Abstract: South-Eastern Serbia is with traditionally acknowledged intake of spicy and energy-dense food. For the purposes of this study we presented official socio-economic determinants for the three counties within South-Eastern Serbia: jablanički, niški and pirotski. The objective of this study was to assess nutritional status of randomly selected 40 subjects (11-74 years) residing in South Eastern Serbia within the counties. By means of validated food and dietary data methodology, we interviewed subjects and analyzed in a
cross-sectional design. Our data indicate low-carbohydrate-high-fat like dietary pattern within the subjects, irrespective of age and gender in the region. Demonstrated likely improper nutritional status of sodium, calcium, vitamins A, C, D and B9 (folate) warrants public health initiatives towards optimization of the intake within the region. Our results indicate inadequate micronutrient intake within the adolescent group. Overall our results underline the necessity of raising education and awareness of healthy nutrition benefits and sustainable food choices within South Eastern Serbia and foster the necessity of implementation of an individualized approach in nutrition healthcare in this middle-income country.

Keywords: low carbohydrate high-fat diet, micronutrients, nutritional assessment, personalized nutrition, South Eastern Serbia

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grants: European Food Safety Authority (EFSA): Support to National Dietary Surveys in Compliance with the EU Menu methodology (sixth support) “The children’s survey”, Reference number: OC/EFSA/DATA/2016/02; European Food Safety Authority (EFSA): Support to National Dietary Surveys in Compliance with the EU Menu methodology (sixth support) “The adult’s survey” Reference number: OC/EFSA/DATA/2016/03.

Abbreviations

%TE, total daily energy intake; AI, Adequate Intake; AR, Average Requirement; DRV, Dietary Reference Values; EAR Cut Point method, Estimated Average Requirement Cut Point Method; EFSA, European Food Safety Authority; GBD, Global Burden of Diseases, Injuries, and Risk Factors Study; MUFA, Monounsaturated Fatty Acids; PRI, Population Reference Intake; PUFA, Polyunsaturated Fatty Acids

INTRODUCTION

World Health Organization data underline an increasing trend in chronic disease prevalence world-wide (World Health Organization [WHO], 2013), pronounced in countries going through economic transition, inseparable from transitions in dietary habits. Serbia is a middle-income country in South Eastern Europe, with dietary factors along with tobacco use and high blood pressure, representing the leading risks for death and disability in the country (Institute for Health Metrics and Evaluation [IHME], 2017). According to Global Burden of Disease study data, ischemic heart disease and stroke, both potentially modulated by nutrition factors, represent leading causes of death in the country (IHME, 2017). There is also substantial prevalence of overweight in the country of up to 35.1% (Ministry of Health of Republic of Serbia, 2013).

Food and eating habits are an important socio-habitual aspect of living in our country. Although socialization around meals is highly supported, in Serbia it is often followed by exaggeration in meal sizes and intake of palatable food with acknowledged health disadvantages. In South Eastern Serbia dietary patterns are often composed of high-calorie, spicy and picante food. Addition of salt, frying and baking in oil,
are common practices in this part of our country. Offal, red meat and processed meat, such as salami, sausages, and hams are staple and consumed rather to white and lean meat. The awareness is low on the benefits of fiber-rich food, complex carbohydrates, quality of dietary fats, deleterious effects of added sugars, salt, necessity of prudent intake of microelements. In addition, as part of the cultural heritage Rakia alcoholic drink is a staple in the country, but especially in South Eastern Serbia with high plum fruit yield. Due to an inseparable bond between socio-economic status and nutrition habits, coupled with subsequent health implications, public health and nutritional strategies in the region must adhere to specific socio-economic context.

For the purposes of this study we extracted and presented official socio-economic data for the three counties within South-Eastern Serbia: jablaníčki, niški and pirotški. The main objective of this study was to comprehensively present and analyze the quality of dietary intake in terms of intake of macronutrients, microelements and vitamins, among randomly selected 40 subjects (10-74 year of age) residing within the three counties in the cities of Niš, Pirot, Aleksinac and Leskovac. For the secondary objective we analyzed total and macronutrient-specific distribution of crucial food groups within habitual intake of the subjects.

