal status than the neighbouring countries for several MNs [30] with suboptimal Se [31], Fe, and Zn status [30], and suboptimal vitamin D status, with low 25(OH)D plasma levels being common in various regions of the country [32]. Further, the intake of omega-3 polyunsaturated fatty acids (PUFA), which are required for the mitigation of inflammation [6], has also been shown to be low in Switzerland [33].

The present project is an ancillary study of the *SeroCOVID* project, which aimed at understanding the transmission of the virus, the evolution of herd immunity in the population, and the prevalence of IgG and IgA antibodies against SARS-CoV-2 in the canton of Vaud, Switzerland. The aim of the present analysis was to investigate the link between the status of these selected micronutrients and the SARS-CoV-2 status, using a case–control design from early pandemics data [34]. We hypothesized that anti-SARS-CoV-2 IgG and IgA seropositivity would be associated with alterations of circulating copper, selenium, zinc, and $25(OH)D_3$ levels.

2. Methods and participants

2.1. Study design

We conducted a case–control study using as *cases* PCR confirmed COVID-19 cases in the Canton of Vaud (from February 27 to April 1, 2020) and as *controls* a simultaneous random sample of the general population of the same region from the Federal Office of Statistics, who were seronegative for both IgG and IgA.

SeroCOViD is a sero-epidemiological study comprising multiple cross-sectional surveys which took place every 3–8 months since May 2020. As part of a Swiss national program named Corona Immunitas [35], it aims to assess the evolution of anti-SARS-CoV-2 immunity, to estimate the impacts of prevention and vaccination policies, and to evaluate new anti-SARS-CoV-2 immunity measurement methods in the population of the Canton of Vaud (French-speaking region of Switzerland). The data analysed in the current study were collected during the first wave [34], which took place in May and June 2020 and comprised a self-administered questionnaire as well as the collection of venous blood samples.

While almost all PCR confirmed COVID-19 cases developed anti-SARS-CoV-2 antibodies, the general population presented low levels of viral exposure at that time. We used, as a replication sample, seropositive and seronegative close contacts, selected from people who shared their household with laboratory-confirmed COVID-19 cases and people identified via contact tracing. Unlike the general population, these close contacts were all highly exposed to the virus, as illustrated by an anti-SARS-CoV-2 seropositivity close to 50%. At that time, a close contact was defined by local public health authorities as having had an exposure of at least 15 min within 2 m of an infected individual during his or her infectious period.

2.2. Participants

Participants were eligible for inclusion in the survey and blood sample reuse if they were aged 14 years or older, resided in the Canton of Vaud and excluded if they could not give their informed consent. The study flowchart is shown in Fig. 1: study participants with missing data on variables of interest were excluded (IgG and IgA serostatus, age, gender, anthropometric data, smoking status, Zn, Se, Cu, $25(OH)D_3$ and CRP plasma levels), resulting in a total sample of $N = 961$ individuals. Participation rates were estimated earlier at 49.0% for index cases, at 71.7% and 50.4% for household and outside household close contacts, respectively [34], and at 32.9% for the general population [36].

The current study was approved by the Ethics Commission of the Canton of Vaud (ID 2020–00887), and written informed consent was obtained from all participants.

2.3. Measurements

Participants first filled an online questionnaire (available in French and English), which allowed the collection of sociodemographic characteristics, medical history, and symptoms. Participants then visited one of the four study centres (Epalinges, Nyon, Rennaz and Yverdon-les-Bains) for a venous blood sample collection, with aliquots stored at −80 °C in the institutional biobank, as previously described [34]. The whole process took place in May and June 2020.

Anti-SARS-CoV-2 IgG and IgA levels against the native trimeric spike protein were measured using a locally developed Luminex

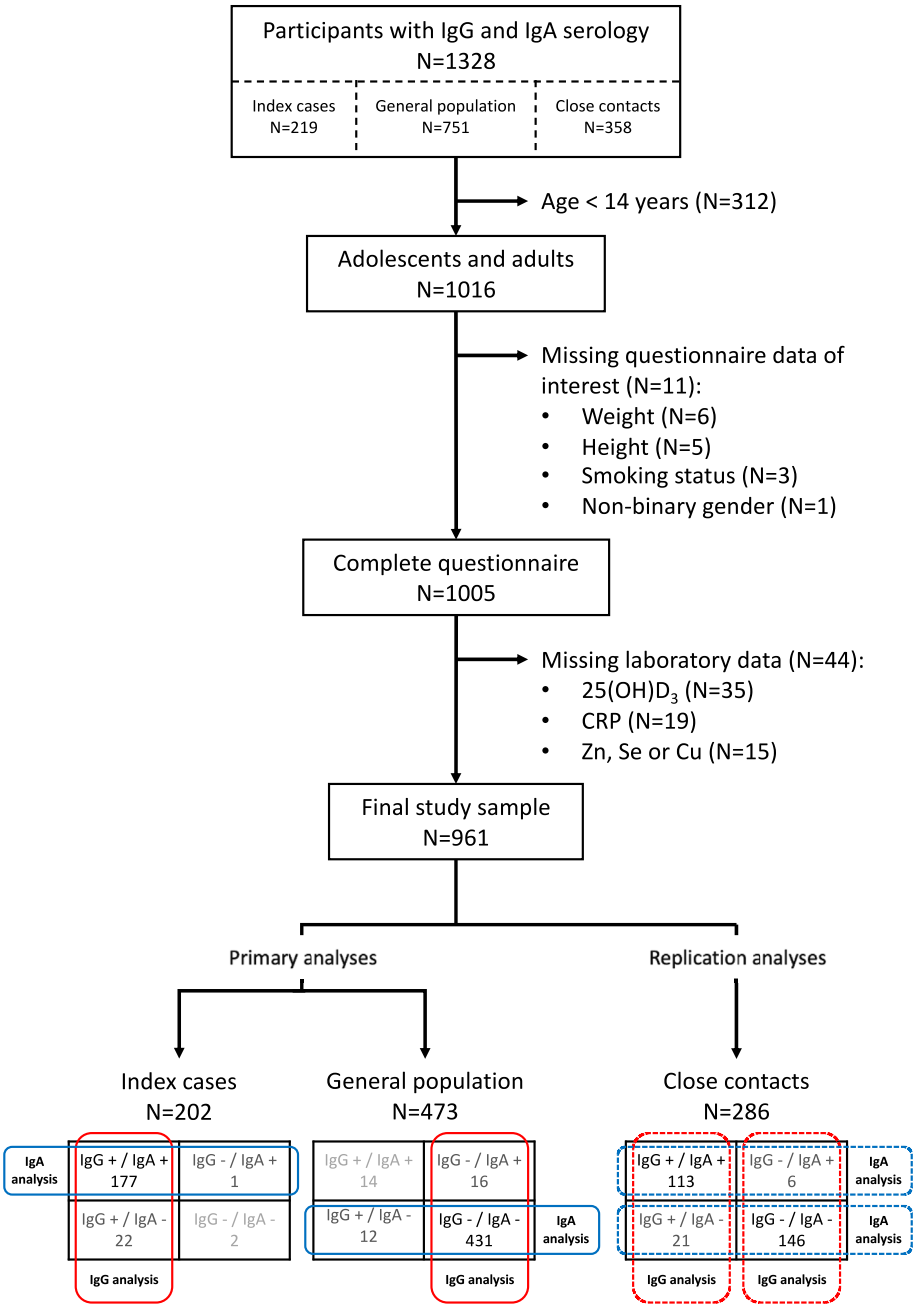


Fig. 1. Flowchart of the study. The Flowchart shows all steps between participants' recruitment from a population-based sample to the final study sample.

immunoassay [37]. Seropositivity was defined as antibody Mean Fluorescence Intensity (MFI) ratios of >6 for IgG and ≥6.5 for IgA, relative to mean controls. With these defined thresholds, the specificity of the test was estimated at 99.7% for IgG and 98.5% for IgA, using prepandemic blood samples of healthy and non-SARS-CoV-2 coronavirus-infected individuals. Sensitivity analyses were performed in hospitalized patients with moderate to severe symptoms and resulted in a sensitivity of 96.6% and 90.0%, for IgG and IgA, respectively, 16–33 days post-symptoms onset [37].

