



# Fueling an epidemic of non-communicable disease in the Balkans: a nutritional survey of Bosnian adults

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## Abstract

**Objectives** Dietary surveys are essential for guiding national efforts to reduce the burden of non-communicable disease, but individual-level dietary data are lacking in many low- and middle-income countries. We aimed to estimate the prevalence of inadequate and excessive intakes of specific nutrients in Bosnia and Herzegovina.

**Methods** A dietary survey among 853 adults using two 24-h recalls.

**Results** The majority of men (73%) and women (66%) were overweight/obese, and > 50% of participants had elevated blood pressure. Low intakes of N-3 polyunsaturated fatty acids, specifically  $\alpha$ -linolenic acid (men: 94.4 mg/day among, women: 96.6 mg/day) and DHA + EPA (men: 18.2 mg/day, women: 16.0 mg/day), low fiber intake (women: 21.5 g/day), and high sodium (men: 3244 mg/day, women: 2291 mg/) and saturated fatty acids intakes (men: 29.2 g/day) were reported. There was also a suggestion of low intakes of vitamins A, B6, C and D (in both sexes), and of riboflavin, folate, B12 and calcium (in women).

**Conclusions** Our findings provide initial evidence on the Bosnian population's dietary habits and identify aspects that need attention. As the survey evolves into a continuing surveillance system, it will allow evaluation of dietary changes over time.

**Keywords** Diet survey · Nutrition assessment · Nutrient intake · Low- and middle-income countries · SFA · PUFA · Usual nutrient intakes

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## Introduction

Dietary factors account for more deaths and disease globally than smoking, drugs, alcohol use and unsafe sex combined (Ezzati and Riboli 2013; Forouzanfar et al.

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2015). Hence, a national dietary surveillance system is a critical element for effective and efficient efforts to prevent premature mortality and reduce burden of disease. Dietary surveys enable governments to monitor population intakes of nutrients and foods, identify disparities among population subgroups, detect areas of concern, plan policies and interventions, and monitor the effectiveness of these interventions. Individual-level dietary data, when converted to nutrients using a local food composition database, provide important insights into intakes that are inadequate (e.g., iron, folate or polyunsaturated fatty acids/PUFAs) or excessive (e.g., sodium, sugar or saturated fatty acids/SFAs) for well-being and prevention of non-communicable diseases (NCDs) (WHO/FAO 2002). Furthermore, these data allow for analyses of heterogeneity of dietary quality by demographic and socioeconomic indicators, enabling policymakers to focus more closely on populations at risk and help reduce health inequalities. The UN's Secretary-General's Independent Expert Advisory Group (IEAG), the WHO Europe and the Rome Declaration on Nutrition signatory governments have called member states to undertake action toward strengthening and expanding their surveillance capacities, including those in diet and nutrition (UNIEAGS 2014; FAO 2014; WHO 2014). Recent global and Europe-wide reviews (Lachat et al. 2013; Huybrechts et al. 2017; Rippin et al. 2017) indicated a lack of dietary surveys and policy responses in many low- and middle-income countries (LMICs) and specifically in the Central and Eastern Europe Countries (CEECs). One barrier to conducting such surveys has been the cost of implementing them and a lack of adequate food and nutrition research infrastructure. Over the past decade, there have been regional initiatives for strengthening research capacities and nutrition surveillance systems in the CEEC and the Balkans that included creation of the Balkans food composition database and development of a dietary assessment software (Gurinovic et al 2016a).

Bosnia and Herzegovina (B&H), a middle-income country in the CEEC, has not historically had a system for periodic monitoring of population dietary intake. The Global Burden of Disease, in part using imputed data (Powles et al. 2013; Micha et al. 2014), and the Bosnian Health Survey from 2012 (ZJZFBiH 2012) suggested high intakes of sodium, saturated fats, refined grains, dairy and sweets, and low intakes of whole grains, fruits, vegetables, fish and nuts among the adult population. Health statistics (FMHFBiH/IPHFBiH 2012; WHO 2015a, b) indicate B&H is at an advanced stage of epidemiologic transition: Cardiovascular diseases (CVDs) account for over 50% of all deaths, type 2 diabetes and diet-related cancer rates are increasing, and over one half of adults are either overweight or obese. The first dietary population-based survey of the adult population in the Federation of B&H was

conducted in collaboration with the Institute for Statistics of the Federation of B&H. The overall goal of this collaboration was to develop an efficient survey method that could be used in follow-up surveys. In this study, we aimed to evaluate nutrient intakes among the country's adult population and estimate the prevalence of inadequate and excessive intakes of specific nutrients.

## Methods

### Study design and population

A dietary survey was conducted among the adult population of the Federation of B&H using a two-stage cluster sample of 980 households, drawn as a subsample of units participating in the 2015 Household Budget Survey (BHAS 2015). One male and one female per household were invited to participate in the study between December 2016 and December 2017. Seeking to develop a sustainable surveillance model, we aimed for a modest sample size of adults (18+ years of age) that was large enough to achieve 5–10% standard error or less of the mean intake for each nutrient of interest (Willett 2012a). For the majority of nutrients, such precision can be achieved with sample sizes of 100–250 individuals. (Vitamin A requires larger sample sizes due to high variability.) A sample size close to 1000 would hence provide good precision for the overall sample and reasonable precision for subgroups defined by age-groups (18–40, 41–60, and > 60), gender, urban/rural residence or other characteristics. The final sample included 853 individuals from 613 households (371 men and 482 women, household response rate = 66%) and was representative of the general adult population in the FB&H in terms of sex, age, geographic location (north/south) and the place of residence (urban/rural) (Supplementary Table 1).

### Data collection

We used 2 days of 24-h recall (the first one collected at the household visit and the second one over the phone 4–30 days later) with a multiple-pass-method approach (Blanton et al. 2006) to collect dietary data. Dietary recalls were collected uniformly across all four seasons with a weekday-to-weekend ratio of 70:30. To determine intake quantities, we used DIET ASSESS Food Atlas (Gurinovic et al. 2016b; Nikolic et al. 2018), which uses color images of eating utensils, dishes, foods and meals to aid in the estimation of portion sizes during the in-home interviews. A short version of this food atlas was left with respondents to be used during phone interviews. Demographic, lifestyle, anthropometric and chronic disease data were collected during the home visit using an interviewer-

administered questionnaire. While anthropometric data were self-reported, participants were given instruments (measuring tapes and scales) and verbal and illustrated instructions to take these measures themselves during the interview. Self-measured waist and hip circumference was successfully validated by comparison with interviewer assessment on a subsample of 87 persons. Intra-class correlation coefficients between participant and interviewer measures were 0.97 ( $p < 0.0001$ ) for waist and 0.91 ( $p < 0.0001$ ) for hip circumference. Blood pressure was measured using an automated M6 Comfort Omron device, validated for epidemiological research (Topouchian et al. 2014; Marazzi et al. 2012). The study protocol was approved by both the Ethics Committee of the Public Health Institute of the Federation B&H and the Harvard T.H. Chan School of Public Health's Office of Human Research Administration.