METHODS

Socio-economic indicators

Geo-specific, socio-economic and demographic parameters for the Republic of Serbia and South Eastern Serbia were obtained using analytical service of the Public Policy Secretariat of the Republic of Serbia, and by the Statistical Office of the Republic of Serbia. A set of indicators was summarized:

a) Total number of subjects is an annual average number of subjects residing within the areas, estimated based on census data, data on population growth and migrations.

b) Population density is calculated as total number of subjects residing per km².

c) Aging index represents ratio between total number of subjects of more than 60 years of age, to total number of subjects of 0-19
years of age, estimated during the middle of the given year. Aging index of 12-20 points indicates young population, while the aging index above 40 indicates old population.

d) Mortality rate is presented as total number of deaths per 1000 subjects in a given year.

e) Vitality index is the number of born babies to premature deaths in a given year.

f) Working age population are subjects of 15-64 years of age. Working population is total number of employed subjects within companies and entrepreneurships.

g) Number of unemployed and unemployment rate are obtained according to National Employment Service evidence, as a fraction of total working age population.

All data are presented for the year of 2017 for the three counties. Average income is related to the given period of the year of 2017.

Assessment of dietary habits and nutritional status

Study subjects and study design

Analysis of nutritional status within the region is a part of larger studies designed to assess the quality of diet and dietary and nutritional habits of subjects residing within the territory of Republic of Serbia. Larger studies include European Food Safety Authority (EFSA): Support to National Dietary Surveys in Compliance with the EU Menu methodology (sixth support) “The children’s survey” and European Food Safety Authority (EFSA): Support to National Dietary Surveys in Compliance with the EU Menu methodology (sixth support) “The adult’s survey”. Survey subjects were selected based on three stratification layers: age, gender and geographical region. Recruitment was conducted by the project team members at the household level, with not more than one individual recruited per household. The subjects were selected by use of national census data from 2011. For the purposes of assessing nutritional status, we included 40 subjects residing in South Eastern Serbia.

Assessment of study variables

For the assessment of dietary intake, demographic and lifestyle data, a package of validated questionnaires was applied based on EU Menu
Methodology. Subjects underwent two 24-hours dietary recalls, using multiple-pass methodology procedure. All the recalls were performed face-to-face in an in-depth interview manner by trained researchers. Data collection was organized in participants home or in study regional examination center based on participants’ preference and availability. In order to provide a better estimate of intrapersonal dietary variability assessment of food consumption was performed for two non-consecutive days with a minimal time-lapse of one week. Observation days were planned in accordance with the survey calendar so that adequate proportion of weekdays and weekend days are captured at population group level. In addition, study subjects were interviewed by retrospective method of three-month food frequency consumption survey. To estimate portion size subjects were offered a photo booklet depicting a range of reference portions for 125 items for simple foods and composite dishes (Gurinović et al., 2018, p. 186-194). We used DIET ASSESS & PLAN to analyze questionnaires, that is advanced nutritional platform for standardized food consumption data collection and diet evaluation (Gurinović et al., 2016a, p. 173–180, Gurinović et al., 2018, p. 186-194). Interviewers were obliged to enter dietary and non-dietary data in the software within three days after the interview. Each questionnaire was double-checked by members of the study steering committee. Total energy and specific nutrient intakes were calculated by use of Serbian Food Composition Database, compliant with EuroFIR standards (Gurinović et al., 2016b, p. 30–38).

For anthropometry data, subjects were measured by bioimpedance analyzer Tanita BC 545N (TANITA Health Equipment H.K. Ltd), in light clothing and in a private room. Waist circumference was assessed by flexible measurer. The BMI was calculated as weight (kg)/(height [m])² and adult subjects were stratified as normal weight if the BMI was 18.5–25 kg/m²; and overweight for BMI≥25 kg/m².

Trained personnel interviewed and measured the subjects.

