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The influence of food consumption and socio-economic factors on the relationship between zinc and iron intake and status in a healthy population

Marija Knez[1,2,*], Marina Nikolic[2], Milica Zekovic[2], James CR Stangoulis[1], Mirjana Gurinovic[2] and Maria Glibetic[2]
[1]School of Biological Sciences, Flinders University, Sturt Road, Bedford Park, Adelaide, SA 5042, Australia: [2]Centre of Research Excellence in Nutrition and Metabolism, Institute for Medical Research, University of Belgrade, Belgrade, Serbia

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Abstract

Objective: To examine Zn and Fe nutritional status of a healthy population by means of anthropometric, dietary and biochemical measurements and to investigate the relationship of usual Zn and Fe dietary intakes to Zn and Fe status. In addition, to examine the impact of food choices and socio-economic factors on Fe and Zn dietary intakes and status with the aim to identify groups at risk of dietary deficiency and suggest factors that may influence the status of these nutrients.
Design: Food consumption was assessed by 24 h recall questionnaires. Twenty biochemical parameters were measured, of which Hb, haematocrit, erythrocyte count and plasma concentrations of Fe and Zn were directly related to Fe and Zn nutrition. The prevalence of study participants with inadequate micronutrient intakes was calculated using the Estimated Average Requirement cut-point method.
Setting: Serbia, Europe.
Subjects: Apparently healthy adults (25–65 years of age).
Results: Mean daily Zn and Fe intakes were 9·1 mg and 11·6 mg for males and 7·3 mg and 9·4 mg for females, respectively. Five per cent of the study population had inadequate dietary Fe intake and 15–25 % had inadequate Zn intake. Lower Hb concentrations were measured in women with lower Zn intakes. No differences in Fe and Zn intakes and status among various socio-economic groups were observed, except for Fe intake between the low-income and affluent groups.
Conclusions: Regular follow-ups are needed to ensure that potential deficiencies of Zn and Fe do get recognized and addressed in a timely manner.

Keywords
Zn
Fe
Nutritional status
Minerals
Adults
Healthy population
Dietary intake
Socio-economic factors

Zn and Fe are essential micronutrients for human health and are involved in many complex enzyme systems that function in various biological processes[1–3]. Deficiencies of these two nutrients remain a global problem, with the worldwide prevalence of Fe deficiency estimated to be about 30 %[4] while approximately 20 % of the world's population is affected by Zn deficiency[5].

Although severe Zn deficiency is uncommon in developed countries, marginal deficiency is likely to be much more prevalent[6] and is associated with certain functional outcomes; for instance, delayed wound healing, disturbances of taste and smell acuity, decreased work capacity and diminished immunological response[6–8].

The burden of Fe and Zn deficiencies is most common in low-income countries; however, inadequate intake of these micronutrients is also seen in resource-rich areas of the world where overeating becomes a public health concern. Recent results from nutritional surveys conducted in the USA, Great Britain and Germany indicated that the recommended intakes of Fe and Zn are not always achieved[9,10]. The population-based data from Europe suggested substantial variability in micronutrient intakes among healthy adults[9,10]. Data from the National Health and Nutrition Examination Survey 2001–2002 showed that 12 % of the US population had Zn intake below the Estimated Average Requirement (EAR)[11]. Similarly, 10–35 % of Canadians from most age and sex groups consumed Zn in inadequate amounts[12]. Between 11 and 21 % of the population from Greece, the UK and Finland had Fe intake below the EAR[13–15]. Similarly, a high percentage (39 %)

*Corresponding author: Email marijaknez186@gmail.com; knez0004@flinders.edu.au

of the population in the USA was also found to be at risk of inadequate Fe intake[16]. Women aged 14–50 years are at particular risk of low Fe intake, as confirmed recently by results obtained from six European countries[10]. The prevalence of inadequate intakes of Fe and Zn is not consistent between studies performed in different developed countries. For Serbia in particular, the relevant data for adults are either old or scarce, therefore requesting additional investigation.

Zn is found in similar food sources to Fe, so insufficient dietary Fe and Zn intakes usually occur simultaneously[17,18]. Considering these diet-based similarities, there is a possibility that Fe and Zn status are also positively correlated. Zn metabolism interacts with the metabolism of Fe[19–21]. Similarly, it was noted that Zn and Fe nutriture are affected by many of the same dietary factors (i.e. Ca, phytate and polyphenols)[22,23]. The simultaneous occurrence of Zn and Fe deficiencies in man has been known since the discovery of Zn deficiency. Zn deficiency associated with Fe-deficiency anaemia was first recorded by Prasad *et al.*[24,25].

A number of studies over the years have demonstrated that Zn intake is correlated with Hb concentration[26,27]. However, it is not established if suboptimal Zn intake or slightly lower Zn status infers low Fe intake *per se* or low Fe status. Therefore, our objective was to identify relationships between Zn and Fe intakes and status in an apparently healthy population, where only marginal Fe and Zn deficiencies may occur.

The influence of socio-economic and demographic factors on Zn and Fe nutriture has been investigated to some degree[28,29]. However, information on the influence of educational level, marital and socio-economic position on mineral status in healthy populations is scarce, especially for Zn intake.

The current cross-sectional study reports on the current nutritional intakes of Zn and Fe in apparently healthy adults (25–65 years of age) living in Serbia and examines the relationship between dietary Zn and Fe intakes and related biochemical parameters. In addition, the study investigates the impact of food choices and socio-economic factors on Fe and Zn dietary intakes and status with an aim to identify groups at risk of dietary deficiency and to suggest factors that may influence the status of these nutrients.