### Statistical analysis

To convert dietary data to nutrients, we used DIET ASSESS and PLAN (Gurinovic et al. 2016b) and the Balkans Food Composition Database (FCDB). Prior to the analysis, we performed a thorough revision and update of the FCDB for all required nutrients, adding any new food items (commodities and local recipes) using analytical data, other country nutrient databases, local cookbooks, and industry data. Data on trans-fatty acids and free/added sugars were not available in the database; hence, we excluded these nutrients from our analyses. Dietary outliers were detected using the %esd macro for performing generalized extreme Studentized deviate many-outlier detection (Rosner 1983). The method is designed to identify extreme values while overcoming the problem of masking that occurs when two outliers are close to one another. All extreme values were individually checked and any identified data errors corrected as appropriate. We performed sensitivity analyses by excluding the extreme values for each log-transformed nutrient where such values were detected and compared the results to those from the full sample. The extreme values were found for MFA, linoleic acid (LA) and vitamins A, B12 and C (up to twelve values for each nutrient). Geometric means and standard errors were estimated using only day 1 data, consistent with previous nutrition surveys (Moshfegh et al. 2009). We estimated distributions of usual intake using both days of data with the National Research Council (NRC) method (IOM 2003a), a simple approach suitable for use with ubiquitously consumed dietary components (i.e., nutrients). Within-person coefficients of variation ( $CV_w$ ) were calculated as the  $100\% \times \text{Root MSE}$  (Rosner 2011) on the raw scale, with MSE calculated from ANOVA using the log scale, and between-person coefficients of variation ( $CV_b$ )

as a  $\text{Root}(\text{Model MS} - \text{MSE}) \times 100\%$ . Given nutrient intakes were not normally distributed, log transformation was applied before partitioning the variance, with subsequent back-transformation to calculate usual mean intakes. Energy-adjusted macronutrient intakes were calculated as a percentage of total energy intakes and as a nutrient density (per 1000 kcal) for micronutrients and cholesterol. Estimated values were obtained using PROC SURVEY procedures in SAS with appropriate weights to account for the sampling and participation effects. *T* and *F* tests were used in PROC SURVEYREG procedure to compare means of log-transformed energy-adjusted nutrient intakes by sex and across age-groups.

The European Food Safety Agency's (EFSA) Dietary Reference Values (DRVs) for nutrients (EFSA 2017), specifically the average requirements (ARs), served as cut-points for identifying proportions of the population at risk of inadequate/excessive intakes. The probability method, along with the IOM tables (IOM 2001) of requirement distribution and probabilities of inadequate intakes, was employed to calculate inadequacy of iron intakes in women (IOM 2003a). For nutrients without an AR, adequate intakes (AIs) (for micronutrients) and EFSA-recommended nutrient intake ranges (IRs) (for fat and carbohydrates) were utilized instead. While AIs cannot serve to estimate prevalence of inadequate intakes, (IOM 2003b), groups with mean intakes at or above the AI can be assumed to have low prevalence of inadequate intakes (EFSA 2010). As EFSA has not set a DRV for sodium or SFA, we used the WHO recommendations (WHO 2012).

The proportion of the population with adequate level of physical activity was defined as having either  $\geq 150$  min of moderate or  $\geq 75$  min of vigorous physical activity per week, or an equivalent combination of the two (WHO 2010). Among alcohol consumers, we defined light-to-moderate drinkers as women consuming no more than 7 drinks/week and men consuming 14 drinks/week or less (USHHS/USDA 2015). High blood pressure in this population was defined as either the use of a physician-prescribed antihypertensive drug or having a blood pressure measurement on site of  $> 140/90$  mmHg. Finally, the WHO cutoffs were used to estimate the proportions of the population with high-risk waist circumferences and waist-to-hip ratios (WHO 2008). All analyses were performed in SAS 9.4.

### Results

Our dietary survey conducted in Bosnia and Herzegovina included data from 853 adults ( $N_{\text{day1}} = 853$ ,  $N_{\text{day2}} = 836$ ). The majority of participants in this survey had a high school diploma, medium socioeconomic status (SES), were

married or living with a partner and did not drink alcohol. There was a fairly equal distribution of participants across genders and age-groups as well as those living in urban and rural settings and those with and without a history of smoking (Table 1). In this sample, women were on average less active than men, suffered more from chronic disease, had a higher prevalence of supplements use and had a

lower prevalence of current smoking and alcohol consumption. The estimated prevalence of high blood pressure in our sample was slightly higher among men (57%) than women (51%) (Table 2). Over two-thirds of all participants were categorized as overweight or obese, with overweight more common among men, and obesity among women. High-risk waist circumference was more common among

**Table 1** Characteristics of participants in 2017 Bosnia and Herzegovina dietary survey

	Male ( <i>n</i> 371)		Female ( <i>n</i> 482)	
	<i>n</i>	%	<i>n</i>	%
Age				
18–40	111	30	130	27
41–60	157	42	227	47
> 60	103	28	125	26
Socioeconomic status (SES)				
Low SES	121	32	172	36
Medium SES	218	59	280	58
High SES	32	9	30	6
Residence				
Urban	167	45	206	43
Education level				
< 12 years (less than high school)	53	14	213	44
12–14 (high school diploma/some college)	267	72	216	45
> 15 years (higher education diploma)	51	14	53	11
Marital status				
Single	61	16	55	11
Married/living with partner	291	78	329	68
Divorced/widowed	19	5	98	20
Smoking status				
Never smoker	143	39	265	55
Past smoker	86	23	53	11
Current smoker	142	38	164	34
Alcohol use				
Nondrinker	214	58	430	89
Light-to-moderate drinker <sup>a</sup>	132	36	38	8
Heavy drinker	25	7	14	3
Physical activity <sup>b</sup>				
Activity level below WHO recommendation	86	23	158	33
Chronic disease	113	30	195	40
Diabetes	19	5	38	8
Cardiovascular disease	66	18	122	25
Special diet (yes/no)	17	5	24	5
Supplements' use <sup>c</sup> (yes/no)	29	8	82	17