For the current study, we reused the biobanked blood samples to measure trace elements, 25(OH)D_3, as well as CRP levels (required to exclude active inflammation and related blood level alterations) [38]. The plasma concentrations of Zn, Se and Cu were measured using inductively coupled plasma mass spectrometry (ICP-MS; 7700 Series; Agilent, Palo Alto; see Appendix Tables 4–6) [39], while the Masstrak Vitamin D kit (from Waters) allowed the simultaneous determination of 25(OH)D_3 and D_2 plasma levels, by ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS): D_2 could only be detected in 36 patients, leading to only D_3 being used in analysis. Finally, CRP plasma levels were obtained by immuno-turbidimetry on latex particles.

The micronutrient reference ranges were: Zn 608–1007 ng/ml, Se 84–151 ng/ml, Cu 639–1706 ng/ml, and 25(OH)D_3 50–125 nmol/l (20–50 ng/ml). For Cu, Zn and Se, these ranges correspond to percentiles 5 and 95 from the SKIPOGH study, a population-based cohort of adults recruited in Switzerland between 2009 and 2012 [40,41]. The 25(OH)D_3 reference ranges are provided by the Swiss Federal Office of Public Health (https://www.bag.admin.ch). For CRP, plasma levels <10 mg/l are considered normal values.

2.4. Statistical analyses

We calculated the associations of log_2-transformed levels of Zn, Se, Cu, and 25(OH)D_3 with anti-SARS-CoV-2 (1) IgG and (2) IgA seropositivity, which, taken one at-a-time, were the two outcome variables of interest. We used five different logistic regression models for the population-based case–control design as well as for the close contact design (replication study).

Four unadjusted models separately included plasma Zn, Se, Cu, and 25(OH)D_3 levels as independent variables of interest. In a fifth model, we included all four micronutrients while further adjusting for age, gender, log_2-transformed BMI, smoking status and square root-transformed CRP levels.

Since micronutrients were log-transformed on base 2 in the logistic regression models, the corresponding estimated odds-ratios gave information on the increased (or decreased) odds of being seropositive when micronutrient levels were doubled.

All analyses were conducted using Stata/BE, version 17.0. A two-sided P value < 0.05 was considered as statistically significant.

3. Results

Altogether 932 participants were included for the IgG analyses (Fig. 1): 646 for the population-based case–control study (199 cases and 447 controls) and 286 for the replication analyses in close contacts (134 IgG positive and 152 IgG negative). The IgA analyses included in 907 participants, 178 of them as cases, 443 as controls (corresponding to 621 participants for the primary analyses), and the same 286 close contacts (119 IgA positive and 167 IgA negative).

Selected general, serological, and micronutrient characteristics of the cases and controls included in the IgG analyses, as well as of IgG seropositive and seronegative close contacts, are summarized in Table 1 (Appendix Table 1 shows the slightly different numbers for the IgA analyses). On average, seropositive subpopulations tended to present higher BMI and lower smoking prevalence, but similar gender and age distributions, compared to seronegative groups.

Anti-SARS-CoV-2 IgG and IgA levels, as displayed in Fig. 2, were similarly high in cases and seropositive close contacts, and comparatively low in controls and seronegative close contacts. Appendix Figure 1 illustrates the bimodal distribution of IgG and IgA levels in close contacts.

Most of the participants presented with no inflammation, as CRP plasma levels exceeded 10 mg/l in only 30 participants, with the highest value at 32 mg/l. While Zn and 25(OH)D_3 levels did not vary much with CRP concentrations, measured Se levels, respectively Cu levels, were significantly lower, respectively higher, in these 30 participants (see Appendix Figure 2).

Figure 3 shows the distribution of plasma Zn, Se, Cu and 25(OH)D_3 levels across cases, controls, seropositive and seronegative close contacts, for both IgG and IgA. Overall, the proportion of participants with deficiency (i.e. with a plasma level below the lower reference range) was 12.1% for Zn, 8.5% for Se, 0.8% for Cu, and 45.2% for 25(OH)D_3. Cases and seropositive close contacts had similarly lower Zn distributions compared to controls and seronegative close contacts. No major difference between subpopulations was observed for both Se, Cu and 25(OH)D_3 distributions. Worth noting is that median 25(OH)D_3 plasma levels approached 50 nmol/l in all groups, meaning that almost half of the participants were deficient according to current definitions [42].

Appendix Figure 3 displays the distribution of plasma Cu/Zn ratios across IgG and IgA status and subpopulations, showing globally median Cu/Zn ratios above the normal ratio of 1:1, and the highest ratios in the COVID cases. Appendix Figure 4 shows the Se/Cu ratio distributions across the same subpopulations, with no significant difference between them.

3.1. Associations of micronutrients with anti-SARS-CoV-2 serostatus

The associations of log_2-transformed Zn, Se, Cu, and 25(OH)D_3 plasma levels with anti-SARS-CoV-2 IgG and IgA seropositivity are detailed in Tables 2 and 3. In unadjusted models, only Zn levels were significantly negatively associated with seropositivity, both for IgG and IgA. This association remained significant after adjusting for other micronutrients and confounding parameters, and could be replicated in close contact analyses.

Other micronutrients levels were not associated with anti-SARS-CoV-2 seropositivity. Concerning covariables, IgG and IgA seropositivity were positively associated with BMI and negatively

Table 1
Participants' general, serological and micronutrient characteristics across subpopulations (IgG analysis, N = 932).

	Primary analyses			Replication analyses		
	Cases	Controls	*P* value (statistic)	Close contacts IgG +	Close contacts IgG -	*P* value (statistic)
N	199	447		134	152	
Age (years), mean (SD)	49.3 (18.7)	50.4 (22.3)	*0.56 (T_{644} = -0.59)*	46.9 (18.2)	43.8 (16.1)	*0.13 (T_{284} = 1.52)*
Gender, n (% women)	109 (54.8)	244 (54.6)	*0.96 (χ^2_1 = 0.00)*	69 (51.5)	82 (53.9)	*0.68 (χ^2_1 = 0.17)*
BMI (kg/m^2), mean (SD)	25.9 (5.1)	24.7 (4.5)	***0.003*** *(T_{644} = 2.95)*	25.3 (5.1)	24.6 (4.4)	*0.21 (T_{284} = 1.26)*
Smoking, n (% current smoker)	23 (11.6)	94 (21.0)	***0.004*** *(χ^2_1 = 8.33)*	20 (14.9)	33 (21.7)	*0.14 (χ^2_1 = 2.17)*
CRP (mg/l), median (IQR)	1 (0; 2)	1 (0; 2)	*0.43 (T_{644} = 0.79)*	0 (0; 2)	0 (0; 2.5)	*0.26 (T_{284} = -1.13)*
SARS-CoV-2 serology						
IgG (semi-quantitative[a]), median (IQR)	67.0 (38.7; 95.2)	0.4 (0.2; 0.8)	***<0.001*** *(T_{644} = 40.4)*	60.9 (41.6; 89.0)	0.4 (0.2; 0.9)	***<0.001*** *(T_{284} = 25.6)*
IgG positivity [b], n (% positive)	199 (100.0)	0 (0.0)	*n.s.*	134 (100.0)	0 (0.0)	*n.s.*
IgA (semi-quantitative[a]), median (IQR)	22.6 (10.5; 51.7)	0.7 (0.4; 1.4)	***<0.001*** *(T_{644} = 9.24)*	22.5 (9.9; 60.6)	0.6 (0.3; 1.1)	***<0.001*** *(T_{284} = 6.18)*
IgA positivity [b], n (% positive)	177 (88.9)	16 (3.6)	***<0.001*** *(χ^2_1 = 479)*	113 (84.3)	6 (3.9)	***<0.001*** *(χ^2_1 = 189)*
Micronutrients						
Serum Zn (ng/ml), median (IQR)	707 (625; 791)	733 (670; 798)	***0.004*** *(T_{644} = -2.86)*	692 (639; 766)	733 (662; 801)	***0.032*** *(T_{284} = -2.15)*
Serum Se (ng/ml), median (IQR)	103 (94.7; 115)	103 (93.1; 113)	*0.88 (T_{644} = 0.16)*	103 (94.6; 115)	107 (98.3; 115)	*0.20 (T_{284} = -1.29)*
Serum Cu (ng/ml), median (IQR)	971 (875; 1123)	970 (840; 1101)	*0.45 (T_{644} = 0.76)*	959 (871; 1127)	958 (848; 1103)	*0.97 (T_{284} = 0.04)*
25(OH)D_3 (nmol/l), median (IQR)	52.5 (39.4; 68.4)	51.4 (36.2; 66.2)	*0.31 (T_{644} = 1.02)*	51.3 (38.0; 65.9)	55.2 (42.2; 66.4)	*0.15 (T_{284} = -1.43)*
Cu/Zn ratio, median (IQR)	1.41 (1.17; 1.67)	1.30 (1.10; 1.58)	***0.024*** *(T_{644} = 2.27)*	1.37 (1.18; 1.66)	1.30 (1.16; 1.57)	*0.36 (T_{284} = 0.91)*
Se/Cu ratio, median (IQR)	0.104 (0.089; 0.124)	0.107 (0.091; 0.128)	*0.50 (T_{644} = -0.68)*	0.109 (0.087; 0.124)	0.112 (0.094; 0.126)	*0.26 (T_{284} = -1.12)*