*Instruments for the assessment of nutritional status*

Total energy intake was evaluated in MegaJoules and compared to age- and gender-specific requirements proposed by European Food and Safety Authority (EFSA) (European Food Safety Authority [EFSA], 2017) and further assigned as suboptimal, adequate or excessive.
For the purposes of evaluation of nutritional intake, we used protocols and Dietary Reference Values (DRV) proposed by EFSA. We also compared nutrient-specific data to the guidelines developed by WHO or other relevant regulatory body. Following nutrient-specific DRV were considered:

a) Population Reference Intake (PRI), indicating level of intake of a nutrient optimal for almost all subjects in the population.
b) Average Requirement (AR), indicating the level of intake of a nutrient, meeting requirements of almost 50% subjects in the population.
c) Adequate Intake (AI), which is an empirical value indicating the likely sufficient level of particular nutrient intake, established if the previous two values are not evidence-supported.

If AR is established, we applied Estimated Average Requirement Cut Point method to evaluate proportion of the population based on group estimates, likely to be with inadequate intake of the nutrient (Murphy & Poos, 2002). Shortly, for a certain nutrient the method counts number of subjects with daily intake lower or higher than the proposed AR. Percentage of subjects with daily intake falling below established AR, virtually represents percentage of population with likely inadequate intake of the nutrient, and vice versa.

If the mean daily intake within a subgroup falls above proposed PRI or AI, the nutrient intake was assigned as likely optimal; and likely suboptimal if the mean daily intake within a subgroup was below proposed PRI or AI. Although this approach suffers from limitations due to individual requirement that is challenging to ever determine (Institute of Medicine [IOM], 2000), we considered the approach as the most feasible in depicting dietary and nutritional status of the group, raising awareness on the necessity of personalization of nutrition-based healthcare strategies.

Where applicable, we commented on reference intake ranges proposed for certain nutrient.

Instruments for the assessment of macro- and micronutrient intake

We applied a descriptive approach to evaluate whether macronutrient intake (carbs, fats and differential fat groups) falls within the range proposed by a regulatory body. We used the Estimated Average Require-
ment Cut Point (IOM, 2000) method to evaluate the proportion of the group likely to be with (in)adequate daily intake of proteins, regardless of protein source (animal or plant). In line with trending research orientated towards relevance of dietary patterns instead of single nutrient and food choices (U.S. Department of Health and Human Services and U.S. Department of Agriculture, 2015), we commented on the percentage of macronutrients from total daily energy intake (%TE), deriving the picture on the distribution of nutrient consumption within the group, rather than absolute intake levels.

Intake of micronutrients was evaluated based on proposed Scientific Opinion by EFSA (EFSA, 2017), except for the sodium intake that was compared to WHO recommendations (WHO, 2012). For the purposes of the clarity of the document, we did not present systematically established DRVs for each microelement. The reader is invited to refer to relevant regulatory documents and scientific opinions, used in this paper for dietary assessment of nutritional intake (EFSA, 2017; Elmadfa & Kornsteiner, 2009; Uauy & Dangour, 2009; WHO, 2012).

**Statistical Analysis**

Data are presented as mean±SD for continuous variables, and as a percentage for categorical variables. To compare with established reference ranges (EFSA, 2017), total energy intake per group was adjusted to BMI=22. Between-group differences were analyzed by use of parametric and non-parametric tests: analyses of variance and Mann-Whitney/Kruskall-Wallis tests, respectively, and depending on the number of groups. Statistical threshold of 0.05 was used to assign significant difference. Data were analyzed by SPSS Software Package 24.

**RESULTS AND DISCUSSION**

**Socio-economic status of South Eastern Serbia**

Socio-economic and demographic determinants for South Eastern Serbia are presented in Table 1.
Table 1 Socio-economic and demographic parameters in South-Eastern Serbia