<sup>a</sup>Up to one drink for women and up to two drinks for men per day (USDA Dietary Guidelines for Americans 2015–2020)

<sup>b</sup>The World Health Organization defines sufficient amount of physical activity per week as  $\geq 150$  min of moderate-intensity aerobic physical activity throughout the week, or  $\geq 75$  min of vigorous-intensity aerobic physical activity throughout the week, or an equivalent combination of moderate- and vigorous-intensity activity

<sup>c</sup>Primarily vitamin C, calcium or magnesium, with less than 2% consuming B complex, vitamin D or omega 3 fatty acids

**Table 2** Hypertension and adiposity, by sex and age, 2017 Bosnia and Herzegovina dietary survey

	Sex				Age					
	Male (n 371)		Female (n 482)		18–40 (n 241)		41–60 (n 384)		> 60 (n 228)	
	Mean or n	SE or %	Mean or n	SE or %	Mean or n	SE or %	Mean or n	SE or %	Mean or n	SE or %
<i>Hypertension</i>										
Systolic blood pressure (mmHg) (n = 849) <sup>a</sup>	134	1.6	129	1.8	121	1.7	134	1.7	144	2.7
Diastolic blood pressure (mmHg) (n = 849) <sup>a</sup>	85.1	0.9	84.1	0.9	80.6	1.0	86.6	1.0	87.4	1.1
Elevated blood pressure (%) (> 140/90 mmHg) <sup>b</sup>	183	49	189	39	44	18	176	46	152	67
Receiving therapy for hypertension (%) <sup>b</sup>	74	20	154	32	3	1	90	23	135	59
Estimated prevalence of hypertension (%) <sup>b</sup>	210	57	245	51	45	19	215	56	195	86
<i>Adiposity</i>										
Body mass index (n = 851) <sup>a</sup>	27.3	0.3	26.9	0.5	24.2	0.4	28.3	0.4	29.5	0.5
Overweight (%) (BMI 25–29.9 kg/m <sup>2</sup> ) <sup>b</sup>	190	51	175	36	89	37	188	49	88	39
Obese (%) (BMI ≥ 30.0 kg/m <sup>2</sup> ) <sup>b</sup>	83	22	144	30	19	8	113	29	95	42
<i>Central adiposity</i>										
Waist circumference (cm) (n = 845) <sup>a</sup>	94.9	0.9	87.7	1.3	82.8	1.2	94.3	1.0	98.8	1.4
WC <sup>d</sup> > 80 cm (F)/> 94 cm (M) (%) <sup>b</sup>	204	55	331	69	81	34	277	72	177	78
WHR <sup>e</sup> ≥ 0.85 (F)/≥ 0.90 (M) (%) <sup>b</sup>	258	70	246	51	102	42	245	64	173	76

<sup>a</sup>Mean (SE)

<sup>b</sup>n (%)

<sup>c</sup>Either blood pressure > 140/90 at the time of measurement, or on antihypertensive medication

<sup>d</sup>Waist circumference

<sup>e</sup>Waist-to-hip ratio

women (69% vs. 55% for men) and high-risk WHR among men (70% vs. 51% for women). Both hypertension and adiposity prevalence increased with age.

**Energy and macronutrients**

The percentages of energy from macronutrients were similar across sexes and age-groups (Table 3). While consumption of all PUFAs was generally low across sexes and age-groups, women consumed a significantly higher percentage of energy from ALA than men (0.04% vs. 0.02%). Women also tended to consume more energy from sugar (13.0%) than men (10.6%). Participants in the youngest group had significantly higher energy intakes from sugar (13.2%) compared to the middle-aged (10.8%) and the eldest (11.4%) groups. In the usual intakes analysis, the major type of PUFAs was linoleic acid (LA) with over 89% of participants consuming adequate amounts (Table 4). On the other hand, we did not identify any participants with adequate usual intakes of ALA, DHA or EPA. Almost half of all women (45.8%) and two-thirds of all men (69.1%) consumed excessive energy from SFA

intake (greater than 10% of energy). Mean fiber intakes were below the 25 g recommendation among women (21.5 g). On average, protein intakes in both men and women were adequate.

**Micronutrients**

The middle-aged group had the lowest intakes of calcium (638 mg), selenium (62.7 µg), potassium (2569 mg) and vitamins A (356 µg), C (61.4 mg) and D (2.4 µg) (Table 5). Men had significantly higher usual intakes for the majority of nutrients unadjusted for energy intake (Table 6). After adjusting for energy, intakes of magnesium, selenium, potassium, vitamins A and B12 remained significantly higher among men. Prevalence of inadequate intake of calcium and zinc was 41.2% and 40.7% among men, and 73.1% and 44.8% among women (Table 4). The distributions of usual intakes also suggested low consumption of selenium among women, potassium among men and magnesium among both sexes because mean intakes were below AIs. Reported sodium intake, on the other hand, was high, with over 90% men, and two-thirds

**Table 3** Macronutrient intakes from foods and beverages: mean amounts<sup>a</sup> consumed per individual, by sex and age, 2017 Bosnia and Herzegovina dietary survey

