



Zinc intake and status in Austria in the light of different reference values

Ibrahim Elmadfa, Alexa L. Meyer, Timo Kuen, Karin Wagner, and Verena Hasenegger

Department of Nutritional Sciences, Faculty of Life Sciences, University of Vienna, Vienna, Austria

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Abstract: Zinc has been identified as a critical micronutrient also in high-income countries. There is still some uncertainty about the evaluation of zinc sufficiency due to divergent daily intake reference values. We wanted to exemplify this issue using data from the Austrian Study on Nutritional Status 2012. Plasma zinc concentrations were measured in a nationally representative sample of 872 persons aged 6–80 years (55.5 % female). Dietary zinc intake was estimated from two 24h dietary recalls. Additionally, parameters of the antioxidative status (plasma malondialdehyde (MDA), total antioxidative capacity) and activities of alkaline phosphatase (AP), superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) were determined. Zinc status was marginal in schoolchildren (40 % of boys and 22 % of girls) and in elderly (28 % of men and 33 % of women). Dietary zinc intake was also unsatisfactory in these groups with 38 % of boys and 32 % of girls and 64.5 % of older men below the nationally recommended intake levels. However, the adequacy of zinc intake varied with different reference values. Adults were more likely to meet the D-A-CH reference values and those from the European Food Safety Authority than the recommendations of the International Zinc Nutrition Consultative Group (IZiNCG) and the Institute of Medicine, whereas children met the IZiNCG values best. Zinc status correlated weakly with AP activity ($r = -0.298$, $p < 0.001$) and some antioxidant status markers (CAT, MDA, GSH-PX, SOD), especially in the elderly (MDA: $r = -0.527$, $p < 0.001$, and SOD: $r = -0.466$, $p = 0.002$). Our results suggest a suboptimal zinc supply in Austria particularly among schoolchildren and older adults.

Keywords: Zinc, status, dietary intake, reference values, antioxidant status

Introduction

Zinc is an essential trace element with a wide range of functions in the human body. It is an integral component of zinc proteins of which more than 3000 have been identified so far and which act as enzymes and transcription factors (estimated over 900 each), in cell signalling and DNA repair and replication etc. [1]. Moreover, due to their unique property of maintaining their oxidation state in varying redox environments, zinc ions in themselves are crucial in cell signalling and the functional regulation of proteins, acting as redox transducers [2]. Zinc ions also play a role in the regulation of the cell cycle and apoptosis [3]. This emphasises the necessity of a tight control of the cellular Zn^{2+} homeostasis [4].

Indeed, its central role in major physiological processes makes Zn an important factor in the pathogenesis of many diseases. Part of this arises from the antioxidative functions of Zn in physiological concentrations and the fact that disruptions of the homeostasis in deficiency as well as overload cause oxidative stress [2]. Accordingly, Zn deficiency has been associated with metabolic diseases like diabetes mellitus, cardiovascular diseases and certain cancer types

among others. It has also been linked to impairments in immune function, cognitive function such as Alzheimer's disease and depression [5]. In this context, it is notable that even slight deviations from the normal physiological range of zinc concentration might have effects on the body's functions and health. This is suggested by a Korean study in which fasting blood glucose levels and insulin resistance indices were slightly higher in normal weight individuals whose plasma Zn^{2+} concentration was in the lowest quartile compared to those with plasma Zn^{2+} concentration in the highest quartile (Fasting plasma glucose (FPG) = 101 mg/dl and insulin resistance according to the Homeostasis Model Assessment, HOMA-IR: 2.44 for plasma $Zn^{2+} \leq 17.88 \mu\text{mol/l}$ vs. FPG = 94 mg/dl and HOMA-IR: 2.20 for plasma $Zn^{2+} \geq 23.48 \mu\text{mol/l}$, $p = 0.0019$ between all quartiles for FPG and $p = 0.0139$ between all quartiles for HOMA-IR). Lower plasma Zn^{2+} was also associated with a higher risk for impaired glucose metabolism with 19.7 % and 11.7 % of participants, respectively, suffering from impaired fasting glucose and type 2 diabetes in the lowest Zn quartile compared to 11.7 % and 6.6 %, respectively, in the highest Zn quartile. In participants in the lowest plasma Zn^{2+} quartile the OR to be categorized as metabolically obese normal weight was 4

compared to those in the highest quartile. These results are noteworthy as even in the lowest quartile mean plasma zinc levels were in the normal reference range ($15.77 \mu\text{mol/l}$) [6].