Comparisons between case and controls, respectively seropositive and seronegative close contacts, were made using independent T-tests for continuous variables and χ^2 for binary variables. Significant differences are indicated in bold characters.

[a] Anti-SARS-CoV-2 IgG and IgA seropositivity levels expressed as Mean Fluorescence Intensity (MFI) signals relative to negative controls.

[b] IgG and IgA positivity defined as MFI > 6, and MFI ≥ 6.5, respectively.

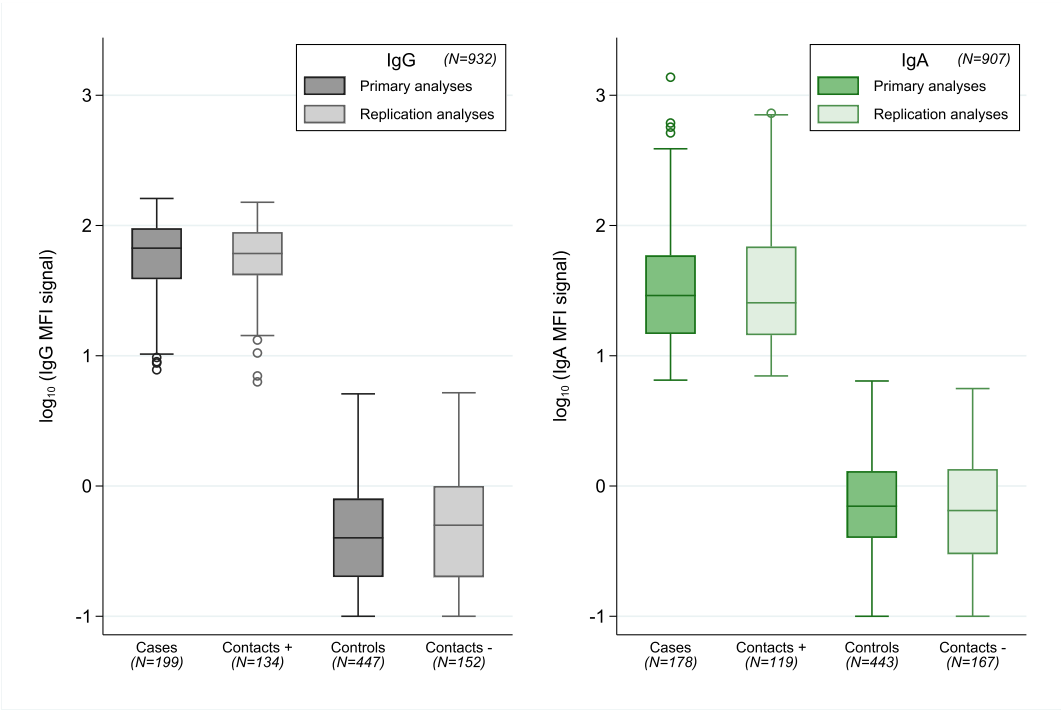


Fig. 2. Distribution of log-transformed anti-SARS-CoV-2 IgG and IgA levels across subpopulations. Note: Anti-SARS-CoV-2 IgG and IgA seropositivity levels are expressed as Mean Fluorescence Intensity (MFI) signals relative to negative controls. Box plot settings: horizontal lines within each box represent the 25th percentile, the median, and the 75th percentile. The maximal length of the whiskers is defined as the interquartile range multiplied by 1.5.

associated with smoking in the case–control analyses, but these associations did not reach statistical significance in close contacts, although keeping the same directionality. Age also showed marginal and inconsistent associations with anti-SARS-CoV-2 serostatus.

Of note, the Cu/Zn ratio was positively associated with anti-SARS-CoV-2 seropositivity in primary analyses for both IgG and IgA, with direction-consistent, yet not significant, results in the replication analyses (Appendix Table 2). Finally, we found no association between the Se/Cu ratio and anti-SARS-CoV-2 seropositivity (Appendix Table 3).

4. Discussion

We found lower plasma Zn levels, to be significantly associated with higher anti-SARS-CoV-2 IgG and IgA seropositivity in the population of the canton of Vaud, Switzerland, aged 14 years and over. Our results are in line with experimental and other observational evidence showing that Zn status plays a key role for proper immune function in the general population and may help protect against SARS-CoV-2 infection, at least for the initially circulating variants before vaccines were available. For both IgG and IgA, the results from the primary case–control analyses using laboratory confirmed COVID-19 cases and population-based seronegative controls were replicated in the close contacts analyses. In this pre-vaccination study, it is likely that most seronegative controls had not been exposed to the virus at the time of the study, unlike the close contacts. At the time of study recruitment, participants were not acutely sick, as confirmed by the absence of inflammation. The present study adds to prior evidence in vitro [43,44] and in clinical settings [15,45–48] suggesting an important role of Zn status in protecting against respiratory viral infections. The public health relevance of our results is high in that what we observed applies to the general population aged 14 years and over. Recently a multicentre double-blind placebo-controlled randomized trial including 470 patients tested 15-day elemental Zn treatment (50 mg/day) within 4.6 days of the onset of symptoms [49],a dose that corresponds to about 5 times the DRI (dietary reference ranges) [38]. The results were a reduction in ICU admission, symptom duration and length of hospital stay. These relatively high Zn doses may well have been a repletion therapy, as Zn deficiency has been previously identified in the Tunisian population [50]. Further experimental studies are needed to explore whether Zn repletion in Zn deficient subjects may prevent SARS-CoV-2 infection. The high proportion of participants with plasma zinc levels below our inferior reference level, which is lower than current international level, was a public health relevant finding, confirming previous observations in Switzerland [30].

Another important finding is the high prevalence of Zn and 25(OH)D_3 deficiencies reflected by plasma levels below reference values in the absence of systemic inflammation. Low levels were observed for Zn (12.1%), Se (8.5%), Cu (0.8%) and 25(OH)D_3 (45.2%). The participants were healthy adolescents and adults (no malnutrition), with normal access to food. Serum Zn is currently considered to be a reliable biomarker of population Zn status [51,52], and levels in this study indicates a high risk of deficiency in the population of the canton of Vaud. Seasonal impact should be limited for vitamin D status as the study was conducted in May and June only, and a real deficiency state is therefore likely.