Nutrient	Sex				Age-group					
	Males		Females		18–40		41–60		> 60	
	(n 371)		(n 482)		(n 241)		(n 384)		(n 228)	
	Mean <sup>f</sup>	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Energy (kcal)	2415	89.2	1858	60.0	2517	100	1921	68.9	1870	89.0
Carbohydrate (g)	272	10.9	219	7.8	295	13.4	219.7	8.4	214	10.6
Carbohydrate (% TEI) <sup>b</sup>	46.3	0.9	48.1	0.9	47.9	1.0	47.0	1.0	46.7	1.3
Protein (g)	85.0	3.3	63.3	2.3	85.3	3.7	67.5	2.7	65.1	3.6
Protein (% TEI) <sup>b</sup>	14.5	0.3	13.9	0.3	13.8	0.3	14.4	0.4	14.2	0.4
Total fat (g)	94.3	4.3	70.3	2.9	97.8	4.6	73.0	3.6	71.9	4.6
Total fat (% TEI) <sup>b</sup>	36.1	0.8	34.7	0.8	35.7	0.8	35.1	0.9	35.4	1.2
MFA (g)	28.2	1.4	20.6	0.9	29.1	1.5	21.6	1.0	21.0	1.4
MFA (%TEI) <sup>b</sup>	11.0	0.4	10.8	0.3	11.0	0.3	10.9	0.4	10.7	0.6
SFA (g)	28.6	1.5	21.9	1.0	30.1	1.6	22.7	1.2	21.7	1.6
SFA (%TEI) <sup>b</sup>	10.8	0.3	10.2	0.3	10.6	0.3	10.4	0.3	10.3	0.5
PUFAs (total) (g)	20.6	1.4	17.3	0.8	22.9	1.3	16.3	1.1	17.6	1.7
PUFAs (total) (%TEI) <sup>b</sup>	7.9	0.4	8.5	0.3	8.4	0.4	7.9	0.4	8.6	0.6
Omega 3 PUFAs (g)	0.65	0.1	0.52	0.05	0.71	0.05	0.5	0.1	0.6	0.1
Omega 3 PUFAs (%TEI) <sup>b</sup>	0.24	0.02	0.25	0.02	0.25	0.01	0.23	0.02	0.27	0.03
DHA+EPA (mg)	15.4	2.9	11.9	2.2	12.7	2.8	11.5	2.3	19.2	5.6
DHA + EPA (%TEI) <sup>b</sup>	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
ALA (mg) <sup>c</sup>	16.7	6.2	38.1	10.6	26.8	10.4	24.8	9.3	25.4	11.2
ALA (%TEI) <sup>b</sup>	0.02	0.01	0.04*	0.01	0.03	0.01	0.03	0.01	0.03	0.01
LA (g) <sup>d</sup>	16.4	1.3	13.3	0.8	18.2	1.2	12.5	0.9	13.9	1.5
LA (%TEI) <sup>b</sup>	6.1	0.4	6.5	0.3	6.5	0.3	5.9	0.3	6.7	0.6
Cholesterol (mg)	241	14.8	181	11.4	242	18.0	203	12.5	173	17.2
Cholesterol (mg) <sup>e</sup>	100	5.2	97.6	5.1	95.9	5.5	106	5.8	92.4	8.2
Fiber (g)	23.9	1.0	21.7*	0.8	24.1	1.1	21.8	1.0	22.4*	1.2
Total sugars (g)	63.7	4.4	60.2	3.2	81.9	6.3	52.1	3.2	53.5	4.2
Total sugars (%TEI) <sup>b</sup>	10.6	0.6	13.0*	0.6	13.2	0.8	10.8	0.6	11.4*	0.7

\*Statistically significant differences in means of log-transformed energy-adjusted nutrients

<sup>a</sup>Only day 1 data used

<sup>b</sup>As a percentage of the total energy intake

<sup>c</sup> $\alpha$ -linolenic acid

<sup>d</sup>Linoleic acid

<sup>e</sup>Per 1000 kcal

<sup>f</sup>Geometric mean and standard error calculated using  $\exp(\log\text{-mean})$  of nutrients

of women consuming over the recommended 2000 mg/day (WHO 2012). Reported  $\beta$ -carotene intakes were strikingly low, especially in the youngest age-group (622  $\mu\text{g/day}$ ). There was also a high prevalence of inadequate intake of vitamin A (96.9% among women and 97.4% among men), vitamin C (66.9% among women and 81.5% among men) and vitamin B6 (73.2% among men and 81.3% among women), and mean intakes of vitamin D among both sexes were generally below the AI. Only among women, there was also high prevalence on inadequate intake of folate

(63.9%), calcium (73.1%) and riboflavin (73.8%), as well as a mean intake of vitamin B12 below the AI.

### Variance components, variance ratios

Within-person variation in nutrient intake was higher than between-person variation for all nutrients, with variance ratios being the lowest ( $\leq 1.5$ ) for energy, carbohydrates, sugar and fiber, and the highest for vitamin A (6.2 for men and 5.0 for women), DHA and EPA (4.2 for men), and for

**Table 4** Usual nutrient intakes<sup>a</sup> from food and beverages compared to the European Food Safety Agency's<sup>b</sup> Dietary Reference Values (DRVs), 2017 Bosnia and Herzegovina dietary survey

Usual nutrient intakes compared to average requirements (AR) <sup>c</sup>	Percentiles of usual intake					AR	Below AR %
	10th	25th	50th	75th	90th		
<b>Vitamin A (µg RE)</b>							
Male	388	416	449	485	511	570	97.4
Female	325	354	389	421	452	490	96.9
<b>Thiamin (mg)+</b>							
Male	0.9	1.1	1.3	1.5	1.8	0.3/1000 kcal	0.0
Female	0.8	0.9	1.0	1.2	1.3	0.3/1000 kcal	0.0
<b>Riboflavin (mg)</b>							
Male	1.0	1.2	1.4	1.7	2.0	1.3	33.7
Female	0.8	0.9	1.1	1.3	1.5	1.3	73.8
<b>Niacin (mg NE)+</b>							
Male	14.7	17.3	20.5	24.1	28.1	5.5/1000 kcal	2.5
Female	12.2	13.6	16.2	18.4	21.7	5.5/1000 kcal	2.1
<b>Vitamin B6 (mg)</b>							
Male	0.9	1.0	1.3	1.5	1.8	1.5	73.2
Female	0.8	0.9	1.1	1.2	1.4	1.3	81.3
<b>Folate (µg DFE)</b>							
Male	188	229	284	345	402	250	34.0
Female	165	189	233	276	319	250	63.9
<b>Vitamin C (mg)</b>							
Male	36.8	48.3	64.4	83.0	98.5	90	81.5
Female	43.0	54.3	68.0	88.7	109.1	80	66.9
<b>Calcium (mg)</b>							
Male	558	670	814	946	1124	750	41.2
Female	444	522	620	764	862	750	73.1
<b>Iron* (mg)</b>							
Male	9.1	11.5	13.2	15.7	19.2	7	0.0
Female	7.8	8.9	10.4	12.1	14.1	4.49–14.39	24.6*
<b>Zinc (mg)++</b>							
Male	7.0	8.5	10.3	13.0	15.1	9.4	40.7
Female	5.4	6.4	7.8	9.1	10.6	7.5	44.8
<b>Protein (g)</b>							
Male	59.5	72.7	86.8	97.2	113	0.66 g/ kg of body weight	8.4
Female	46.6	54.0	62.7	73.2	80.8		19.8
<b>Usual nutrient intakes compared to adequate intakes (AIs)<sup>d</sup></b>						<b>AI</b>	<b>Above AI %</b>
<b>Vitamin B12 (µg)</b>							
Male	1.9	2.6	3.5	5.2	6.5	4	42.4
Female	1.4	1.8	2.2	2.7	3.1	4	2.8
<b>Vitamin D (µg)</b>							
Male	1.9	2.6	3.3	4.1	5.3	15	0.0
Female	1.5	2.2	2.9	3.6	4.5	15	0.0
<b>Vitamin E (mg)</b>							
Male	11.8	15.1	18.2	21.3	23.9	13	86.6
Female	10.9	12.6	14.7	16.4	19.2	11	89.2
<b>Potassium (mg)</b>							
Male	2178	2566	2948	3435	3827	3500	22.9
Female	1880	2214	2536	2855	3230	2300	66.6
<b>Phosphorus (mg)</b>							
Male	882	1042	1213	1431	1600	550	96.8
Female	655	785	930	1094	1246	550	98.4
<b>Magnesium (mg)</b>							
Male	230	260	311	362	402	350	31.5