Zinc deficiency has been recognised as a global health issue mentioned among the ten leading risk factor causes of disability-adjusted life years (DALYs) in low-income countries in 2004 by the World Health Organisation [7]. It is a particularly critical nutrient in children under 5 years of age in whom zinc deficiency causes stunting [8].

According to a Technical Document on the Assessment of the Risk of Zinc Deficiency in Populations and Options for Its Control from the International Zinc Nutrition Consultative Group (IZiNCG) [9], Austria like other industrialized European countries is considered to have a low risk of zinc deficiency. This was based on the average intake (13.0 mg/d), the comparatively high proportion of animal source foods in the diet (which contribute 34.4 % of energy intake) and the moderate amount of phytate in the diet (1.24 mg/d) resulting in a rather low phytate:zinc molar ratio of 9.4. However, inadequate zinc intake in high-income countries has been reported for some special at-risk populations such as young children, adolescents and elderly, for instance [10–12]. Furthermore, concerns have

been voiced that a vegetarian or, notably, a vegan diet might increase the risk of inadequate Zn intake [13].

Assessing the status and dietary intake of zinc at population level was therefore part of the Austrian Study on Nutritional Status 2012 that comprised biochemical analyses in the blood besides the collection of dietary data [14].

Subjects and Methods

Subjects

The Austrian Study on Nutritional Status is an ongoing project to regularly evaluate the nutritional status and dietary habits of the Austrian population through a cross-sectional, randomized design. In 2012, biochemical analyses of major nutrients were included. A quota sample of 872 persons aged 6–80 years (55.5 % female) from all nine federal provinces was included in the here-presented analysis of zinc status (see Table 1 for sample characteristics). The sample was weighted to better represent the age, sex and regional distribution of the Austrian population [14].

Table 1. Characteristics of the sample.

Population group	Schoolchildren (6–14 y)		Adults (18–64 y)		Elderly (65–80 y)	
	Boys	Girls	Men	Women	Men	Women
n	169	163	147	232	72	89
Age (y)	$9.8 \pm 2.1^*$	$10.2 \pm 2.0^*$	40.9 ± 12.8	41.0 ± 13.1	71.5 ± 4.5	71.7 ± 4.4
Body mass index (kg/m^2)	18.8 ± 3.5	18.5 ± 3.5	$25.8 \pm 3.4^*$	$23.7 \pm 4.2^*$	$27.6 \pm 3.9^*$	$29.2 \pm 4.7^*$
Smokers (current/former) (%)	0.0/3.0	2.9/4.1	18.6/21.3	21.8/18.7	6.9/25.3	5.3/15.8
Energy intake (kcal/d)	$1942 \pm 427^*$	$1796 \pm 415^*$	$2225 \pm 603^*$	$1855 \pm 478^*$	$1941 \pm 486^*$	$1720 \pm 388^*$
Protein intake (% of total energy)	13.7 ± 2.7	13.4 ± 2.4	$15.3 \pm 4.2^*$	$14.5 \pm 3.2^*$	14.4 ± 3.5	15.0 ± 3.9
Fat intake (% of total energy)	34.3 ± 5.4	34.0 ± 5.5	36.4 ± 6.5	35.8 ± 6.7	34.9 ± 6.9	36.9 ± 7.1
Carbohydrate intake (% of total energy)	50.8 ± 6.4	51.4 ± 6.2	$43.7 \pm 7.8^*$	$47.0 \pm 7.3^*$	44.7 ± 6.9	44.4 ± 7.4
Dietary fibre intake (g/d)	17.7 ± 5.4	16.6 ± 5.0	$20.7 \pm 8.2^*$	$22.2 \pm 8.1^*$	$21.0 \pm 5.6^*$	$19.5 \pm 7.1^*$
Alkaline phosphatase (U/l)	$227.6 \pm 58.6^*$	$211.0 \pm 58.1^*$	72.8 ± 17.9	69.9 ± 20.2	$74.7 \pm 23.6^*$	$84.0 \pm 19.4^*$
Erythrocyte superoxide dismutase (U/g Hb [†])	1600 ± 449	1720 ± 367	1730 ± 462	1704 ± 387	1503 ± 183	1549 ± 196
Erythrocyte catalase (U/g Hb [†])	227 ± 54	223 ± 50	224 ± 51	226 ± 49	$222 \pm 44^*$	$240 \pm 35^*$
Erythrocyte glutathione peroxidase (U/g Hb [†])	$23.9 \pm 7.0^*$	$27.2 \pm 6.5^*$	28.0 ± 6.1	29.0 ± 6.3	27.8 ± 6.7	30.2 ± 7.0
Total antioxidative capacity (TAC) (mmol trolox equivalent/l)	0.71 ± 0.14	0.70 ± 0.16	$0.81 \pm 0.22^*$	$0.68 \pm 0.17^*$	$0.93 \pm 0.21^*$	$0.81 \pm 0.23^*$
Malondialdehyde (MDA) ($\mu\text{mol/l}$)	$1.66 \pm 0.65^*$	$1.48 \pm 0.56^*$	1.29 ± 0.58	1.27 ± 0.66	1.84 ± 0.61	1.87 ± 0.75

Data are means $\pm$ SD. The nutritional data represent means of two 24h recalls. * $p < 0.005$ between sexes (oneway ANOVA). [†]Hb: haemoglobin.