Materials and methods

Study participants

The study participants (*n* 754) were apparently healthy 25–65-year-old male and female volunteers recruited within an 18-month period from June 2013 to January 2015 through two different projects: National project III 41030 and the BACCHUS project (EU 7th Framework Programme (FP7), project no. 312090). The study participants were recruited through primary health-care facilities (community pharmacies and health centres) where flyers about participation in the study were distributed. Eligible subjects included apparently healthy people living in Serbia (as indicated by brief questionnaires, measure of blood pressure and blood glucose). Subjects without any clinical signs of an acute condition or chronic disease and without the need for medical treatment were included in the study. Pregnant and lactating women were excluded. All subjects went through the informed consent process, both verbal and written. The study protocols were approved by the Clinical Hospital Centre Zemun, Belgrade, Serbia (ethics committee approval number: 2125, 2013) and by the Southern Adelaide Clinical Human Research Ethics Committee (SAC HREC EC00188 approval number: 96.15), Adelaide, South Australia.

The protocols and procedures were in agreement with the ethical guidelines on biomedical research on human subjects of The Code of Ethics of the World Medical Association's Declaration of Helsinki (1964) and its further amendments.

Assessment of dietary zinc and iron intakes

Dietary intake data were collected for three non-consecutive days, two working days and one weekend day, using the 24 h recall technique. All interviews were done face to face and led by a trained professional according to a standardized protocol. The estimated time of data collection was 15–30 min. During the interview, a food atlas with coloured photographs of 125 items (foods and composite dishes), illustrating a range of portion sizes in increasing order, was used in order to enhance the accuracy of portion size estimation[30]. In addition, to enhance interpretation of dietary estimates, general questions related to education, previous medical conditions, detailed food supplements, medication use, and lifestyle habits regarding smoking, physical activity/exercise, alcohol as well as coffee and tea consumption were also incorporated in the questionnaire. All questionnaires were checked for errors/omissions together with study participants.

The Zn and Fe contents of foods were determined using the Serbian food composition database, harmonized with EuroFIR standards and embedded in the EuroFIR food platform and Balkan food platform[30,31]. DIETS ASSESS & PLAN, a nutritional tool validated in different national and regional surveys and international projects, and evaluated in the European Food Safety Authority project, was used to obtain comprehensive dietary intake assessments[32]. Dietary data were entered in duplicate to ensure accuracy of data entry and to identify potential inconsistencies. Both the dietary intake assessment software used and the food composition data management system have integrated quality control mechanisms including mandatory fields, standard templates and error warnings[30,31].

The dietary intakes from administered questionnaires were calculated by multiplying the frequency of consumption of each food item by the composition of that food using adequate portion sizes. In addition to Zn and Fe dietary intakes, data were obtained for energy, macronutrients and main food groups.

Zn and Fe contents of participants' diets were verified using the EAR, as defined in the Dietary Reference Intakes provided by the Institute of Medicine[33]. To check for under-reporting, the ratio of reported energy intake to predicted BMR was used, where BMR was estimated according to the Schofield equations[34] taking into account age, sex and body weight.

Collection of blood samples, socio-economic data and anthropometric measurements

Blood samples were collected from a random sub-sample of 177 participants (23 % of the study population) between 08.00 and 09.00 hours after an overnight fast (>10 h fasting). Whole-blood samples were collected from participants in a seated position into trace-mineral-free tubes by venepuncture from an antecubital vein using butterfly needles (Sarstedt, Inc.). All samples were centrifuged (1000***g*** for 15 min). The serum and plasma samples were removed and 1 ml aliquots were stored at −80°C until further analysis.

Data on the socio-economic and demographic characteristics of participants were collected via questionnaires. The risk of poverty was defined as 'the share of people with an equalised disposable income (after social transfer) below the at-risk-of-poverty threshold, which is set at 60 % of the national median equalized disposable income'[35,36].

Anthropometric variables, height and weight, were measured to the nearest 0·1 cm and 0·1 kg, respectively. Body weight and body composition (muscle mass and fat tissue mass) of participants were measured using a TANITA UM072 balance (TANITA Health Equipment H.K. Ltd). BMI was computed as the ratio of weight to height squared (kg/m^2). BMI was used to assess the prevalence of overweight (25·0–29·9 kg/m^2) and obesity (≥30·0 kg/m^2) according to WHO criteria[37].

Biochemical analysis

The biochemical parameters were measured using a Cobas e411 clinical chemistry analyser (Roche Diagnostics) and using Roche Diagnostics kits according to the manufacturer's instructions.

Plasma Fe, Hb, haematocrit and erythrocyte count were used as markers of Fe status and plasma Zn was used for Zn status. Individuals were considered to be at risk of Fe-deficiency anaemia if they had below-normal values for Hb (<120 g/l).

Determination of plasma zinc and iron concentrations

The assessment of Fe and Zn mineral status was performed for eighty-two participants (approximately 10 % of the study population). The analysis of plasma Zn and Fe was conducted at the Institute of Public Health, Pozarevac, Serbia, using flame atomic absorption spectrometry on a Varian SpectrAA-10 instrument with instrument parameters: wavelength 213·9 nm, slit width 1·0 nm and air–acetylene flame, according to the method described by Jian Xin[38].