**Table 4** (continued)

Usual nutrient intakes compared to adequate intakes (AIs) <sup>d</sup>	AI						Above AI %
Female	185	215	248	289	326	300	18.2
Selenium (µg)							
Male	50.9	63.7	78.9	97.7	116	70	62.9
Female	37.1	45.7	53.7	63.4	72.2	70	14.2
Fiber (g)							
Male	15.1	19.6	23.9	29.2	34.2	25	55.8
Female	14.2	17.5	21.4	24.8	28.2	25	22.4
DHA + EPA (mg)							
Male	6.9	11.2	14.6	23.5	31.5	250	0.0
Female	3.5	6.6	7.5	11.6	21.9	250	0.0
ALA (%E) <sup>g</sup>							
Male	0.01	0.02	0.03	0.05	0.07	0.5	0.0
Female	0.02	0.03	0.04	0.06	0.07	0.5	0.0
LA (%E) <sup>g</sup>							
Male	4.0	5.0	6.0	6.9	8.0	4	89.6
Female	4.2	5.0	6.1	7.1	8.2	4	90.8
Usual nutrient intakes compared to intake ranges (IR) <sup>e</sup>	IR <sup>e</sup>						Within IR %
Carbohydrate (%E) <sup>g</sup>							
Male	42.6	44.9	47.7	50.1	52.4	45–60	73.8
Female	43.2	46.4	49.2	51.6	54.0	45–60	85.6
Fat (%E) <sup>g</sup>							
Male	30.0	32.7	35.2	38.0	39.2	20–35	48.0
Female	29.5	31.4	34.6	37.3	39.5	20–35	55.0
Usual nutrient intakes compared to recommended reduction of intake (RRI) <sup>f</sup>	RRI						Above RRI %
SFA (%E)							
Male	8.7	9.7	10.6	11.8	12.5	10	69.1
Female	8.2	8.8	10.1	11.3	12.6	10	45.8
Sodium (mg)							
Male	2198	2640	3156	3790	4382	2000	92.2
Female	1691	1920	2283	2565	2910	2000	69.4

\*Calculated using the IOM Distribution of iron requirements for women and the probability approach to inadequate intakes, using ranges for mixed population in Tables 1–7 (IOM 2001)

<sup>a</sup>Usual nutrient intakes calculated using the National Research Council (NRC) method and both days of intake

<sup>b</sup>European Food Safety Authority

<sup>c</sup>AR—the level of nutrient intake that is adequate for half of the people in a population group, given normal distribution of requirement

<sup>d</sup>AI—the value estimated when a Population Reference Intake cannot be established because an average requirement cannot be determined. An adequate intake is the average observed daily level of intake by a population group (or groups) of apparently healthy people that is assumed to be adequate

<sup>e</sup>IR—the intake range for macronutrients, expressed as % of the energy intake. These apply to ranges of intakes that are adequate for maintaining health and associated with a low risk of selected chronic diseases

<sup>f</sup>RRI—recommended reduction of intake was determined for those nutrients which have been known to have adverse health effects in large quantities but for which UL has not been defined. WHO recommends a reduction in sodium intake to < 2 g/day and consumption of SFA less than 10%TEI

<sup>g</sup>Proportion of total energy intake per day from this macronutrient

**Table 5** Micronutrient intakes from foods and beverages: mean amounts<sup>a</sup> consumed per individual, by sex and age, 2017 Bosnia and Herzegovina dietary survey

Nutrient	Sex				Age-group					
	Males		Females		18–40		41–60		> 60	
	(n 371)		(n 482)		(n 241)		(n 384)		(n 228)	
	Mean <sup>b</sup>	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
<i>Minerals</i>										
Calcium (mg)	793	36.1	638	27.0	804	40.4	638	29.0	692*	43.8
Magnesium (mg)	311	12.5	261*	8.7	338	14.7	258	9.6	253	12.1
Phosphorus (mg)	1201	49.8	935	37.0	1273	58.3	944	40.6	948	53.8
Selenium (µg)	79.5	3.7	55.5	2.8	69.5	4.4	62.7	3.7	66.2*	3.8
Potassium (mg)	2941	105	2575*	88.4	3035	131	2569	88.9	2624*	135
Iron (mg)	13.3	0.5	10.2	0.4	13.3	0.7	10.8	0.5	10.6	0.6
Zinc (mg)	10.5	0.5	7.7	0.4	10.1	0.5	8.2	0.5	8.4	0.6
Sodium (mg)	3174	131	2286	94.4	3181	151	2434	121	2405	125
<i>Vitamins</i>										
Beta-carotene (µg)	751	77.9	795*	72.3	622	70.9	849	87.0	926*	140
Vitamin A (µg RE)	419	25.6	356	20.0	390.5	23.0	356	28.2	429*	29.6
Thiamin (mg)	1.3	0.1	1.0	0.1	1.3	0.1	1.1	0.1	1.1	0.1
Riboflavin (mg)	1.4	0.1	1.1	0.1	1.4	0.1	1.1	0.1	1.1	0.1
Niacin (mg NE)	20.4	1.0	16.1	0.7	21.4	1.2	17.6	0.9	14.6*	0.9
Vitamin B6 (mg)	1.3	0.1	1.1	0.1	1.4	0.1	1.1	0.1	1.1	0.1
Vitamin B12 (µg)	3.6	0.3	2.0*	0.2	3.2	0.4	2.3	0.3	2.7	0.3
Folate (µg)	282	13.4	232	10.8	281	15.1	248	14.0	230	13.7
Vitamin C (mg)	61.6	5.5	70.1*	5.3	66.9	6.8	61.4	5.2	72.2*	9.0
Vitamin D (µg)	3.2	0.3	2.6	0.2	3.1	0.3	2.4	0.2	3.3*	0.5
Vitamin E (mg)	18.3	1.2	15.6	0.8	19.5	1.2	15.1	0.9	16.2	1.4