5. Role of zinc in immunity and COVID-19

The essential role of Zn in immune defence has long been recognized [53], for maintenance and development of immune cells of both the innate and adaptive immune system. A disrupted Zn homeostasis leads to impaired formation, activation, and

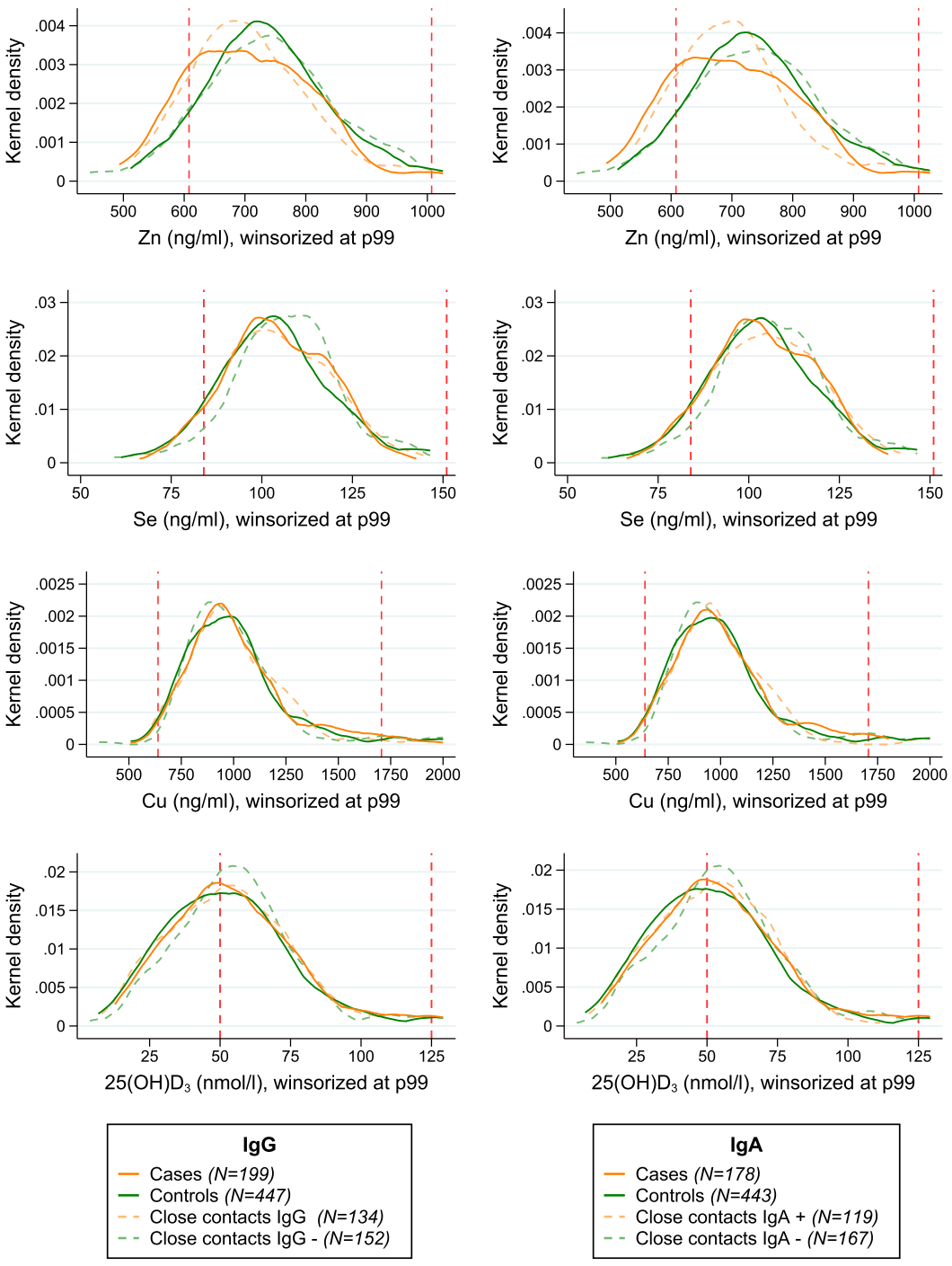


Fig. 3. Distribution of plasma Zn, Se, Cu, and 25(OH)D_3 levels, across IgG and IgA status and subpopulations. Note: Vertical dashed red lines represent reference ranges for each micronutrient: 608–1007 ng/ml for Zn, 84–151 ng/ml for Se, 639–1706 ng/ml for Cu and 50–125 nmol/l for 25-hydroxy-vitamin D_3 (25(OH)D_3). Micronutrients levels distributions are winsorized at percentile 99 (i.e. all values above percentile 99 are set to percentile 99).

maturation of lymphocytes, disturbed intercellular communication via cytokines, and weakened innate host defence via phagocytosis and oxidative burst [54]. Zn deficiency is estimated to affect over two billion subjects [55]. Mild Zn deficiency, decreases thymulin activity in Th1 cells, decreases mRNAs of IL-2 and IFN-gamma genes, and decreases activity of natural killer cells (NK) and T cytotoxic T cells [53]. Its mechanism of action includes stimulation of the Toll-like receptor 4, which regulates Zn homeostasis [56].

A strong interrelationship of Zn status with survival odds and mortality risk of patients affected by the ongoing COVID-19 pandemic has been described in several observational studies [43,57–59], a finding which is supported by in vitro studies

Table 2
Associations of selected micronutrients with anti-SARS-CoV-2 IgG seropositivity (N = 932).

IgG seropositivity	Primary analyses					Replication analyses				
	Unadjusted				Adjusted	Unadjusted				Adjusted
N	646	646	646	646	646	286	286	286	286	286
Log_2 (Zn)	**0.250***** (0.110; 0.568)				**0.196***** (0.0831; 0.465)	**0.306*** (0.0990; 0.948)				**0.294*** (0.0893; 0.968)
Log_2 (Se)		1.17 (0.544; 2.51)			1.38 (0.612; 3.10)		0.515 (0.177; 1.50)			0.554 (0.172; 1.78)
Log_2 (Cu)			1.24 (0.756; 2.04)		1.39 (0.742; 2.60)			1.10 (0.543; 2.22)		1.46 (0.578; 3.66)
Log_2 (25(OH)D_3)				1.19 (0.924; 1.52)	1.25 (0.956; 1.64)				0.806 (0.564; 1.15)	0.837 (0.573; 1.22)
Age (years)					**0.989*** (0.980; 0.997)		s			1.01 (0.991; 1.02)
Gender female					0.929 (0.623; 1.38)					0.784 (0.435; 1.41)
Log_2 (BMI)					**4.35***** (1.97; 9.59)					1.60 (0.538; 4.74)
Current smoker					**0.495**** (0.298; 0.822)					0.653 (0.343; 1.24)
Sqrt (CRP)					0.916 (0.742; 1.13)					0.817 (0.625; 1.07)

Data are odds ratios and 95% confidence intervals from multiple logistic regression models using IgG seropositivity as the outcome variable of interest.
Units: Zn, Cu and Se levels are expressed in ng/ml, 25-hydroxy-vitamin D_3 (25(OH)D_3) levels in nmol/l, CRP levels in mg/l, and BMI in kg/m^2.
*p < 0.05, **p < 0.01, ***p < 0.001; Significant differences are indicated in bold characters.

Table 3
Associations of selected micronutrients with anti-SARS-CoV-2 IgA seropositivity (N = 907).