The concentrations of Zn and Fe were measured after dilution 1:10 with high-purity Milli-Q water (>18 MΩ resistivity). To verify the accuracy of the method for Zn, control serum ClinChek-Control (Recipe, Chemical + Instruments GmbH; catalogue number 8880) with Zn concentration of 889 (sd 178) μg/l (Level I) and 1738 (sd 261) μg/l (Level II) was analysed. Likewise, for Fe, control serum ClinChek-Control (Recipe, Chemical + Instruments GmbH; catalogue number 8882) with Fe content of 612 (sd 122) μg/l (Level I) and 1538 (sd 154) μg/l (Level II) was used. Method performances were monitored by analysis of the same control serum within each series. The mean results for Zn concentration were 914 (sd 23) μg/l (Level I) and 1801 (sd 35) μg/l (Level II) which is in accordance with the certified values. In order to avoid Zn contamination, all tubes and utensils were either soaked in HNO_3 (25 %, w/w) for 16 h, or were known from previous studies to be Zn-free. The mean results obtained for Fe content were 598 (sd 24) μg/l (Level I) and 1529 (sd 55) μg/l (Level II) which was in agreement with the certified values.

Statistical analysis

The distribution of data was tested using the Shapiro–Wilk's test for normality. Non-parametric statistical methods were applied for data that were not normally distributed.

The associations of Zn and Fe intakes and status with biochemical parameters were established by Spearman rank correlation. The effect of demographic and socio-economic factors on Fe and Zn intakes and status was analysed using the Kruskal–Wallis test for factors that were divided in more than two categories and the Wilcoxon test for independent samples (Wilcoxon rank-sum test) for factors with two categories. Data are presented as means and sd and results are considered significant at $P < 0·05$. All analyses were performed using the R software package[39].

Results

Macronutrient, iron and zinc intakes of study participants

The study sample included 754 participants, 128 males (17 %) and 626 (83 %) females. Table 1 shows mean intakes of macronutrients, Fe and Zn together with their percentage contributions to total energy intake.

Energy, protein and mineral crude intakes were significantly higher in men than in women ($P = 0·001$). Under-reporting of food consumption was estimated to occur in 34 % (260/754) of our study participants. The mean dietary Zn intake was 9·1 mg/d for males and

Table 1 Macronutrient, iron and zinc intakes of apparently healthy Serbian adults, June 2013–January 2015

	Males (25–65 years, *n* 128)				Females (25–65 years, *n* 626)			
	Mean	SD	%TE	SD	Mean	SD	%TE	SD
Macronutrients								
Energy (kJ/d)	8937	3055	–	–	7135	1802	–	–
Energy (kcal/d)	2135·9	730·1	–	–	1705·3	430·7	–	–
Protein (g/d)	83·4	26·0	16·5	4·2	64·3	17·7	15·7	3·5
Total carbohydrate (g/d)	207·7	99·0	39·1	10·1	183·5	55·4	44·5	7·5
Total fat (g/d)	94·8	36·6	40·9	8·4	69·6	24·3	37·4	7·1
Minerals								
Zn (mg/d)	9·1	4·6	–	–	7·3	3·8	–	–
					Females (<50 years, *n* 501)		Females (≥50 years, *n* 125)	
Fe (mg/d)	11·6	5·4			9·4	4·0	10·0	5·7

%TE, percentage of total energy intake.

Table 2 Percentage of apparently healthy Serbian adults with iron intake below recommendations, June 2013–January 2015

	Males (25–65 years, *n* 128)		Females (<50 years, *n* 501)		Females (≥50 years, *n* 125)	
	Fe RNI (mg/d)	% below RNI	Fe RNI (mg/d)	% below RNI	Fe RNI (mg/d)	% below RNI
FAO/WHO[53]	9·1	39·8	19·6	97·8	7·5	32·8
IOM[33]	8	23·4	18	96·6	8	36·7
IOM (EAR)[33]	6	7·0	8·1	38·3	5	11·8
NNR[52]	9	35·9	15	94·6	9	50·0

RNI, Recommended Nutrient Intake (the daily intake that meets the nutrient requirements of almost all (97·5 %) apparently healthy individuals in an age- and sex-specific population group); IOM, Institute of Medicine; EAR, Estimated Average Requirement (the daily intake that meets the nutrient needs of 50 % of the healthy individuals in a specific population's group); NNR, Nordic Nutrition Recommendations.

7·3 mg/d for females, while mean dietary Fe intake was 11·6 mg/d for males and 9·4 mg/d for females. Zn intake correlated with energy ($r = 0·45$), protein ($r = 0·64$), carbohydrate ($r = 0·32$) and Fe ($r = 0·57$) intakes (all $P < 0·001$).

Energy, protein, carbohydrate, Fe and Zn intakes were adequate according to the Dietary References Intakes for age and sex of the adult population[40]. However, the percentage of energy coming from fat was higher than the recommended amount: 40·9 and 37·4 % of energy for males and females, respectively, compared with the recommended 20–35 %[40].

Percentage of population with iron and zinc intakes below dietary recommendations

The percentage of the study population with low Zn intake was greater than the percentage with low Fe intake. After taking under-reporting into account, we estimated that 5 % of the study population was at risk of inadequate Fe intake and 15–25 % was at risk of inadequate Zn intake. Females were at much higher risk of inadequate Fe intake, while males were at higher risk of inadequate Zn intake. Females below 50 years of age were at the highest risk of inadequate Fe intake. There were significant differences in the level of inadequacy observed when using dietary recommendations proposed by different expert groups (Tables 2 and 3).

Anthropometric, socio-economic and biochemical data of study participants

Main anthropometric and demographic characteristics of the study participants are presented in Tables 4 and 5.

Anthropometric parameters measured in the present study fell within the reference ranges for the healthy adult population. The mean BMI of our study population was 26·8 kg/m^2 for males and 24·0 kg/m^2 for females. About 30 % of males and 70 % of female participants in our study (Table 4) had BMI values in the normal range (*n* 476), while about 30 % of participants were overweight (*n* 277) and 10 % were obese.

An assessment of marital status and education showed that 50 % of participants were married and 34 % were tertiary educated (Table 5).