As approximately 70 % of the zinc in the blood is bound to albumin, the concentration of this latter also influences the zinc concentration. However, in the present collective, only one adult man presented with marginal hypoalbuminaemia (34 g/l) and was excluded from the analysis.

Blood samples were taken after an overnight fast, refrigerated during transport to the study centre and there immediately processed to separate the plasma, serum and erythrocytes. These samples were stored at -80°C until analysis.

This survey was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethical Committee of the City of Vienna (EK_10_037_0310). Written informed consent was obtained from all participants.

Anthropometric measurements and dietary assessment

Body height was measured to 0.1 cm with a stadiometer (Seca 214, Seca Vogel & Halke, Hamburg, Germany), body weight was determined to the nearest 0.1 kg using a digital scale (Seca Bella 840, Seca Vogel & Halke, Hamburg, Germany) with participants lightly dressed and wearing no shoes. Body mass index was calculated by dividing each subject's weight in kg by the squared height in m^2 .

Food consumption was ascertained from two 24h recalls in the adult and elderly participants with the first recall obtained in a direct interview by trained personnel, the second in a telephone interview after two weeks. The interviews were conducted following a modified version of the Automated Multiple-Pass Method (AMPM) developed by the US Department of Agriculture (USDA) [15]. The children were asked to fill out a three-day estimated food record on three consecutive days. Portion sizes were determined using a photo collection from the Second Bavarian Food Consumption Survey (BVS II) based on the EPIC-SOFT picture book [16, 17]. Energy and nutrient intake was calculated with the programme "nutritional software (nut.s) science" based on the German food composition database Bundeslebensmittelschlüssel 3.01 (<https://www.blsdb.de/bls>) complemented by typical Austrian foods (dato Denkwerkzeuge, Vienna, Austria).

Additionally, participants had to fill out a food frequency questionnaire on their habitual consumption of major food groups.

Biochemical analysis

Zinc in the plasma was determined by flame atomic absorption spectrophotometry using a Perkin Elmer 5100-PC AA spectrometer with a Perkin Elmer AS-90 auto-sampler and a Perkin Elmer FIAS 400 flow injection system

(Perkin Elmer, Waltham, MA) according to the method by Fuwa et al. [18]. Intraassay and interassay coefficients of variation (CV) were 6.77 % and 8.53 %, respectively. The limit of detection was at $1.308\text{ }\mu\text{mol/l}$, the limit of quantification at $2.615\text{ }\mu\text{mol/l}$.

Malondialdehyde (MDA) in the plasma, a marker of lipid peroxidation, was analysed by HPLC after complexation with thiobarbituric acid according to the method by Wong et al. [19].

For the determination of the activities of the antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase the photometrical methods by Marklund and Marklund [20] modified by Beutler [21], Beutler [21] and Aebi [22] were used, respectively. For SOD, intraassay and interassay CVs were 4.1 % and 4.9 %, respectively, for GSH-Px 3.8 % and 5.9 %, respectively, and for catalase 4.6 % and 6.2 %, respectively.

The total antioxidative capacity was determined according to Miller et al. by measuring the oxidation of 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) by hydrogen peroxide in the sample against a Trolox standard [23]. Activity of plasma total alkaline phosphatase was determined photometrically in a Vitros 250 autoanalyzer (Ortho-Clinical Diagnostics, Inc., Rochester, NY, intraassay CV: 0.89 %, interassay CV: 2.32 %).

Statistical analysis

The cut-off values to identify misreporters of dietary intake were calculated according to Goldberg et al. 1991 [24]. Based on a mean physical activity level of 1.55 and a confidence interval of 99.7 %, these cut-offs were determined as ≤ 0.81 or ≥ 2.98 times the estimated basal metabolic rate (BMR) in children and ≤ 0.76 or ≥ 3.16 times the estimated BMR in adults and elderly. Misreporters were excluded from the analyses.