IgA seropositivity	Primary analyses					Replication analyses				
	Unadjusted				Adjusted	Unadjusted				Adjusted
N	621	621	621	621	621	286	286	286	286	286
Log_2 (Zn)	**0.217***** (0.0929; 0.507)				**0.185***** (0.0759; 0.452)	**0.283*** (0.0900; 0.890)				**0.228*** (0.0660; 0.788)
Log_2 (Se)		0.937 (0.429; 2.05)			1.12 (0.490; 2.57)		0.676 (0.232; 1.97)			0.658 (0.197; 2.20)
Log_2 (Cu)			1.17 (0.696; 1.96)		1.27 (0.656; 2.45)			0.864 (0.422; 1.77)		1.33 (0.504; 3.53)
Log_2 (25(OH)D_3)				1.22 (0.942; 1.59)	1.32 (0.996; 1.75)				0.788 (0.551; 1.13)	0.835 (0.563; 1.24)
Age (years)					0.992 (0.983; 1.001)					**1.02*** (1.003; 1.03)
Gender female					0.900 (0.592; 1.37)					0.627 (0.340; 1.16)
Log_2 (BMI)					**5.05***** (2.22; 11.5)					2.16 (0.683; 6.81)
Current smoker					**0.468**** (0.271; 0.807)					0.552 (0.276; 1.10)
Sqrt (CRP)					0.950 (0.765; 1.18)					**0.745*** (0.557; 0.996)

Data are odds ratios and 95% confidence intervals from multiple logistic regression models using IgA seropositivity as the outcome variable of interest.
Units: Zn, Cu and Se levels are expressed in ng/ml, 25-hydroxy-vitamin D_3 (25(OH)D_3) levels in nmol/l, CRP levels in mg/l, and BMI in kg/m^2.
*p < 0.05, **p < 0.01, ***p < 0.001; Significant differences are indicated in bold characters.

assessing the effects of Zn on SARS-CoV replication and RNA polymerase activity [44]. Importantly, Zn preserves the natural tissue barriers such as the respiratory epithelium [57]. A meta-analysis including 49 studies showed that COVID-19 patients had significantly lower circulating Zn ($p < 0.001$), Fe ($p = 0.023$), and Se ($p < 0.001$) levels than healthy controls, and circulating low levels were associated with the disease severity in COVID-19 patients [16]. In addition, a potential direct interrelation of Zn was also proposed for SARS-CoV-2 maturation [60,61]. The studies on immune responses to anti-viral vaccines in relation to Zn status are inconclusive at present [62–64]. One of the reasons may be that Zn supplementation is only effective in presence of true deficiency, and biological effects requires several days to appear. In a cohort of 126 healthcare workers who received 2 doses of anti-SARS-Cov2 vaccine (BNT162B2) [64], total serum Zn levels and Zn supplementation were not associated with SARS-CoV-2 antibody levels and vaccination response. Yet, free Zn levels, were significantly positively correlated with the anti-SARS-CoV-2 IgG levels as well as with the neutralizing activity of the virus [64].

We here used, as laboratory references for Zn and Se, the percentiles 5 and 95 from the SKIPOGH population-based cohort [65], which are lower, with 608 ng/ml, than those considered in the international literature (784 ng/ml = 12 μmol/l) [51]: with the latter values 688 participants (71.6%) would have been below the cut and considered as deficient. A previous Swiss cross-sectional study, based on a convenience sample, had shown a large proportion of people with low Zn status [66] (from 10.8% in omnivorous up to 47% in vegans). The large proportion of participants with low Zn levels in this Swiss region possibly explains the observed strong association with anti-SARS-CoV-2 seropositivity.

6. Absence of association with selenium

We observed no association of Se levels with anti-SARS-CoV-2 seropositivity. Most participants presented with a normal plasma Se level, which is in line with previous data from Switzerland [31,67]. In a German study investigating Se and its carrier protein selenoprotein P in COVID-19 patients [38], low blood Se and selenoproteins in both survivors and non-survivors was observed, which is expected in the presence of inflammation, with higher Se values in survivors. Se deficiency has been associated with worse outcome after COVID-19 [12,68], which has been confirmed by a systematic review [69]. The authors cautiously concluded that Se supplements might be considered but that interventional studies were needed. In this study, only 8.5% of participants had plasma levels below our population-based inferior range, which does not mean that such level reflects Se adequacy from a physiological point of view. For Se, the normal activity of plasma or erythrocyte glutathione and selenoprotein P are the signature of an adequate level: levels ≥95 ng/ml are required for optimal function [70]. A normal Se status is associated with a normal antioxidant capacity, which favours defence against viral infections [71].

7. Absence of association with copper

We observed no association of Cu levels with anti-SARS-CoV-2 seropositivity. Studies exploring the association of plasma copper levels with SARS-CoV-2 related outcomes are few. Copper is a positive acute phase reactant meaning that it increases in the context of inflammation. A small German study showed high Cu values in COVID-19 patients, which is the normal response to an inflammatory disease [72], and no serum levels alluding to a deficiency in their patients. In the present study, the proportion of participants with values below the Swiss population-based reference (639 ng/l) was small with 0.8%. Reference values (P5–P95) for copper levels were 795–1949 ng/ml for serum levels in Germany [73] and 794–2023 ng/ml for plasma levels in 100 healthy French volunteers [74], which are higher than the one we used based on a Swiss population-based sample including over 1000 people aged 18 years and over (639–1706 ng/ml). Copper was included in the model because of its antiviral properties [75]. Cu participates in the host response to bacterial infection and is broadly implicated in regulating immunity [5,76], being involved in the function and maintenance of Th cells, B cells, neutrophils, NK cells and macrophages [77]. It has been hypothesized that correction of Cu deficit might improve antiviral defences [78].

Elevated Cu with low Zn is one of the most common trace element imbalances and is determined by dietary intake. The ratio of Cu to Zn is clinically more important than the concentration of either of these trace metals [79]. The normal Cu/Zn ratio, in children and adults, is close to one and a high ratio is associated with mortality in old adults [80]. The imbalance is associated with changes in immune defence, inflammation and stress response [81]. The Cu/Zn ratio has been shown to increase with aging [81], which is interpreted as a repair and maintenance mechanism, is correlated positively with biomarkers of inflammation [82], and might contribute to aggravate the inflammation present in COVID-19 patients. In this study, the Cu/Zn ratio was positively associated with seropositivity. High Cu/Zn ratios have been reported with infections [83], but it is not clear if this only reflects inflammation redistribution or if it is a causal relationship. As some authors have observed an association of higher Cu/Se ratio with oxidative stress [84], we also investigated the association but could find none.

8. Absence of association with 25-hydroxy-vitamin D_3

An unexpected finding of this study was the absence of association of 25(OH)D_3 levels with IgG nor IgA seropositivity status, which contrasts with results from studies and meta-analyses in COVID-19 patients [21–26]. This study however included people with mainly mild courses of infection or disease reflected by absence of inflammation. Overall, 45.2% of the entire study population had 25(OH)D_3 status below 50 nmol/l, considered to be a cut-off towards deficiency, and hence a potential cause of altered immunity [42]. These global low values may result, in part, from the COVID-19 confinement, as the study took place in Switzerland in Spring 2020, a time of the year with higher vitamin D status than in winter. But low serum values of 25(OH)D_3 have been shown to be present across the year in Switzerland [32], and particularly in pregnant women [85]. Women constituted 53% of the participants, with slightly lower 25(OH)D_3 levels in women than in men. The absence of association of 25(OH)D_3 levels with IgG and IgA status is therefore new.

9. Strengths and limitations

The main strength of the current study resides in its population-based design, which, unlike clinical studies that mainly focus on the most severe forms of COVID-19, allowed to observe micronutrient associations with anti-SARS-CoV-2 IgG and IgA seropositivity at the population level in a still SARS-CoV-2 “naïve” population. Moreover, by also studying the close contacts subsample, we were able to replicate our results in highly exposed individuals. However, the generalizability of our results is limited by the population studied, as we only observed Swiss inhabitants aged 14 years and older, during the early pandemic. Another important consideration is that immunoglobulin levels are indirect and imperfect markers of host immunity, which is illustrated in the current study by the fact that not all index cases were seropositive, and by the imperfect correlation between IgG and IgA seropositivity. Hence, any relationship between micronutrient status and immunity discussed in relation

with the current results should be regarded with caution. Further we censored 55 participants due to missing values, which might be a bias. Finally, as the nature of the present study is observational, one cannot infer any causation mechanism.