Of the 754 participants included in the study, biochemical data were obtained for 23 % of our study population (Table 5). Different biochemical and haematological parameters were assessed (twenty different indicators) with only parameters related to Fe status being presented (Table 5).

Dietary intakes of zinc and iron of study participants

Different food groups in relation to iron and zinc intakes

Grains (mainly wheat, rye and rice) were the most important source of dietary Fe. The proportion of total dietary Fe coming from grains was about 30 % for both genders.

Table 3 Percentage of apparently healthy Serbian adults with zinc intake below recommendations, June 2013–January 2015

	Males (25–65 years, *n* 128)		Females (25–65 years, *n* 626)	
	Zn RNI (mg/d)	% below RNI	Zn RNI (mg/d)	% below RNI
FAO/WHO[53]	4·2	10·2	3	3·7
IOM[33]	11	73·4	8	69·4
IOM (EAR)[33]	9·4	60·2	6·8	50·6
FAO/WHO[53]	9	58·6	7	53·8
IZiNCG[92]	10	66·4	6	38·9

RNI, Recommended Nutrient Intake (the daily intake that meets the nutrient requirements of almost all (97·5 %) apparently healthy individuals in an age- and sex-specific population group); IOM, Institute of Medicine; EAR, Estimated Average Requirement (the daily intake that meets the nutrient needs of 50 % of the healthy individuals in a specific population's group); NNR, Nordic Nutrition Recommendations; IZiNCG, International Zinc Nutrition Consultative Group.

Table 4 Anthropometric measurements of apparently healthy Serbian adults, June 2013–January 2015

	Males (*n* 128)	Females (*n* 626)
Mean weight (kg)	88·9	68·0
SD	14·0	11·8
Mean height (cm)	181·8	168·4
SD	0·1	0·1
Mean BMI (kg/m^2)	26·8	24·0
SD	3·5	4·0
Mean WC (cm)	95·4	78·5
SD	16·9	14·3
Nutritional status (by BMI; %)		
Underweight (<18·5 kg/m^2)	0·8	2·1
Normal range (18·5–24·9 kg/m^2)	30·5	69·2
Overweight (≥25·0 kg/m^2)	68·8	28·8
Pre-obese (25·0–29·9 kg/m^2)	50·8	20·0
Obese class 1 (30·0–34·9 kg/m^2)	18·0	6·5
Obese class 2 (35·0–39·9 kg/m^2)	0·0	1·8
Obese class 3 (≥40·0 kg/m^2)	0·0	0·5
Obesity co-morbidity risk (by WC; %)		
Level 1 (females >80 cm; males >94 cm)	57·0	32·9
Level 2 (females >88 cm; males >102 cm)	32·0	19·0

WC, waist circumference.

Other important sources of Fe were meats (17 %) and vegetables (mainly beans and legumes, 16–21 %; Table 6). Similarly, red meats were the most essential contributor to dietary Zn intake in the study population (30 % of total Zn intake), followed by grains and grain products and vegetables, with a contribution of 17 and 12 %, respectively. Good sources of Zn were also milk and milk products, where more than 20 % of Zn intake was coming from products such as cheese and yoghurt (see online supplementary material, Supplemental Table 1). The intakes of both Fe and Zn were correlated with the intakes of certain food groups.

The most noteworthy correlations among the various food groups were those for grain products ($r = 0{\cdot}43$ for Fe and $r = 0{\cdot}28$ for Zn, both $P < 0{\cdot}001$), vegetables ($r = 0{\cdot}33$ for Fe and $r = 0{\cdot}22$ for Zn, both $P < 0{\cdot}001$) and meat ($r = 0{\cdot}25$ for Fe and $r = 0{\cdot}41$ for Zn, both $P < 0{\cdot}001$). The intakes of Fe and Zn were correlated with energy intake ($r = 0{\cdot}56$ and $r = 0{\cdot}45$, respectively, both $P < 0{\cdot}001$).

Contribution of grains and pulses to total iron and zinc intakes

Phytate in the diet comes mainly from cereal grains, grain products and legumes. White bread and wheat flour are two of the most consumed cereal foods in the Serbian diet and beans and peas are the most commonly used legumes by our study participants (see online supplementary material, Supplemental Tables 2 and 3 for the top ten foods from the cereals and legumes food groups contributing to Fe and Zn intakes).

The negative effect of phytic acid on Zn absorption follows a dose-dependent response, and the molar ratio of phytate to Zn of a diet can be used to predict the proportion of absorbable dietary Zn[41].

Due to inadequate dietary data on the phytate content of foods in Serbian databases, we were not able to calculate phyate:Zn molar ratios. However, we employed the method by Jati *et al.*[42] to determine the bioavailability (Table 7) and confirmed that there is a high bioavailability of both minerals in participants' diets.

Correlation between zinc and iron intakes and status and iron biochemical parameters

Mean plasma Zn and Fe status of our study population appeared adequate in spite of the lower dietary intake of these minerals (Table 5).

Intakes of Fe and Zn were strongly correlated ($r = 0{\cdot}57$, $P = 0{\cdot}001$), while the correlations between biochemical parameters and Fe and Zn dietary intakes were weak. The plasma Zn concentration did not reflect Zn intake ($P = 0{\cdot}81$). Women below 50 years of age are the most vulnerable group for development of Fe deficiencies due to increased requirements (menstrual bleeding) and increased need for Fe, and also very often poor intake and poor bioabsorption of Fe. Out of 142 women for whom biochemical data were available, about 23 % had lower Hb concentration (<120 g/l).

There was a statistically significant difference in the intake of Zn between the groups of women with different Hb concentrations ($P = 0{\cdot}01$; Table 8) with no variation in any biochemical or anthropometrical indicators measured.