\*Statistically significant differences in means of log-transformed energy-adjusted nutrients

<sup>a</sup>Only day 1 data used

<sup>b</sup>Geometric mean and standard error calculated using exp(log-mean) of nutrients

vitamin B12 (3.3) and β-carotene (3.9) among women (Table 6). Ratios were higher among women for the majority of nutrients, and increased after energy adjustment. The exceptions were vitamin A (6.2 and 3.6 for men, 5.0 and 3.2 for women), β-carotene (2.1 and 2.0 for men, 3.9 and 2.8 for women) and vitamin C (2.6 and 2.4 for men and 2.3 and 2.2 for women), for which ratios decreased after energy adjustment.

### Discussion

In this manuscript, we have reported the results of the first-to-date nutrient-based dietary survey conducted among an adult population in the Federation of B&H. The survey was designed to describe the overall dietary habits of the Bosnian population and evaluate dietary variations by sex and age-groups. In general, Bosnian adults had an alarmingly high prevalence of overweight, obesity and hypertension across both sexes, and these prevalence rates increased with age. The diet quality of participants was also generally

very poor with low intake of omega 3 fatty acids from both marine and plant sources, high intake of SFA and sodium, and very few men and women consuming adequate intakes of key micronutrients.

Our findings on DHA and EPA were in line with other studies on availability and consumption of dietary fats in this region (Micha et al. 2014; Petrova et al. 2011). This is an important public health concern as both adequate intakes of n-3 fatty acids (Willett 2012b) and a reduction of sodium intake (Sacks et al. 2001) are needed for prevention of CVDs. While fish is eaten episodically (and would not necessarily be captured by 2 days of recall), the mean values of our sample should still be valid. ALA-rich foods, such as walnuts and rapeseed or soybean oil, should be consumed more ubiquitously. However, over 90% of households in this sample reported using sunflower oil as their main cooking oil and reported intakes of nuts were low (Gicevic et al. unpublished results). As a result, intakes of N-6 linoleic acid were sufficient, but those of N-3 fatty acids fell below EFSA recommendations. While both fatty acids are essential, public health efforts are needed to

**Table 6** Means, within-person and between-person coefficients of variation (CV%)<sup>a</sup> and variance component ratios<sup>b</sup>, 2017 Bosnia and Herzegovina dietary survey