In conclusion, lower plasma Zn levels were associated with anti-SARS-CoV-2 IgG and IgA seropositivity in the population of a Swiss region aged 14 years and over, at a time when the initial viral variant was circulating, and no vaccination was available. The low Zn levels observed in this Swiss region are of concern as they reflect suboptimal Zn status, which may confer a low immunity against viral infections. These results highlight the importance of maintaining a healthy and balanced diet during pandemic crises and more efforts should be devoted to informing the general population of the benefit of a healthy diet in general and especially against respiratory viral infections. Further studies are needed to explore whether these results also apply to other viral variants, whether Zn supplementation may offer protection against SARS-CoV-2 infection in the general population, and whether Zn status has an influence on the response to vaccination.

Authors's contribution

	Design	Data acquisition	Analysis	Drafting manuscript	Final approval	Integrity
A Equey	x	x	x	x	x	x
MM Berger	x	x	x	x	x	x
S Gonseth	x	x			x	x
M Augsburger		x	x	x	x	x
S Rezzi		x	x	x	x	x
A Hodgson		x	x		x	
S Estoppey		x	x		x	x
G Pantaleo	x		x		x	
C Pellaton		x	x	x	x	
S Lenglet		x	x		x	
M Perrais		x	x		x	x
V Rousson		x	x	x	x	x
V D'Acremont	x			x	x	x
M Bochud	x	x	x	x	x	x

Funding

The study received funding from the Swiss Society of Public Health + (SSPH+) foundation as part of the Corona Immunitas research group (ISRCTN18181860), the Federal Office of Public Health, Switzerland; the Department of Health and Social Action, Canton of Vaud, Switzerland; the Leenaards Foundation, Switzerland; the Lausanne University Foundation, Switzerland; the Center for Primary Care and Public Health - Unisante, Lausanne, Switzerland; DSM Biomedical Health.

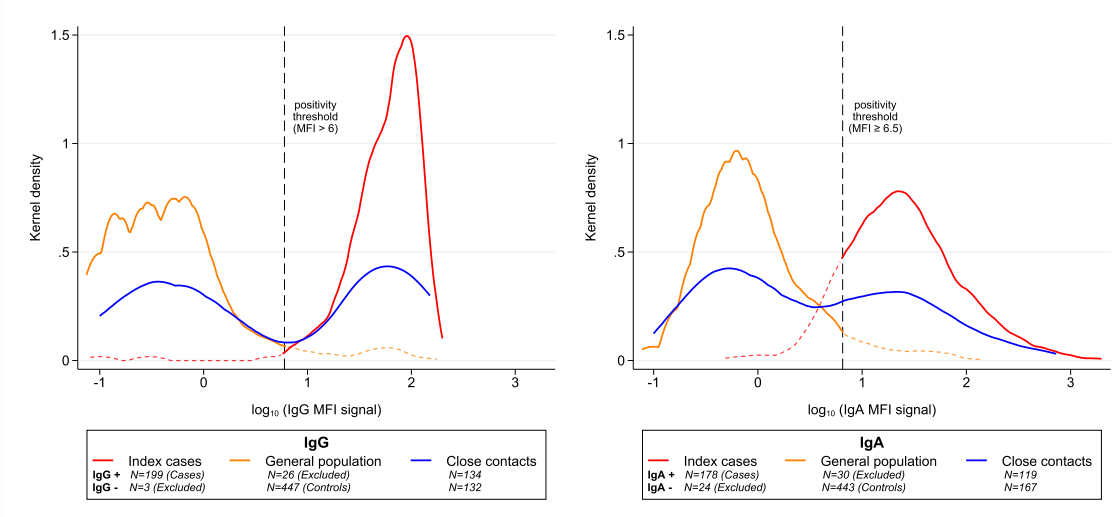
Conflicts of interest

The authors declare no conflict of interest related to the present study.

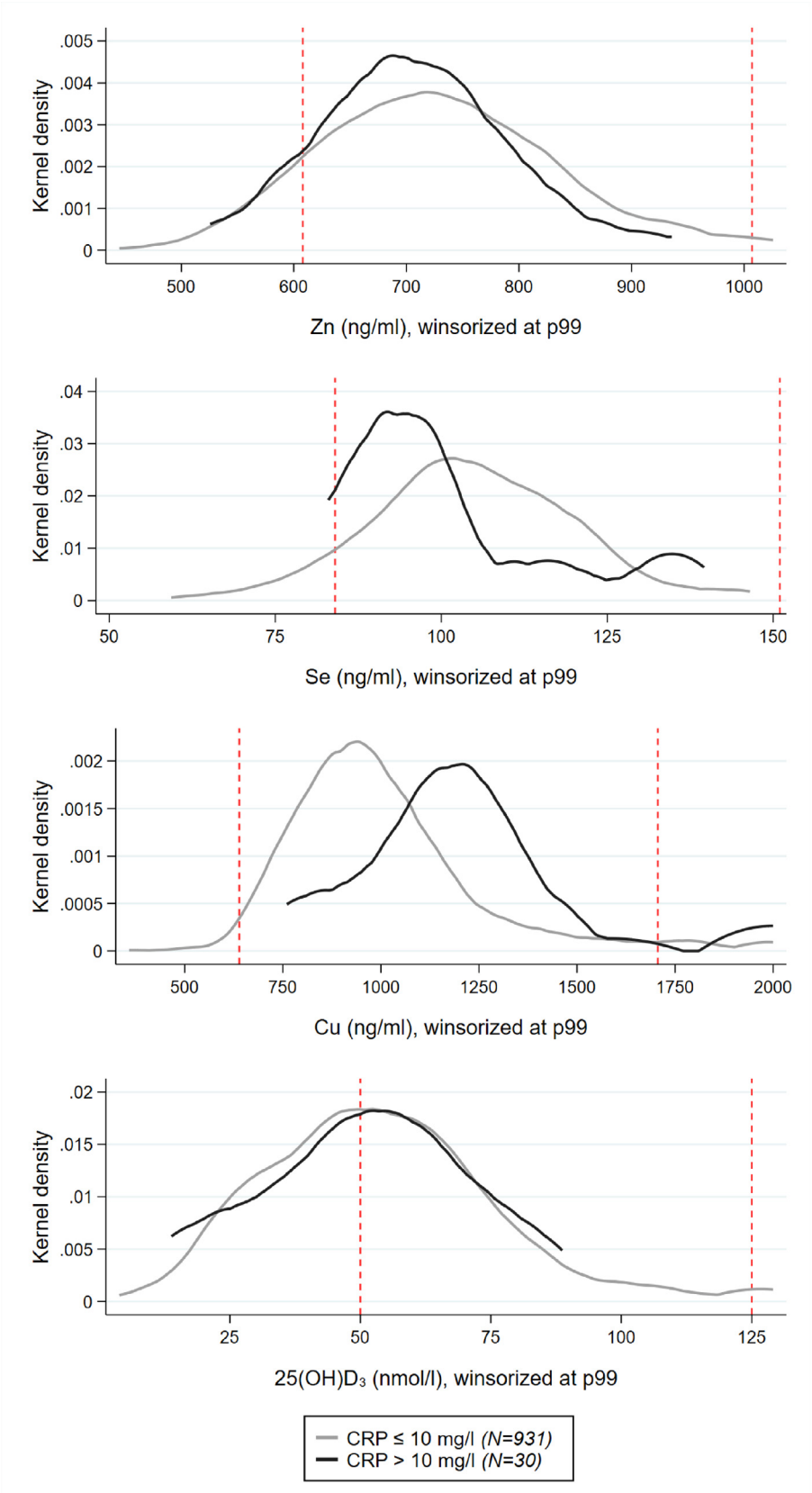
Acknowledgments

The authors would like to express their gratitude to the participants for making the study possible with their participation.