Effect of lifestyle factors on iron and zinc intakes and status

Plasma Zn levels ranged from 0·72 to 1·67 mg/dl in males and from 0·78 to 1·64 mg/dl in females. Mean plasma concentrations of Fe and Zn were different between males and females ($P < 0{\cdot}05$ and $P = 0{\cdot}02$, respectively). Likewise, the Fe and Zn dietary intakes were gender-dependent. Generally, mean dietary intake levels of Fe and Zn were significantly higher in males compared with

Table 5 Effect of age, gender, educational level, obesity, marital status, socio-economic status, and iron and zinc intake adequacy on mean daily crude iron and zinc dietary intakes, iron-related biochemical parameters and plasma concentrations of iron and zinc in apparently healthy Serbian adults, June 2013–January 2015

	Dietary intakes						Biochemical parameters											
			Fe (mg/d)		Zn (mg/d)				Haematocrit (l/l)		Hb(g/l)		Erythrocyte count (×10^{12}/l)		Plasma Fe (mg/dl)		Plasma Zn (mg/dl)	
	n	%	Mean	SD	Mean	SD	*n*	%	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Gender																		
Male	128	17	11·6***	5·4	9·1***	4·6	31	38	0·4***	0·04	150·6***	10·9	5·1***	0·4	1·3*	0·4	1·1*	0·2
Female	626	83	9·5	4·4	7·3	3·8	51	62	0·4	0·03	127·1	11·4	4·5	0·4	1·3	0·9	1·0	0·2
Age years)																		
20–29	231	31	9·5	3·5	7·5	2·9	3	4	0·4	0·03	131·3	9·1	4·3	0·5	1·6	0·6	1·0	0·1
30–39	197	26	10·0	5·2	7·4	3·3	28	34	0·4	0·1	141·6	18·1	4·9	0·6	1·1	0·5	1·1	0·2
40–49	154	20	9·7	4·4	7·8	4·5	38	46	0·4	0·03	133·7	13·1	4·7	0·4	1·3	0·7	1·1	0·2
50–59	118	16	10·4	5·5	8·2	5·9	13	16	0·4	0·05	131·8	18·2	4·5	0·6	1·6	1·1	1·0	0·1
60–69	54	7	10·2	5·3	6·9	3·5	0											
Obesity (BMI)																		
Obese (≥30·0)	79	10	10·4*	4·7	8·2	4·3	26	32	0·4	0·04	138·5	17·8	4·8	0·6	1·3	0·4	1·1	0·2
Non-obese (<30·0)	675	90	9·8	3·9	7·6	3·9	56	68	0·4	0·05	134·9	15·1	4·7	0·5	1·2	0·8	1·1	0·2
Fe intake adequacy																		
Adequate	538	11·3***	4·7	8·4***	4·3	70	85	0·4*	0·04	137·5*	16·6	4·8	0·5	1·3	0·6	1·1	0·2	
Inadequate	215	6·3	1·4	5·5	1·9	12	15	0·4	0·03	127·4	7·3	4·5	0·4	1·3	1·3	1·1	0·2	
Zn intake adequacy																		
Adequate	360	11·5***	5·2	10·1***	4·3	52	63	0·4*	0·05	137·1*	16·8	4·8	0·5	1·3	0·6	1·0	0·2	
Inadequate	393	8·3	3·4	5·3	1·5	30	37	0·4	0·04	134·2	14·7	4·6	0·5	1·3	0·9	1·1	0·2	
Socio-economic status																		
Low income	247	33	10·5*	5·5	7·5	3·4	0	0	–		–		–		–		–	
Affluent	507	67	9·5	4·1	7·7	4·2	82	100	0·4	0·04	136·0	16·0	4·7	0·5	1·3	0·7	1·1	0·2
Education																		
Primary	58	8	10·5	5·4	7·2	3·4	1	1	0·4		125		4·5		0·6		1·1	
Secondary	443	59	9·7	4·7	7·3	3·0	17	21	0·4	0·04	132·6	13·7	4·7	0·4	1·3	0·6	1·1	0·2
Tertiary	253	34	9·9	4·2	8·2	5·3	64	78	0·4	0·05	137·1	16·6	4·7	0·5	1·3	0·7	1·1	0·2
Marital status																		
Married	383	51	9·9	4·7	7·7	4·7	55	67	0·4	0·04	134·6	16·5	4·7	0·5	1·3	0·8	1·1	0·2
Single	203	27	9·7	4·1	7·5	2·9	18	22	0·4	0·05	138·7	16·9	4·8	0·5	1·3	0·5	1·0	0·2
Other	168	22	10·1	6·2	7·3	3·3	9	11	0·4	0·03	139·6	9·9	4·9	0·4	1·0	0·3	1·1	0·2

Plasma Fe and Zn concentrations determined by flame atomic absorption spectrometry analysis.
Between-factor comparison (using the Kruskal–Wallis test for factors with more than two categories and the Wilcoxon test for factors with two categories): *$P < 0.05$, ***$P < 0.001$.