Nutrient	Males						Females									
	Mean <sup>d</sup>	SE	Unadjusted			Energy-adjusted <sup>c</sup>			Mean	SE	Unadjusted			Energy-adjusted <sup>c</sup>		
			CV <sub>w</sub>	CV <sub>b</sub>	S <sub>w</sub> <sup>2</sup> /S <sub>b</sub> <sup>2</sup>	CV <sub>w</sub>	CV <sub>b</sub>	S <sub>w</sub> <sup>2</sup> /S <sub>b</sub> <sup>2</sup>			CV <sub>w</sub>	CV <sub>b</sub>	S <sub>w</sub> <sup>2</sup> /S <sub>b</sub> <sup>2</sup>	CV <sub>w</sub>	CV <sub>b</sub>	S <sub>w</sub> <sup>2</sup> /S <sub>b</sub> <sup>2</sup>
Energy (kcal)	2442	47.1	30.9	25.4	1.2	–	–	–	1898 <sup>§</sup>	34.7	32.8	21.9	1.5	–	–	–
Protein (g)	86.3	1.6	36.2	25.9	1.4	23.9	9.7	2.5	63.6 <sup>§*</sup>	1.0	38.3	22.6	1.7	25.8	11.9	2.2
Total fat (g)	92.5	1.8	46.6	28.1	1.7	29.0	12.1	2.4	71.5 <sup>§</sup>	1.4	46.6	26.8	1.7	28.9	13.5	2.1
SFA (g)	29.2	0.7	56.7	32.9	1.7	37.3	15.6	2.4	23.2 <sup>§*</sup>	0.5	57.3	32.2	1.8	37.2	22.4	1.7
MFA (g)	28.2	0.6	52.7	30.3	1.7	42.2	20.8	2.0	21.2 <sup>§</sup>	0.5	51.5	33.0	1.6	44.2	16.5	2.7
PUFAs (total) (g)	20.1	0.4	70.4	24.1	2.9	58.3	11.8	4.9	16.3 <sup>*</sup>	0.3	66.8	27.3	2.4	54.3	19.9	2.7
Omega 3 PUFAs (g)	0.7	0.1	99.5	38.9	2.6	92.4	23.9	3.9	0.5 <sup>§</sup>	0.1	90.6	47.9	1.9	85.4	35.6	2.4
DHA+EPA (mg)	18.2	1.0	239	57.3	4.2	235	51.3	4.6	16.0 <sup>§</sup>	0.9	213	77.0	2.8	209	71.3	2.9
ALA (mg)	94.4	7.6	180	94.4	1.9	159	72.5	2.1	96.6 <sup>e*</sup>	6.1	163	82.5	2.0	150	67.5	2.2
LA (g)	16.2	0.4	73.9	33.3	2.2	63.8	26.2	2.4	12.7 <sup>§</sup>	0.3	77.4	34.9	2.2	66.1	31.0	2.1
Cholesterol (mg)	253	6.0	93.3	40.7	2.3	85.1	28.1	3.0	185 <sup>§</sup>	3.8	104	38.2	2.7	96.3	27.9	3.5
Carbohydrate (g)	282	6.1	33.8	27.8	1.2	21.7	9.5	2.3	228 <sup>§*</sup>	4.8	37.3	25.4	1.5	21.8	9.5	2.3
Fiber (g)	24.5	0.6	38.0	30.3	1.3	35.2	26.4	1.3	21.5 <sup>§*</sup>	0.4	38.3	25.7	1.5	36.8	21.3	1.7
Total sugars (g)	72.2	2.9	58.3	49.8	1.2	55.0	37.3	1.5	67.8 <sup>*</sup>	2.4	58.0	47.8	1.2	52.4	33.9	1.5
<i>Minerals</i>																
Calcium (mg)	813	17.5	43.5	27.5	1.6	38.5	21.2	1.8	648 <sup>§</sup>	13.4	46.7	25.9	1.8	38.8	20.4	1.9
Magnesium (mg)	313	5.7	35.3	22.7	1.6	30.1	14.3	2.1	256 <sup>§*</sup>	4.8	35.8	23.6	1.5	30.3	13.5	2.2
Phosphorus (mg)	1231	23.8	35.4	26.3	1.4	24.8	14.6	1.6	947 <sup>§</sup>	18.2	39.9	24.3	1.6	29.8	15.4	1.9
Selenium (µg)	81.4	1.9	51.9	33.8	1.5	46.2	18.9	2.4	54.5 <sup>§*</sup>	1.1	58.6	27.6	1.9	51.8	22.6	2.3
Potassium (mg)	2994	52.0	34.7	23.5	1.5	32.4	19.1	1.7	2549 <sup>§*</sup>	43.2	35.6	20.9	1.7	35.3	17.0	2.1
Iron (mg)	13.9	0.3	38.8	29.6	1.3	33.8	15.1	2.2	10.7 <sup>§</sup>	0.2	43.4	23.8	1.7	37.7	15.0	2.5
Zinc (mg)	10.8	0.3	46.6	29.4	1.6	38.9	22.6	1.7	8.0 <sup>§</sup>	0.2	52.6	26.7	2.0	42.9	20.2	2.1
Sodium (mg)	3244	65.8	43.1	30.7	1.4	31.5	13.9	2.3	2291 <sup>§</sup>	41.0	50.9	21.8	2.3	37.9	13.0	2.9
<i>Vitamins</i>																
Beta-carotene (µg)	891	35.4	141	67.2	2.1	146	71.1	2.0	820 <sup>*</sup>	20.6	145	37.3	3.9	143	51.5	2.8
Vitamin A (µg RE)	452	4.6	84.3	13.6	6.2	82.2	23.0	3.6	392 <sup>§*</sup>	4.5	84.1	16.7	5.0	79.0	25.0	3.2
Thiamin (mg)	1.3	0.1	41.8	26.2	1.6	36.1	14.1	2.6	1.0 <sup>§</sup>	0.1	49.8	24.3	2.0	43.4	17.0	2.5
Riboflavin (mg)	1.5	0.1	42.2	34.2	1.2	36.1	24.3	1.5	1.1 <sup>§</sup>	0.1	50.2	22.5	2.2	41.1	17.0	2.4
Niacin (mg)	21.1	0.4	56.9	26.4	2.2	52.5	18.0	2.9	16.4 <sup>§</sup>	0.3	54.7	26.7	2.0	48.8	21.5	2.3
Vitamin B6 (mg)	1.3	0.1	60.8	29.9	2.0	57.5	23.2	2.5	1.1 <sup>§*</sup>	0.1	61.9	28.6	2.2	57.0	19.2	3.0
Vitamin B12 (µg)	4.0	0.2	109	58.6	1.9	104	46.8	2.2	2.3 <sup>§*</sup>	0.1	144	43.4	3.3	134	41.1	3.3
Folate (µg)	300	8.2	55.7	32.0	1.7	54.3	25.6	2.1	240 <sup>§</sup>	5.6	58.8	27.5	2.1	55.8	23.6	2.4
Vitamin C (mg)	67.2	2.1	106	41.1	2.6	109	45.6	2.4	72.5 <sup>*</sup>	2.3	100.2	43.1	2.3	99.1	44.7	2.2
Vitamin D (µg)	3.5	0.1	91.9	40.9	2.3	85.5	34.0	2.5	3.0 <sup>§*</sup>	0.1	90.3	41.1	2.2	86.6	34.6	2.5
Vitamin E (mg)	18.5	0.4	57.1	25.6	2.2	48.8	19.4	2.5	14.9 <sup>§</sup>	0.3	58.7	22.2	2.4	50.0	19.1	2.6

\*Statistically significant differences in log-transformed means of energy-adjusted nutrients ( $p < 0.05$ )

§Statistically significant differences between log-transformed means of unadjusted nutrients ( $p < 0.05$ )

<sup>a</sup>CV<sub>w</sub> is calculated  $\sqrt{\text{MSE}} \times 100\%$ . CV<sub>b</sub> is calculated as  $\sqrt{(\text{MSModel} - \text{MSError})} \times 100\%$ , where MSE is calculated on the log scale in ANOVA

<sup>b</sup>Ratios of within-person and between-person components of variance  $S_w^2/S_b^2$

<sup>c</sup>Energy adjustment as %TEI for main macronutrients and as nutrient density (nutrient intake per 1000 kcal) for cholesterol, all PUFAs and micronutrients

<sup>d</sup>Nutrient variables were log-transformed to partition the variance and back-transformed to calculate the means. Analysis restricted to individuals for whom 2 days of dietary data were available

<sup>e</sup>Women had significantly higher percentages of total energy intake from ALA than men

promote intakes of N-3 fatty acids, possibly including subsidies for production of rapeseed oil as a locally grown and acceptable type of cooking oil rich in ALA, or the cultivation and consumption of more affordable fatty fish, e.g., trout, sardine and mackerel, which are rich in DHA + EPA. Intakes of saturated fatty acids (SFAs) and sodium also exceeded the WHO recommendations, especially among men and persons in the youngest age-group. Even though sodium intakes from dietary assessment may be inaccurate, our findings correspond with the GBD data based on the urinary sodium excretion and earlier diet assessments in Eastern Europe (Powles et al. 2013). Given that the main sources of these nutrients were processed meats and full fat dairy such as salty sheep milk cheese and cream spread (60% fat), public education programs are needed to raise awareness about the importance of limiting intakes of these foods. A significant reduction in CVD risk can be achieved by replacing SFAs by PUFAs (Willett 2012b; Mensink et al. 2003) and reducing sodium in diet (Sacks et al. 2001). Along with CVD, sodium is also a risk factor for stomach cancer, which is the second most common cancer in B&H (FMHFBIH/IPHFBIH 2012).