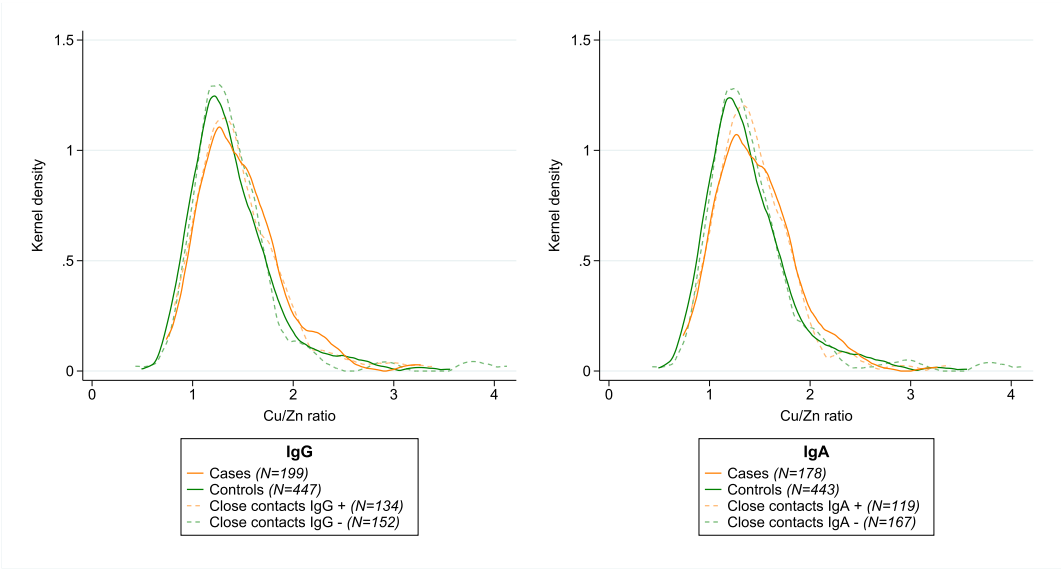
Appendix



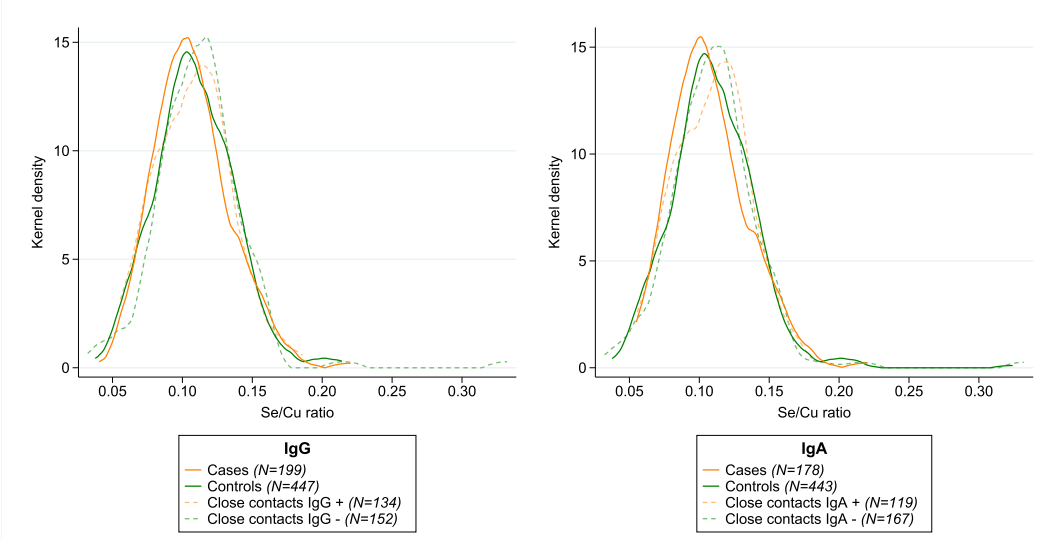
Appendix Fig. 1. Distribution of log-transformed anti-SARS-CoV-2 IgG and IgA levels in the three subpopulations (N = 961). Note: Anti-SARS-CoV-2 IgG and IgA seropositivity levels are expressed as Mean Fluorescence Intensity (MFI) signals relative to negative controls.



Appendix Fig. 2. Distribution of plasma Zn, Se, Cu and 25(OH)D_3 levels by CRP plasma concentration in the total sample (N = 961). Note: Vertical dashed red lines represent reference ranges for each micronutrient: 608–1007 ng/ml for Zn, 639–1706 ng/ml for Cu, 84–151 ng/ml for Se and 50–125 nmol/l for 25-hydroxy-vitamin D_3 (25(OH)D_3). Micronutrients levels distributions are winzorized at percentile 99 (i.e. all values above percentile 99 are set to percentile 99).



Appendix Fig. 3. Distribution of plasma Cu/Zn ratio across IgG and IgA status and subpopulations, showing that the median Cu/Zn in the cohort is superior to the normal value 1:1.



Appendix Fig. 4. Distribution of plasma Se/Cu ratio across IgG and IgA status and subpopulations.

Appendix Table 1
Participants' general, serological and micronutrient characteristics across subpopulations (IgA analysis, N = 907).

	Primary analyses			Replication analyses		
	Cases	Controls	*P* value (statistic)	Close contacts IgA +	Close contacts IgA -	*P* value (statistic)
N	178	443		119	167	
Age (years), mean (SD)	50.5 (18.2)	49.7 (22.3)	*0.65 (T_{619}= 0.45)*	49.4 (17.7)	42.3 (16.2)	***<0.001*** *(T_{284}= 3.52)*
Gender, n (% women)	93 (52.2)	238 (53.7)	*0.74 (χ^2_1 = 0.11)*	56 (47.1)	95 (56.9)	*0.10 (χ^2_1 = 2.69)*
BMI (kg/m^2), mean (SD)	26.1 (5.1)	24.6 (4.5)	***<0.001*** *(T_{619}= 3.59)*	25.5 (4.4)	24.5 (4.9)	*0.076 (T_{284}= 1.78)*
Smoking, n (% current smoker)	19 (10.7)	95 (21.4)	***0.002*** *(χ^2_1 = 9.83)*	15 (12.6)	38 (22.8)	***0.029*** *(χ^2_1 = 4.74)*
CRP (mg/l), median (IQR)	1 (0; 2)	1 (0; 2)	*0.26 (T_{619}= 1.12)*	0 (0; 2)	0 (0; 3)	*0.082 (T_{284}= -1.74)*
SARS-CoV-2 serology						
IgG (semi-quantitative[a]), median (IQR)	72.4 (45.6; 95.9)	0.4 (0.2; 0.8)	***<0.001*** *(T_{619}= 42.7)*	64.2 (41.6; 91.7)	0.6 (0.2; 1.4)	***<0.001*** *(T_{284}= 19.4)*
IgG positivity[b], n (% positive)	177 (99.4)	12 (2.7)	***<0.001*** *(χ^2_1 = 561)*	113 (95.0)	21 (12.6)	***<0.001*** *(χ^2_1 = 189)*
IgA (semi-quantitative[a]), median (IQR)	29.0 (14.7; 58.9)	0.7 (0.4; 1.3)	***<0.001*** *(T_{619}= 10.1)*	25.5 (14.4; 68.9)	0.6 (0.3; 1.3)	***<0.001*** *(T_{284}= 7.07)*
IgA positivity[b], n (% positive)	178 (100.0)	0 (0.0)	*n.s.*	119 (100.0)	0 (0.0)	*n.s.*
Micronutrients						
Serum Zn (ng/ml), median (IQR)	704 (621; 781)	733 (669; 800)	***0.003*** *(T_{619}= s -3.01)*	697 (637; 753)	734 (662; 803)	***0.024*** *(T_{284}= -2.27)*
Serum Se (ng/ml), median (IQR)	103 (94.7; 115)	103 (93.6; 114)	*0.67 (T_{619}= -0.43)*	104 (95.3; 115)	105 (97.2; 115)	*0.42 (T_{284}= -0.81)*
Serum Cu (ng/ml), median (IQR)	969 (867; 1123)	962 (835; 1101)	*0.71 (T_{619}= 0.37)*	958 (871; 1120)	961 (845; 1107)	*0.51 (T_{284}= -0.66)*
25(OH)D_3 (nmol/l), median (IQR)	52.4 (39.4; 68.4)	50.1 (35.9; 65.6)	*0.22 (T_{619}= 1.23)*	51.1 (37.7; 65.9)	54.9 (42.0; 66.4)	*0.10 (T_{284}= -1.65)*
Cu/Zn ratio, median (IQR)	1.41 (1.17; 1.67)	1.29 (1.10; 1.57)	***0.037*** *(T_{619}= 2.10)*	1.38 (1.20; 1.65)	1.30 (1.16; 1.59)	*0.78 (T_{284}= 0.28)*
Se/Cu ratio, median (IQR)	0.104 (0.089; 0.125)	0.108 (0.092; 0.128)	*0.40 (T_{619}= -0.85)*	0.112 (0.089; 0.127)	0.109 (0.093; 0.126)	*0.69 (T_{284}= -0.40)*

Comparisons between case and controls, respectively seropositive and seronegative close contacts, were made using independent T-tests for continuous variables and χ^2 for binary variables. Significant differences are indicated in bold characters. Abbreviation: 25(OH)D_3: 25-hydroxy-vitamin D_3.
[a] Anti-SARS-CoV-2 IgG and IgA seropositivity levels expressed as Mean Fluorescence Intensity (MFI) signals relative to negative controls.
[b] IgG and IgA positivity defined as MFI >6, and MFI ≥6.5, respectively.