Table 6 Contribution (%) of the main food groups to total zinc and iron intakes of apparently healthy Serbian adults, June 2013–January 2015

	Fe			Zn		
	Males	Females	Total	Males	Females	Total
Meat and meat products	17·9	16·7	17·2	35·2	29·2	30·6
Milk and milk products	1·9	2·4	2·3	20·0	22·6	22·2
Grains and grain products	30·1	32·8	32·6	13·9	17·3	16·6
Vegetable and vegetable products	14·9	11·4	12·3	11·2	7·7	8·3
Legumes	6·1	4·8	5·1	3·6	2·9	3·1
Egg and egg products	5·1	3·5	3·8	4·9	3·9	4·1
Nuts, seeds, kernel products	8·4	9·6	9·4	3·1	3·5	3·3
Beverages (non-milk)	4·2	4·9	4·8	2·0	3·2	3·0
Seafood and related products	2·4	2·6	2·6	1·6	3·1	2·7
Fruit and fruit products	3·4	4·6	4·4	1·7	2·4	2·2
Sugar and sugar products	3·3	3·9	2·6	1·9	2·1	2·1
Miscellaneous food products	1·9	2·6	2·5	1·0	2·0	1·8
Fats and oils	0·3	0·3	0·3	0·0	0·1	0·1

Table 7 Assessment of the bioavailability of zinc and iron in the diets of apparently healthy Serbian adults, June 2013–January 2015

	Males	Females
Total energy (kJ)	8916	7182
Total energy (kcal)	2131·0	1716·5
Energy from protein from fish & meat (kJ)	625	417
Energy from protein from fish & meat (kcal)	149·3	99·6
Energy from protein from fish & meat (%TE)	7·0	5·8
Energy from grains & nuts & pulses (kJ)	2923	2654
Energy from grains & nuts & pulses (kcal)	698·6	634·4
Energy from grains & nuts & pulses (%TE)	32·8	36·9
Fe bioavailability (%)	15	15
Zn bioavailability	High	High

%TE, percentage of total energy.
The method of Jati *et al.*[42] was applied.

Table 8 Dissimilarities in various parameters among apparently healthy Serbian women with low and high Hb concentration, June 2013–January 2015

	Females (*n* 142)		Low Hb (*n* 33)		High Hb (*n* 108)		
	Mean	SD	Mean	SD	Mean	SD	*P* value
Age (years)	23·3	0·8	30·2	10·5	23·0	0·8	
Weight (kg)	60·3	5·5	67·2	10·3	61·0	5·0	
Height (cm)	169·9	4·4	169·5	6·0	170·7	4·1	
BMI (kg/m^2)	20·9	1·4	23·4	3·8	20·9	1·3	
WC (cm)	72·0	4·4	76·4	9·8	72·8	4·4	
Hb (g/l)	130·4	10·7	112·4	7·8	135·0	8·1	
Haematocrit (l/l)	0·4	0·0	0·4	0·0	0·4	0·0	
Erythrocyte count (×10^{12}/l)	4·4	0·3	4·2	0·3	4·5	0·3	
Fe (μmol/l)	15·8	4·8	12·5	6·1	16·5	4·5	
Dietary Fe (mg/d)	9·6	5·1	10·2	4·1	9·5	4·5	0·036
Dietary Zn (mg/d)	7·1	2·2	9·0	5·5	7·3	2·2	0·010

Low Hb, Hb value below 120 g/l; WC, waist circumference.
Dietary Fe and Zn data were obtained from 24 h recalls. Only significant *P* values are given.

females, being approximately 20 % higher in males. Similarly, Hb concentration and plasma concentrations of Fe and Zn were higher in males than in females (Table 5). We did not observe any differences in the Zn and Fe intake or status data within different age groups ($P=0·16$).

Fe intake was related to BMI ($P<0·05$), while Zn intake was not different between obese and non-obese individuals ($P=0·07$). No correlation was seen between BMI and Fe and Zn status ($P=0·44$ and $P=0·25$, respectively). We estimated the degree of association between biochemical parameters (plasma Fe, Hb, erythrocyte count and plasma Zn), energy and protein intakes and lifestyle factors, and did not detect any significant associations.

No association was found between dietary Fe and Zn intakes and education level or marital status (Table 5). Similarly, components of socio-economic status (SES) measured in the study showed no correlation with any of the biochemistry-related parameters, including Fe and Zn status. However, there was a statistically significant difference in Fe intake between the low-income and affluent participants ($P=0·034$), while no such difference was observed for Zn intake ($P=0·084$).

Differences were also observed in the intake of Fe between the affluent and low-income female groups ($P=0·005$; Table 5). Finally, there was a statistically significant difference in Hb concentration between the groups of women with Zn adequate and Zn inadequate diets.

Discussion

The results of the current study demonstrate that inadequate intake of Zn was present in a healthy population cohort. There was no strong relationship between dietary Zn intake and Fe biochemical parameters in this healthy cohort. However, there was a statistically significant difference in Zn intake among groups of women with dissimilar Hb concentration. Zn and Fe dietary intakes were strongly correlated. Grains and meat were identified as major sources of dietary Fe intake and meat and dairy products as main foods consumed contributing to Zn intake. Generally, no differences were seen for Fe and Zn intakes and status among various SES groups, except for Fe intake between those on low income and affluent. Plasma Zn and Fe concentrations were within the reference ranges despite the insufficient dietary intakes of these minerals. However, a prolonged inadequate intake of Zn and Fe may contribute to the development/manifestation of Zn and/or Fe deficiencies, so regular monitoring of mineral intake and status in this population cohort is necessary.

The mean Fe and Zn intakes of our study population were similar to the values for these elements reported for the adult population in other developed countries: Hungary, Croatia, Eastern and Central European countries, Australia and Canada[29,43–45].

A recent review of available micronutrient intake and status data in Europe[29] showed that Fe and Zn intake data for a number of countries are very limited. More current measurements of adult Fe and Zn intake and status data are rare[46]. In this sense, results presented in the current study can be considered a valuable resource.