Partial correlation analyses were used to study relationships between zinc plasma concentration, dietary zinc intake, activities of antioxidative enzymes and biomarkers of oxidative status as well as indicators of dietary pattern that were derived from the FFQ results. The data were controlled for the participants' age, sex, BMI, and their smoking status as well as the plasma concentrations of haemoglobin, iron, selenium. Correlations between zinc status and enzyme activities were also adjusted for haemoglobin concentration, and iron and selenium status, as these trace elements are co-factors of some of the measured enzymes. Those with dietary components were controlled for total energy intake.

Data were tested for normal distribution using the Kolmogorov-Smirnov test with Lilliefors correction. As the data for dietary zinc intake as well as those for the plasma

zinc concentration in the adult subgroup were not normally distributed, non-parametric tests (Mann-Whitney-U test and Kruskal-Wallis test to study differences between the subgroups, Spearman coefficient for correlation analyses) were chosen for further analyses. Between group comparisons in the distribution of the adequacy of zinc intake and status were done with the χ^2 -test. All analyses were performed with SPSS Statistics for Windows Version 24.0 (IBM Corp., Armonk, NY).

Results

Zinc status in the Austrian population by age and sex

The mean concentration of zinc in the plasma ($\mu\text{mol/l}$) showed a slight but statistically significant variation across the age groups ($p < 0.001$ in men and $p = 0.001$ in women, Kruskal-Wallis-test) (see Table 2). It was highest in the adults (18–64 years) of both sexes ($14.4 \pm 3.2 \mu\text{mol/l}$ in the men and $13.1 \pm 2.8 \mu\text{mol/l}$ in the women, $p < 0.001$, Mann-Whitney-U-test) and lowest in the children (6–14 year) ($11.3 \pm 2.8 \mu\text{mol/l}$ in the boys and $12.6 \pm 3.0 \mu\text{mol/l}$ in the girls, $p < 0.001$, Mann-Whitney-U-test). With the exception of the children aged 6–9 years and 10–12 years, male participants had higher plasma concentrations than women although this was not significant for the groups of the 13–14 year-old and the elderly.

The average plasma Zn^{2+} concentrations were above the thresholds for low Zn status of $11.3 \mu\text{mol/l}$ in men and boys ≥ 10 y, $10.7 \mu\text{mol/l}$ in non-pregnant women and girls ≥ 10 y, and $9.9 \mu\text{mol/l}$ in children < 10 y in fasting plasma samples taken in the morning as proposed by the International Zinc Nutrition Consultative Group (IZiNCG) [9]. However, a low Zn status was found in 40.0% and 21.7% of the male and female schoolchildren, in 13.4% and 18.1% of the adult men and women, and in 27.6% and 33.3% of the elderly men and women, respectively. The highest prevalence was found in the 10–12 year-old boys (48.1%) and in the 13–14 year-old boys and girls (42.9% and 38.9%, respectively) (see Table 2 for all age groups).

Recently, a cut-off of $7.65 \mu\text{mol/l}$ for severe Zn deficiency was suggested by Wessells et al [25]. This value was exceeded by most of the participants in the current survey with a total of 35 individuals (4.1% of the total sample) showing lower Zn concentrations. The prevalence by age and sex was 4.3% in the girls aged 6–14 years, 4.8% and 2.6% in the male and female adults, 2.3% in the female and 0% in the male elderly but rising to 7.5% in the 6–14 year-old boys. Again, the older children aged 13–

14 years were most affected (14.3% of the boys and 5.6% of the girls), but also 7.6% of the male 6–9 year-olds.

Zinc intake

As can be seen in Table 2, zinc intake and its adequacy varied between sexes and age groups. Intake of elderly adults aged 65 to 80 years was significantly lower than that of the younger adults of the same sex except for the 51–64 year-old women. With the exception of 65–80 year-old men, the median intake was higher than the respective value recommended by the German, Austrian and Swiss Nutrition Societies (D-A-CH reference values) in all age groups [26]. However, 31.6% of the 6–14 year-old girls and 37.6% of the boys did not meet the recommendation. Upon further age categorisation, a particularly unsatisfactory supply was found in the 10–12 year-old and the 13–14 year-old boys of whom respective 45.7% and 50% did not meet the recommended intake level. Among the girls, this percentage was highest for the 10–12 year-old (35.8%).

Adult women were most likely to consume enough zinc with 79.4% meeting the reference intake level, but this was the case for only 61.4% of the men ($\chi^2(1) = 14.65$, $p < 0.001$). The highest percentage of participants with an adequate intake was found in the two younger age subgroups of women (88.5% of the 18–24 year-old and 81.7% of the 25–50 year-old).