Although we did not measure added/free sugars, due to low intakes of sources of intrinsic sugar, it is safe to conclude that the majority of the 'total sugar' in this survey came from added sugar. The mean intake of 10.6–13.0% of energy from total sugar most probably exceeded the WHO recommendation (WHO 2015b) to limit free sugar intake to 5–10% TEI. While mean sugar intake was slightly lower than reported mean total sugar intakes (16–21% TEI) in ten European countries (Azais-Braesco et al. 2017), this is probably due to low consumption of fruits among Bosnians in this sample.

As our data show, micronutrient deficiencies may occur even in food abundant settings due to modern lifestyles (Pietrzik 2012). Low intakes of vitamin B6, folate, vitamin C and  $\beta$ -carotene likely result from low intakes of vegetables and fruits. Low intakes of vitamins A, B12 and D were also noted.  $\beta$ -Carotene intake can be improved in an affordable way by adding more carrots to stews and soups or eaten as fresh carrots. Citrus fruits are affordable and locally acceptable vitamin C sources. Inadequate iron intakes were found among 24.6% women; while dietary intake studies are not the optimum way to assess iron status due to the low bioavailability of this nutrient, these findings are in line with the estimated 24% prevalence of iron deficiency anemia among reproductive age women. Increased consumption of iron combined with vitamin C from both supplemental and food sources, as well as folate to prevent incidence of spina bifida and reduce anemia rates.

Similar to results of our study, a recent systematic review of dietary surveys from 21 countries in Europe

(Rippin et al. 2017) found comparable overall mean intakes of SFA, zinc, iron, calcium, potassium and vitamin D. Bosnians in our sample, however, reported consuming less sugar, protein, MFA, n-3 fatty acids, folate and vitamin B12, and higher consumption of dietary fiber, carbohydrates and PUFAs. These findings suggest that while Bosnia is undergoing nutrition transition, the influence of traditional diets is still stronger than in more developed European countries (Popkin 2004).

For most nutrients, the variance ratios increased, as expected, after energy adjustment due to decrease in between-person variance (Beaton et al. 1979). High ratios for DHA and EPA suggest that many more than 2 days of dietary recall will be needed to estimate the true intakes of marine omega 3 fatty acids for individual participants. Similarly, a high ratio for vitamin B12 among women suggests more sporadic intake of meat, compared to men. Within-person variation is culture specific and depends on food availability; however, our data do not support the common wisdom that diets in LMICs are monotonous from day to day, as the ratios are largely similar to those in HICs (Willett 2012a, b). To some degree, however, such variation in our sample may be explained by the day of the month (Willett 2012a), as we conducted the interviews 4–30 days apart, so at least one of the two was often conducted in the days before the monthly payday, when savings on food are very probable. On the other hand, seasonality was not expected to influence within-person variation as both dietary recalls were conducted within the same season. Data on variance ratios are useful for planning nutrition epidemiology studies in specific populations, as high within-person variation would bias estimates of associations between diet and disease toward the null (Willett 2012a).

Our estimates of hypertension prevalence exceeded the country estimates from 2008 and 2012 (ZJZFBIH 2012; FMHFBIH/IPHFBIH 2012). However, our estimates are plausible, given the positive trend in hypertension and obesity prevalence (WHO 2015a), excessive dietary intake of sodium and inadequate intake of various micronutrients, and high current smoking prevalence in this population. While data on overweight and obesity together corresponded with high-risk WHR and WC estimates across both sexes and age-groups, it is evident that abdominal adiposity, more than obesity, is a cause for concern. As both overall and central adiposity measures have been found to predict heart disease similarly (Flint et al. 2010), these findings provide clear evidence that there is an urgent need for public health action to tackle adiposity.

Our study limitations should also be considered. A modest size of our sample could have lead to loss of precision when estimating mean intakes of some highly variable nutrients such as vitamin A. While it limited our

ability to analyze intake of such nutrients in some subgroups (such as high SES subgroup with less than hundred participants), mean estimates by sex and age-group (containing over two hundred participants) should remain reasonably precise. Due to conscious or unconscious underreporting which is an inherent characteristic to any diet recall method, nutrient database errors and the exclusion of supplement intake from the analysis, it is possible that we overestimated the prevalence of inadequacy for some nutrients (Barr et al. 2002). However, we took care to update the nutrient database beforehand using published analytical and industry data, and we checked against other food composition databases. We also used a multiple pass method for the 24-h recall to decrease the likelihood of errors in reporting due to memory issues (Moshfegh et al. 2008). While excluding supplement use may overestimate the true prevalence of inadequate nutrient intakes, the primary purpose of this study was to evaluate dietary inadequacies in this population. Also, < 13% of participants reported consuming single micronutrient supplements, and < 2% consuming supplemental PUFAs.

To conclude, this study identified some serious concerns in dietary intake. Inadequate diets coupled with a high prevalence of smoking, overweight, and obesity, and hypertension that increases with age are expected to have a negative impact on the health of this population, leading to premature mortality and increased disability (Murray and Lopez 2013). Public health interventions including policies to improve the food supply, promote appropriate supplementation and fortification, and raise awareness about healthy diets are needed to increase intakes of long-chain omega 3 fatty acids, fiber, vitamins A, C, B6, D and folate, as well as magnesium and iron, and limit intakes of sodium, added sugar and SFA. Further research should focus on major food sources of nutrients, socioeconomic variations in intakes and factors influencing food choice.

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### Compliance with ethical standards

**Conflict of interest** Selma Gicevic declares that she has no conflict of interest. Audrey J. Gaskins declares that she has no conflict of interest. Teresa T. Fung declares that she has no conflict of interest. Bernard Rosner declares that he has no conflict of interest. Edin Sabanovic declares that he has no conflict of interest. Mirjana Gurinovic declares that she has no conflict of interest. Agnes Kadvan declares that she has no conflict of interest. Emir Kremic declares that he has no conflict of interest. Walter Willett declares that he has no conflict of interest.

**Ethical approval** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

**Informed consent** Informed consent was obtained from all individual participants included in the study.

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