The intakes of Fe and Zn were different between males and females, and there was a strong correlation between Zn and Fe intakes, which is in agreement with previous reports[47–49]. There was also a positive correlation between mineral intakes and energy and this relationship has been highlighted by others[28,50]. The EAR cut-point method was used to assess the adequacy of Fe and Zn intakes. The EAR cut-point method is identified as an appropriate dietary reference intake to use when assessing the adequacy of group intakes and has been adopted for assessing nutrient inadequacy[51]. In addition, we also compared the intake data with other available dietary recommendations[52,53] and showed that the risk of inadequate intakes was very different. The fact that the method employed can affect the estimation of nutrient intake inadequacy[53,54] has been confirmed again. The Nordic and FAO/WHO recommendations overestimate the true prevalence of inadequate intake compared with the EAR cut-point method. Still, it must be noted that while intakes below the recommendation are not synonymous with deficiency, they certainly indicate increased risk of deficiency[54].

The estimation of and adjustment for bias (such as under- or over-reporting of food intake) is a relatively unexplored field. An evaluation of the effect of misreporting on energy and nutrient intake assessment indicated that low energy reporters had lower mean intakes for several micronutrients (from 25 to 36 %) compared with non-under-reporters[55]. Likely under-reporting of food consumption was estimated to occur in 34 % (260/754) of the present study participants. Of our study population, 28 % had low Fe intake and 52 % had Zn intake below current dietary recommendations[33]. Taking under-reporting into account, it is estimated that 5 % of the study population had inadequate Fe and 15–25 % had unsatisfactory Zn intake. Sixty-five per cent of our study population were women within the age range of 20–50 years. Women in this age group are known as the most vulnerable group for the development of Fe and Zn deficiencies because they are more likely to have excessive menstrual bleeding, infrequently consume sources of bioavailable Zn and Fe, rarely consume rich sources of enhancers and often consume rich sources of inhibitors of Zn and Fe absorption[56]. We found that women with dietary Fe intake below recommendations (EAR of 8·1 mg/d) were at greater risk of inadequate dietary Zn intake: 38 % of women in the present study had inadequate dietary Fe intake and 76 % of these with low dietary Fe intake show concurrent insufficient intake of Zn.

In many developed countries, micronutrient deficiencies are not related to the quantity of food consumed, but rather to the quality of the diet[57]. Inadequate dietary intakes of Zn and Fe that failed to meet the high physiological demands of adolescent girls in Australia and New Zealand were shown to be related to food choices[3]. Relevant to these findings is the observation in fifty-two non-pregnant premenopausal women from Seattle, Washington, USA, whose diets provided similar amounts of Fe, where the consumption of red meat five times per week was more efficacious for body Fe stores than the consumption of lacto-ovo vegetarian foods, or the flesh of chicken and fish[58]. In further support of this are the findings of the National Food Survey of the British population[59] showing that Fe intake and red meat consumption have declined during the past three decades, leading to low Fe stores in females aged 11–64 years.

Being overweight is a worldwide problem that affects developed and developing countries similarly[37,40]. Approximately 30 % of our study population was overweight and 10 % were obese. Other European countries have shown a similar percentage of overweight and obesity[37]. However, even with excess dietary intake of energy and macronutrients, it is still uncertain if people are taking the recommended intakes of micronutrients. For instance, a number of national epidemiological surveys conducted in several developed countries have reported the co-occurrence of obesity with inadequate intakes of certain vitamins and minerals, particularly Ca, Fe, Zn, thiamin, riboflavin, vitamins B_6 and D, and folate[15,60,61].

In the present study we found higher intake of Fe in the obese population than in non-obese individuals. A possible explanation for the higher intake of Fe in obese people is that their energy intake was higher because of the greater consumption of fatty (energy-dense) foods (i.e. meats) and refined grain products (white bread is a staple food). The correlations of energy intake, total grain products and meat with Fe intake support this explanation. However, not only inappropriate intakes, but also impaired bioavailability and utilization of micronutrients may be involved in the inadequate micronutrient status in obesity. The low-level inflammation that accompanies obesity is likely to decrease Fe absorption[62]. This needs to be taken into account when evaluating the adequacy of Fe intake in a population.

Zn and Fe nutrition are often associated. Red meat and grain products are the most important common dietary sources of Fe and Zn. Our findings confirm observations that food sources of Zn are also important food sources of Fe, as well as support the theory that usual low intake of either mineral increases the likelihood of low intake of the other[3,17,18]. Furthermore, other factors influence daily intakes of Zn and Fe: their concentration in food, the amount of food consumed and the consumption of dietary components that delay Fe and Zn absorption[23]. Zn and Fe are most bioavailable from many of the same foods and their absorption is repressed by many of the same dietary substances[56]. Although we were not able to estimate the exact phytate:Zn molar ratio of the diets, by employing the method of Jati *et al.*[42] we showed that there was a high bioavailability of both nutrients in the diets of the people we studied. The food composition database needs to be updated because, as in many other databases, our data did not contain information on the content of phytate.

Food preparation and processing methods can be used to reduce the phytate content of foods based on cereals and legumes. These methods are based on the enzymatic hydrolysis of phytic acid to lower inositol phosphates that are induced by germination and fermentation[26]. Soaking, followed by empting out of the soaking water, can also be used to reduce the phytate content of cereal and legume flours by passive diffusion of the water-soluble sodium, potassium and magnesium phytates[63]. Household-level information was obtained for people where cereals and legumes contributed a large proportion to total Fe and Zn intakes. Almost all of our participants (97 %) employed preparation techniques that are known to reduce phytate content of foods and increase Zn bioavailability (i.e. soaking of beans, fermentation of bread dough).

Overall, the plasma concentrations of Fe and Zn in the present study were within the expected normal ranges for the adult population and similar to reported levels of these elements in healthy individuals elsewhere[64,65]. Similarly, the mean values of Fe-related biochemical parameters were within the expected ranges for a healthy population of subjects[64,66,67]. Mean plasma levels of both elements were significantly different between males and females. Obesity, age, education and marital status did not contribute to the differences in plasma levels of the studied minerals. These findings are in agreement with the results of Sian *et al.*[68], Bailey *et al.*[69] and Sanchez *et al.*[28].