In turn, zinc intake was least satisfactory in the elderly men of whom only 35.5% met the recommended intake level whereas in women aged 65–80 years this was the case in 25.3% ($\chi^2(1) = 25.34$, $p < 0.001$). In 7.9% of the elderly men, zinc intake was even lower than half the recommended intake level of 10.0 mg/d , whereas this was only seen in one boy and one adult man as well as two adult women (data not shown).

Elderly had a lower mean energy intake than the younger adults so that a lower total food intake may have contributed to the lower zinc intake. However, 65–80 year-old men also had a lower zinc intake relative to energy intake compared to the other groups (1.16 mg/MJ vs. 1.26 mg/MJ in the 65–80 year-old women, 1.27 mg/MJ in the 18–64 year old men and 1.25 mg/MJ in the 18–64 year old women). If these values are compared to the respective D-A-CH reference values for Zn and energy intake we obtain a Zn density of 0.96 mg/MJ for a physical activity level (PAL) of 1.6 and of 1.14 mg/MJ for a PAL of 1.4 [26]. Considering the decline in physical activity level with ageing [27, 28], the latter is likely to apply to at least part of the age group of ≥ 65 years, and these male participants in our study would only just achieve the desirable level, while the other age groups were better supplied.

Table 2. Status and intake of zinc by age and sex.

Population group	Schoolchildren		Adults		Elderly	
	Boys (n)	Girls (n)	Men (n)	Women (n)	Men (n)	Women (n)
Zinc concentration in the plasma ($\mu\text{mol/l}$) (mean $\pm$ SD)	all	12.6 $\pm$ 3.0	14.4 $\pm$ 3.2	13.1 $\pm$ 2.8	13.0 $\pm$ 2.6 (72)	12.2 $\pm$ 2.5 ^a (88)
	6–9 y ^d	12.6 $\pm$ 2.9 ^{a,b} (53)	14.1 $\pm$ 2.9 ^{b,c} (17)	13.1 $\pm$ 3.0 ^{a,b} (36)		
	10–12 y ^d	12.8 $\pm$ 3.1 ^{a,c} (78)	14.8 $\pm$ 3.3 ^b (85)	13.5 $\pm$ 2.8 ^c (142)		
	13–14 y	11.2 $\pm$ 2.5 ^b (23)	13.6 $\pm$ 3.1 ^c (43)	12.3 $\pm$ 2.4 ^a (51)		
Prevalence of low zinc levels* (%)	all	21.7	13.4	18.1	27.6	33.3
	6–9 y	19.6	10.7	23.1		
	10–12 y	19.6	13.4	13.0		
	13–14 y	38.9	17.0	27.5		
Dietary zinc intake (mg/d) (median (interquartile range)) [§]	all	8.0 (6.5–9.6)	11.4 (8.8–13.2)	9.3 (7.3–11.6)	8.8 (6.5–11.7) ^a (72)	8.2 (6.9–11.5) ^{a,e} (89)
	6–9 y	7.9 (6.6–10.2) ^{a,e} (57)	11.4 (9.4–14.3) ^b (17)	9.9 (8.1–12.2) ^b (37)		
	10–12 y ^d	8.0 (6.4–9.1) ^a (81)	11.4 (8.4–13.2) ^b (86)	9.5 (7.3–11.5) ^{b,c} (143)		
	13–14 y	8.0 (7.3–10.6) ^{a,c,e} (25)	11.4 (9.3–14.4) ^b (44)	8.7 (6.8–11.5) ^{b,e} (52)		
Prevalence of dietary zinc intake below the D-A-CH recommendations [§] for daily intake	all	31.6	38.6	20.6	64.5	25.3
	6–9 y	27.3	33.3	11.5		
	10–12 y	35.8	40.7	18.3		
	13–14 y	20.0	37.5	28.8		

*Low status defined according to the thresholds proposed by the International Zinc Nutrition Consultative Group (IZINCG): 11.3 $\mu\text{mol/l}$ in men and boys $\geq$ 10 y, 10.7 $\mu\text{mol/l}$ in non-pregnant women and girls $\geq$ 10 y, 9.9 $\mu\text{mol/l}$ in children $<$ 10 y, using fasting plasma samples taken in the morning [10]. [§]Misreporters (defined by an energy intake of $\leq$ 0.81 or $\geq$ 2.98 times the estimated basal metabolic rate (BMR) in children and $\leq$ 0.76 or $\geq$ 3.16 times the estimated BMR in adults and elderly calculated according to Goldberg et al. 1991 [24]) were excluded from the analysis. [†]Daily intake amount of zinc recommended by the German, Austrian, and Swiss Nutrition Societies: 7.0 mg/d in girls $\geq$ 7 years and non-pregnant women of all ages, 7.0 mg/d in 7–9 year-old boys, 9.0 mg/d in 10–12 year-old boys, 9.5 mg/d in 13–14 year-old boys and 10.0 mg/d in male adolescents and adult men ($\geq$ 15 year) [26]. Within each sex, statistically significant differences between the age subgroups are indicated by differing superscript letters. Superscript ^d indicates a significant difference between sexes in the respective age subgroup.