Appendix Table 2
Associations of selected micronutrients with A) anti-SARS-CoV-2 IgG seropositivity (N = 932), and B) anti-SARS-CoV-2 IgA seropositivity (N = 907), using the Cu/Zn ratio.

	IgG seropositivity				IgA seropositivity			
	Primary analyses		Replication analyses		Primary analyses		Replication analyses	
	Unadjusted	Adjusted	Unadjusted	Adjusted	Unadjusted	Adjusted	Unadjusted	Adjusted
N	646	646	286	286	621	621	286	286
Log_2 (Cu/Zn ratio)	**1.75*** (1.14; 2.67)	**2.17**** (1.29; 3.64)	1.46 (0.818; 2.61)	2.01 (0.961; 4.19)	**1.74*** (1.12; 2.69)	**2.11**** (1.23; 3.61)	1.27 (0.710; 2.27)	2.11 (0.984; 4.53)
Log_2 (Se)		1.17 (0.528; 2.58)		0.48 (0.152; 1.50)		0.943 (0.419; 2.12)		0.538 (0.164; 1.76)
Log_2 (25(OH)D_3)		1.24 (0.950; 1.62)		0.846 (0.580; 1.24)		1.31 (0.992; 1.74)		0.847 (0.573; 1.25)
Age (years)		**0.989*** (0.981; 1.00)		1.01 (0.991; 1.02)		0.993 (0.983; 1.00)		**1.02*** (1.003; 1.03)
Gender female		0.834 (0.565; 1.23)		0.723 (0.408; 1.28)		0.794 (0.528; 1.19)		0.558 (0.308; 1.01)
Log_2 (BMI)		**4.08***** (1.86; 8.91)		1.57 (0.530; 4.65)		**4.62***** (2.05; 10.4)		2.10 (0.665; 6.63)
Current smoker		**0.479**** (0.289; 0.795)		0.667 (0.351; 1.27)		**0.445**** (0.258; 0.766)		0.564 (0.283; 1.12)
Sqrt (CRP)		0.876 (0.713; 1.08)		0.793 (0.609; 1.03)		0.909 (0.735; 1.12)		**0.715*** (0.536; 0.953)

Data are odds ratios and 95% confidence intervals from multiple logistic regression models using IgA seropositivity as the outcome variable of interest.
Units: Cu/Zn ratio has no unit.
Se levels are expressed in ng/ml, 25-hydroxy-vitamin D_3 (25(OH)D_3) levels in nmol/l, CRP levels in mg/l, and BMI in kg/m^2.
*p < 0.05, **p < 0.01, ***p < 0.001; Significant differences are indicated in bold characters.

Appendix Table 3
Associations of selected micronutrients with A) anti-SARS-CoV-2 IgG seropositivity (N = 932), and B) anti-SARS-CoV-2 IgA seropositivity (N = 907), using the Se/Cu ratio.

	IgG seropositivity				IgA seropositivity			
	Primary analyses		Replication analyses		Primary analyses		Replication analyses	
	Unadjusted	Adjusted	Unadjusted	Adjusted	Unadjusted	Adjusted	Unadjusted	Adjusted
N	646	646	286	286	621	621	286	286
Log_2 (Se/Cu ratio)	0.893 (0.581; 1.37)	0.911 (0.540; 1.53)	0.766 (0.426; 1.38)	0.634 (0.295; 1.36)	0.873 (0.560; 1.36)	0.900 (0.523; 1.55)	0.980 (0.543; 1.77)	0.713 (0.322; 1.58)
Log_2 (Zn)		**0.213***** (0.0908; 0.499)		**0.286*** (0.0881; 0.926)		**0.193***** (0.0796; 0.467)		**0.224*** (0.0659; 0.764)
Log_2 (25(OH)D_3)		1.27 (0.976; 1.66)		0.83 (0.571; 1.20)		**1.33*** (1.01; 1.77)		0.829 (0.564; 1.22)
Age (years)		**0.989*** (0.980; 0.998)		1.01 (0.991; 1.02)		0.992 (0.983; 1.001)		**1.02*** (1.003; 1.03)
Gender female		0.994 (0.676; 1.46)		0.771 (0.432; 1.37)		0.935 (0.624; 1.40)		0.620 (0.340; 1.13)
Log_2 (BMI)		**4.22***** (1.93; 9.27)		1.56 (0.531; 4.60)		**4.98***** (2.20; 11.3)		2.13 (0.680; 6.69)
Current smoker		**0.497**** (0.300; 0.824)		0.658 (0.347; 1.25)		**0.469**** (0.272; 0.809)		0.554 (0.278; 1.10)
Sqrt (CRP)		0.934 (0.759; 1.15)		0.813 (0.623; 1.06)		0.960 (0.774; 1.19)		**0.743*** (0.556; 0.993)

Data are odds ratios and 95% confidence intervals from multiple logistic regression models using IgA seropositivity as the outcome variable of interest.
Units: Se/Cu ratio has no unit.
Se levels are expressed in ng/ml, 25-hydroxy-vitamin D_3 (25(OH)D_3) levels in nmol/l, CRP levels in mg/l, and BMI in kg/m^2.
*p < 0.05, **p < 0.01, ***p < 0.001; Significant differences are indicated in bold characters.

Appendix Table 4
ICP-MS instrument operating conditions.

ICP-MS spectrometer	Agilent 7700 series
Skimmer and sampler cones	Ni
Spray chamber	temperature stabilized (Peltier cooled)
Sample depth	8 mm
RF Power	1550 W
Plasma gas flow rate	15 L/min
Carrier gas flow rate	0.7–1.1 L/min
Makeup gas flow rate	0.13 L/min
He flow rate (KED mode)	4.3 mL/min
Peristaltic pump tubing	inner Ø 1.02 mm, wall thickness 0.85 mm
Uptake time and nebulizer pump speed	20 s, 0.5 rps
Stabilization time and nebulizer pump speed	55 s, 0.1 rps
Oxyde (CeO/Ce) ratio	0.76–1.21%
Doubly charged (Ce) ratio	1.25–1.68%

Appendix Table 5
Trace element acquisition parameters.

Isotope	Integration time (s)		
	No gas (standard mode)	He (KED mode)	Internal standard
^{63}Cu	–	0.5	^{103}Rh
^{66}Zn	–	0.5	^{103}Rh
^{78}Se/^{82}Se	–	0.5	^{103}Rh

Appendix Table 6
Analytical validation method.

CRM	ClinCkek-Control Plasma level I and II (Recipe Chemicals, München, Germany)				
Isotope	LOD (ng/ml)	LOQ (ng/ml)	Repeatability	Reproducibility	Recovery (I/II)
^{63}Cu	1.15	3.69	1.70%	3.10%	92%/93%
^{66}Zn	0.42	5.54	1.20%	3.90%	105%/96%
^{78}/^{82}Se	0.61	1.68	1.40%	2.50%	114%/114%

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