Our observation of a strong association between usual Fe and Zn intakes was not accompanied by a similar relationship between Fe and Zn status. The lack of association may be due to several factors.

First, the inter-individual variation in biological availability of dietary Fe and Zn is determined by the degree of intestinal absorption that depends on the composition of meals; the absorption from two different meals with similar Fe/Zn content may vary considerably due to the interaction between dietary Fe/Zn and absorption enhancers and inhibitors[70,71]. It could also be that good homeostatic regulation of Zn contributes to these dissimilarities[44,72]. Second, the calculation of reported dietary intake is performed using food composition tables (calculated and chemically measured content of the diet may be different). Finally, the reported intake of dietary Zn and Fe may deviate to some degree from the true intake due to diet reporting error. In the present study we attempted to correct for some of these discrepancies by taking under-reporting into account; however, we need to acknowledge that a certain level of error may still be present.

The correlation of Zn intake and Zn status with Fe biochemical parameters in healthy populations has not been investigated widely. Only a few studies have been initiated and provide inconsistent results[28,73,74]. Various studies in Fe- and Zn-deficient populations have shown a positive link between Zn intake and Fe status indicators[6,27]. In our healthy population cohort we did not find any significant correlation, most likely due to having a small number of people with inadequate Fe status. Still, it was evident that Hb concentration was statistically different between women with appropriate and insufficient Zn intakes. Further work may be needed to examine the role of dietary Zn on Fe status indicators more fully. Measurements of ferritin, transferrin and total Fe-binding capacity may provide more detailed analysis of this relationship as low Hb, which was used in the present study, is not the best indicator of Fe status and is mainly apparent during the late stages of Fe deficiency. In addition, as a number of studies over the years have demonstrated the positive link between Zn intake and Fe status and the role of Zn in the Fe absorption process[21,27,73,75], further work in this area should aim to elucidate the specific point in Fe deficiency progression when inadequate Zn intake is beginning to have its role.

As shown by many, SES, conventionally measured by education, occupation or inco.me, can contribute to inequalities in health[76,77]. Those with limited incomes or lower educational levels are more likely to have poor-quality diets[78]. Energy-dense and nutrient-poor diets are cheap and usually consumed by those with limited budgets[77].

A limited number of studies have shown inconsistent results on the variation in Fe and Zn intakes between SES groups. Higher (2–5 %) Zn levels were found in low-SES groups[28,79], while others found no differences among the groups[80]. Similarly, higher values of plasma Fe have been reported in high-SES groups[81,82], while again there are studies that showed no differences in Fe intake or status among various SES groups[83].

In the current study we looked at the association of marital status, educational level and occupation with Fe and Zn intakes and status, and found no differences in intake or status between SES groups. The only difference was seen in the intake of Fe between the affluent and those on poor income, which is most likely due to higher consumption of grain products and pulses (staple foods) by people in the low-income group.

The findings of the current study should be interpreted in view of its strengths and limitations. The strength of our study is that we investigated a homogeneous population with similar demographic and genetic background. In this way, we were able to look at the influence of nutrition on Fe and Zn intakes and status and their relationship in the healthy adult population. Another strong point of our work is the collection of dietary data. We administered three 24 h recall questionnaires, on three non-consecutive days, which is the gold standard method for assessing the adequacy of Fe and Zn intakes[84].

We identify a few limitations of the present study. Plasma (serum) Zn is a good indicator of Zn deficiency; however, plasma Zn levels do not reflect marginal status and may not reflect dietary intakes[85]. Plasma Zn is insensitive to Zn intakes within this range[71,86], and the Institute of Medicine ruled out plasma Zn as a useful status indicator for evaluating human Zn requirements for Canada and the USA[33]. It is also accepted that plasma Zn concentration is a valid indicator of whole-body Zn status in the absence of confounding factors, such as infection or stress[87]. Nevertheless, with all its limitations, it is the best biomarker currently available. Studies have pointed out that the ratio of blood fatty acids (linoleic acid:dihomo-γ-linolenic acid) may be a more appropriate biomarker of Zn status that is able to differentiate between various Zn deficiency/adequacy states[88,89]; however, additional research is needed to fully determine the efficacy of this biomarker. Another weakness of our study was the inability to calculate the phytate:Zn molar ratios due to the lack of available data on phytate content of foods in our database. However, we believe that this was overcome by estimating the bioavailability of Fe and Zn using the method of Jati *et al.*[42].

In countries where fresh vegetables, fruits and proteins are freely available and well embodied in the average diet, as is the case in many European countries, nutritional deficiencies of Fe and Zn are expected to be rare. However, easy access to food does not ensure healthy food choices required to achieve adequate nutrition[57]. Similarly, lifestyle changes occurring over the last few decades in many developed countries are characterized by increased consumption of low-cost but energy-dense foods and by reduced physical activity levels. These lifestyle changes are the major factors implicated in the aetiology of the current obesity epidemic[90]. It is well known that an unbalanced diet can contribute to micronutrient malnutrition[91]. Dietary decisions made by individuals may lead to Fe and Zn deficiencies. Finally, with the present global trend of people in developed countries to decrease meat intake and increase intake of grains, there is a tendency that more individuals may end up having inadequate intakes of these important minerals.

In conclusion, considering that more than 20 % of our study population had inadequate dietary intake of Zn and bearing in mind the health consequences of prolonged inappropriate Zn intake on both Zn and Fe nutritional status, more regular follow-ups are needed to monitor the intake and status of these nutrients and to make sure that potential deficiencies do get recognized and addressed in a timely manner.

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Supplementary material

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