According to the D-A-CH reference values [26], adult men and women have an estimated average requirement (EAR) of 7.5 mg/d and 5.5 mg/d zinc, respectively. This amount was met by 93 % of the adult women, 87 % of the adult men, 86 % of the elderly women and 62 % of the elderly men (data not shown).

In turn, the European Food Safety Authority (EFSA) recommends staggered population reference intakes for adults depending on the content of phytate in the diet giving four levels covering the range of mean or median phytate intake observed in European adult populations (300–1200 mg/d) [29]. The population reference intake for zinc assuming a daily phytate intake of 600 mg was met by 51 % of the adult women and 41 % of the men and by 34 % of the elderly women and 21 % of the elderly men. There was a rather high percentage of the population consuming less than the estimated average requirement (based on 600 mg/d of phytate) that is higher than the one suggested by the D-A-CH reference values (28 % and 29 % of the adult women and men, respectively, and 42 % and 58 % of the elderly women and men, respectively). Again, intake was particularly unsatisfactory in children between 10 and 14 years with only 35 % and 42 % of the 10–12 year-old girls and boys and 19 % of the 13–14 year-old children meeting the respective EFSA RDIs and the majority (48 % and 35 % of the 10–12 year-old and 62 % and 44 % of the 13–14 year-old girls and boys, respectively) were even below the estimated average requirement levels.

Comparable results were found using the EAR values of the US Institute of Medicine [30] as a reference.

In turn, comparing the data from our sample with the age- and sex-specific EAR values suggested by the IZiNCG [9], the proportion of individuals with Zn intakes below the respective cut-off was lower in children especially in boys (7 % of the 10–12 year-old and 32 % of the 13–14 year-old girls and 11.6 % and 5.6 %, respectively, of the boys), but higher among the adult and elderly men (44 % and 65 %, respectively). An overview of the status based on the intake depending on the choice of reference values is given in Table 3.

Zinc intake showed a weak negative correlation with plasma zinc concentration in the adult ($r = -0.150$, $p = 0.006$) and the elderly ($r = -0.179$, $p = 0.026$) subgroup.

Notably, a low plasma zinc concentration was not more frequent in participants with zinc intakes below the RDI than in those with adequate intakes being found in about a quarter of each subgroup across all age groups (24.9 % of those consuming less than the D-A-CH reference values and 23.9 % of those meeting this amount, n.s.). Very similar results were obtained with the EFSA RDI [29]. Comparing the different age groups and sexes, a higher prevalence of low zinc plasma status combined with a low zinc intake was only observed among the boys aged 6–14 years and the elderly men.

Table 3. Overview of the status based on the intake depending on the choice of reference values.

Population group	EAR by different entities and proportion of the study sample with zinc intakes below the respective cut-offs					
	D-A-CH [26]		EFSA [27]		IZiNCG [10]	
	% below cut-off (m/f)	EAR (m/f) (mg/d)	% below cut-off (m/f)	EAR (m/f) (mg/d) ^a	% below cut-off (m/f)	EAR (m/f) (mg/d)
Schoolchildren						
all	n.i.*	n.d.*	25.7/39.8	4.6 (4–6 y)	9.6/10.3	19.1/31.9
6–9 y	n.i.*		12.3/17.5	6.2 (7–10 y)	8.0/5.2	13.3/15.8
10–12 y	n.i.*		34.6/48.4	8.8/8.9 (11–14 y)	11.6/7.0	24.2/39.0
13–14 y	n.i.*		43.8/61.9	11.8/9.9 (15–17 y)	5.6/32.1	16.7/39.3
Adults						
all		7.5/5.5	29.1/27.8	9.3/7.6	44.0/13.4	36.5/20.6
18–24 y			25.0/15.4		37.9/14.3	27.6/14.8
25–50 y			31.6/26.7		46.0/11.9	39.7/20.6
51–64 y			26.5/34.6		44.4/16.4	33.3/21.8
Elderly (65–80 y)		7.5/5.5	57.9/42.0	9.3/7.6	65.0/20.0	60.0/29.2
				10/6		9.4/6.8

*Not indicated/not determined (The D-A-CH reference values for nutrient intake do not contain EAR values for children). ^aBased on a mean phytate intake of 600 mg/d. ^bAssuming a mixed or refined plant-based diet.

The influence of food pattern

Frequency of red meat consumption was not associated with a plasma zinc concentration or the prevalence of low Zn plasma levels. There was also no statistically significant difference in zinc intake between different levels of red meat consumption frequency.

Information on consumption of wholegrain bread was available for most participants ($n = 624$). In persons with a regular intake (at least once daily, $n = 62$), the frequency of sufficient Zn status was slightly lower than in persons consuming wholegrain bread only occasionally or never (72.6 % vs. 77.4 %) but this difference was not statistically significant. Moreover, a markedly low status was more prevalent in persons with a low consumption of wholegrain bread (4.4 % vs. 1.6 % of those with a high consumption) even though this was also not significant. No clear trend was seen with regards to the adequacy of zinc intake.

Dietary fibre intake was not related to Zn plasma concentration either.

Zinc status and enzyme activities and oxidative markers

Zinc plasma status was negatively correlated to AP activity ($r = -0.298$, $p < 0.001$). There were also weak negative correlations between zinc plasma concentration and the activity of blood catalase activity ($r = -0.150$, $p < 0.001$) and plasma MDA ($r = -0.158$, $p < 0.001$) as well as a weak positive correlation to blood glutathione peroxidase ($r = 0.115$, $p = 0.001$).

In a stratified analysis, the correlation with AP activity was only maintained in the adult subgroup ($r = -0.212$, $p < 0.001$), that with GSH-Px only in the children ($r = 0.167$, $p = 0.003$) and that with MDA in the children ($r = -0.176$, $p = 0.002$) and the elderly ($r = -0.527$, $p < 0.001$), while CAT and Zn concentration were associated in the children ($r = -0.154$, $p = 0.007$) and adults ($r = -0.123$, $p = 0.023$).

Furthermore, in the elderly subgroup, zinc status was negatively associated with erythrocyte superoxide dismutase (SOD) ($r = -0.466$, $p = 0.002$). No correlation was observed between zinc plasma concentration and total antioxidative capacity.

Discussion

Based on the present findings, schoolchildren and elderly in Austria may be considered at-risk groups for sub-optimal Zn intake as the prevalence of low Zn plasma concentration exceeded 20 %. The high prevalence of a marginal

status is also notable although in the young children, it might be due to the threshold being too high as suggested by the lower IZiNCG levels for this age group. This could also be the case for the cut-off for severe Zn deficiency suggested by Wessells et al. that was deduced from studies in adults aged 18–40 years [25]. Nevertheless, this finding underscores the importance of zinc as a critical nutrient all the more as the low zinc status in the older children coincided with an unsatisfactory intake in a high percentage of this sub-population. This was especially the case when taking the higher reference values of the EFSA [29] as the basis despite the fact that considering the low intake of whole grain bread and cereals and pulses, the major sources of phytate, in the present collective, the reference values based on a daily intake of 600 mg were used for this study. Only 19 % of the 13–14 year-old girls and boys met the respective recommended amounts and the majority (62 % and 44 %, respectively) were even below the estimated average requirement levels. The lower D-A-CH reference values [26] were better met, but it has to be considered that zinc is particularly important for growth and might therefore be especially critical in adolescents.

Elderly men are another critical population when it comes to zinc supply, showing the highest prevalence of inadequate Zn intake. Moreover, in this group, zinc nutrient density (i.e. intake amount per energy intake) was also the lowest and might be suboptimal in persons with a low physical activity level and hence, low energy requirement. This suggests that the insufficient intake is not only caused by a generally reduced food intake as it is often observed in this age group but that diet quality is also not optimal [10, 31, 32]. Nevertheless, this group showed a comparatively better Zn status with 72.4 % above the threshold for adequacy.

However, the prevalence of low zinc intake did not always coincide with low plasma level in any of the age groups. It has been argued that plasma zinc level might be too tightly regulated to serve as a good indicator of zinc status at least in the absence of severe zinc deficiency. Moreover, it is also influenced by other factors besides intake such as circadian rhythm, infectious diseases, inflammatory events, exercise, stress and trauma [9, 29, 30, 33, 34]. On the other hand, plasma zinc level does respond to zinc supplementation [9, 35]. It is currently a widely used and accepted biomarker for zinc status but the need for optimal biomarkers of zinc status in humans is acknowledged [29, 34]. Moreover, dietary assessment of zinc intake is also more difficult than for other nutrients due to a paucity of composition data for this nutrient. Nevertheless, the composition database used in the present study is based on data that was used among others to estimate the intake of the European population and to derive reference values for daily zinc intake [29].

Diet composition and quality are important factors influencing Zn absorption. Besides an insufficient Zn intake, a low bioavailability of this trace element may also cause a deficient status. The most important antinutritive factor in this context is phytic acid. A high intake of phytic acid typical of diets consisting mainly of little or unrefined foods can compromise adequate zinc supply [9, 36, 37]. While such diets are particularly consumed in rural parts of many low-income countries [38], low bioavailability of zinc from unrefined diets such as those consumed by vegetarians and vegans or for health reasons might also result in higher risks of trace mineral insufficiencies in industrialised countries [13, 39]. In turn, meat, particularly the red type, is a good source of highly bioavailable zinc [37]. However, none of these factors was significantly associated with zinc status in the present survey. The fact that these analyses were based on the results from the FFQ is likely to have contributed to this failure as the FFQs were not quantitative but only reflected the habitual frequency of consumption of different foods or food groups. This approach was chosen because, if at all, zinc status is more likely to be influenced by long-term food consumption rather than acute consumption that can be subject to high variability.

Due to the role of zinc as a co-factor to many enzymes, some of them involved in redox metabolism, as well as for oxidative balance in general, fluctuations in zinc status may affect the function of these enzymes and markers of oxidative status. However, so far, attempts to associate zinc deficiency states with altered activity of zinc-containing enzymes like alkaline phosphatase (AP), lactate dehydrogenase and plasma ribonuclease have not proved successful [40]. Positive relationships between AP activity and Zn status have previously been reported under conditions of severe Zn deficiency whereas the picture is less clear under zinc sufficiency or marginal status in which positive relationships but also no or negative associations were observed [41–43]. The negative correlation of zinc plasma status with AP activity falls in line with these latter findings.

However, the weak positive and negative correlations to the activity of blood glutathione peroxidase and blood catalase activity, respectively, and the moderate negative correlation to erythrocyte superoxide dismutase (SOD in the elderly population might be related to the role of Zn in redox regulation. Thus, the negative correlations of SOD and catalase in erythrocytes with Zn concentration in plasma could be explained by the fact that a better Zn status in itself contributes to lower oxidative stress so that there is no need for high activity of antioxidative enzymes. In line with this, MDA as a major indicator of lipid peroxidation was negatively correlated to $[Zn^{2+}]$.

A limitation of the current study is the fact that no adjustment was made to estimate the usual intake distribution and thereby the participants' habitual intake. Moreover, as phytate intake was not determined due to a lack of a respective composition database the bioavailability of zinc in the study population can only be approximated.

However, the inclusion of a large study population covering a wide age range and the collection of repeated dietary recalls representing all days of the week and all seasons are strengths that can compensate for some of the day-to-day variability of dietary zinc intake. The identification of zinc as a potentially critical nutrient in certain Austrian population groups is also supported by the biochemical data obtained in the study.

Conclusion

An adequate dietary intake of zinc is important for optimal health and performance. However, the recommended intake amounts are often not met especially in certain at-risk population groups even in wealthy industrialised countries like Austria. Monitoring of zinc status at population level is thus warranted to identify deficiency risks and address them efficiently. In this regard, special relevance comes to the use of appropriate biomarkers and reference values for daily intake. While plasma or serum zinc concentration is a widely used and accepted biomarker, its specificity and sensibility are not optimal. Among other factors, reductions can result from infectious diseases and inflammatory states [9, 34]. Alternative biomarkers, especially of long-term zinc status, have been suggested in the form of intracellular zinc concentrations in erythrocytes, different leukocytes or platelet cells as well as concentrations in hair but these options have a limited usefulness particularly for identifying moderate deficiency states [9, 35]. Thus, there is a need for novel biomarkers of zinc status that have a high specificity and sensibility. Some potential candidates based on new biotechnological approaches have been proposed like the expression of metallothionein and zinc transporters that have been shown to respond to small changes in zinc bioavailability [9, 34]. Notably, decreases in metallothionein mRNA in human buccal samples following depletion also offer the potential of developing a non-invasive biomarker [44]. However, these parameters are also influenced by other factors like other trace and toxic heavy metals as well as oxidative stress [45]. This might be overcome by using a combination of several biomarkers. Furthermore, the costs, requirements for special equipment and trained personnel limit their applicability to the screening at population level.

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Conflicts of interest

The authors are not aware of any conflicts of interest.

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Prof. emer. Ibrahim Elmadfa

Faculty of Life Sciences
Department of Nutritional Sciences,
University of Vienna
Althanstraße 14
1090 Vienna
Austria

ibrahim.elmadfa@univie.ac.at