<table>
<thead>
<tr>
<th></th>
<th>Jablanički county</th>
<th>Nišavski county</th>
<th>Pirotski county</th>
<th>Serbia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Population, total number</td>
<td>203254</td>
<td>364157</td>
<td>85964</td>
<td>7020858</td>
</tr>
<tr>
<td>Population density</td>
<td>73</td>
<td>133</td>
<td>31</td>
<td>90</td>
</tr>
<tr>
<td>Aging index</td>
<td>144.10</td>
<td>158.40</td>
<td>200.70</td>
<td>141.60</td>
</tr>
<tr>
<td>Mean age, years</td>
<td>43.40</td>
<td>43.90</td>
<td>46.40</td>
<td>43.00</td>
</tr>
<tr>
<td>Working age population (15-64 years), % of total population</td>
<td>63.24</td>
<td>63.01</td>
<td>62.36</td>
<td>65.19</td>
</tr>
<tr>
<td>Mortality rate, permiles</td>
<td>16.43</td>
<td>14.97</td>
<td>18.22</td>
<td>14.77</td>
</tr>
<tr>
<td>Vitality index</td>
<td>49.55</td>
<td>60.34</td>
<td>41.19</td>
<td>62.57</td>
</tr>
<tr>
<td>Working age population, total number</td>
<td>40314</td>
<td>96492</td>
<td>21122</td>
<td>1977344</td>
</tr>
<tr>
<td>Average income, RSD</td>
<td>34684</td>
<td>35972</td>
<td>37654</td>
<td>40112</td>
</tr>
<tr>
<td>Unemployed subjects, total number</td>
<td>30281</td>
<td>41688</td>
<td>10708</td>
<td>573266</td>
</tr>
<tr>
<td>Unemployment rate</td>
<td>42.89</td>
<td>30.17</td>
<td>33.64</td>
<td>22.48</td>
</tr>
</tbody>
</table>

RSD, official currency of Republic of Serbia

Figure 1 presents the parameters within South-Eastern Serbia relative to global country parameters in the Republic of Serbia. According to the official data derived by Serbian governmental bodies based on gross domestic product, the units of self-government within the three counties were with the following degree of development: 5 out of 6 units within jablanički county, 6 out of 12 within nišavski county and 3 out of 4 within pirotski county, fell below 60% of republic average, indicating substantial level of poor economic status within the region.

We conclude that the region of South-Eastern Serbia within the three counties is of lower socio-economic status, potentially coupled with malnutrition and poor dietary habits.
Out of 40 subjects, there were 14 males and 26 females; and 5 adolescents (11-18 years of age) and 35 adults (≥18 years of age). Within adolescents there was 1 boy and 4 girls, while within adult category there were 13 men and 22 women. Sixteen subjects were with chronic condition present, and 13 were taking medication. Table 2 summarizes total daily energy intake of the study subjects, with corresponding comment assigning comparison with daily Average Energy Requirement per age and gender category, recommended by recent EU Scientific Opinion (EFSA, 2017). Our subjects presented with moderate physical activity and PAL value (physical activity level) was set at 1.6.

Calculated in daily caloric count, adult subjects in our sample (n=35) were with mean daily intake of 2598.20±864.50 total kcal. The intake was higher in comparison with previous data from Serbian subjects.
with mean intake of 1983.56 ± 532.07 kcal (Pantovic et al., 2018). In 503 healthy Serbian women of reproductive age previously reported mean intake was 1724.62 ± 330.02 kcal (Zekovic et al., 2017).

Table 2  Total daily energy intake in megajoules in 40 subjects residing in South-Eastern Serbia across gender and age groups

<table>
<thead>
<tr>
<th>Age Category</th>
<th>Total Energy Intake MJ</th>
<th>Range</th>
<th>Comment</th>
<th>Age Category</th>
<th>Total Energy Intake MJ</th>
<th>Range</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>11 yrs, n=2</td>
<td>14.08±2.48</td>
<td>12.32-15.83</td>
<td>Excessive</td>
<td>16 yrs, n=1</td>
<td>7.85</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 yrs, n=1</td>
<td>9.00</td>
<td></td>
<td>Adequate</td>
<td>18-29, n=5</td>
<td>11.10±4.27</td>
<td>7.92-18.57</td>
<td>Adequate</td>
</tr>
<tr>
<td>16 yrs, n=1</td>
<td>11.28±2.02</td>
<td>9.46-14.13</td>
<td>Excessive</td>
<td>30-39, n=4</td>
<td>11.10±4.27</td>
<td>7.92-18.57</td>
<td>Adequate</td>
</tr>
<tr>
<td>40-49, n=8</td>
<td>7.77±2.52</td>
<td>4.94-11.53</td>
<td>Adequate</td>
<td>40-49, n=2</td>
<td>10.92±0.33</td>
<td>10.54-11.29</td>
<td>Adequate</td>
</tr>
<tr>
<td>50-59, n=1</td>
<td>9.90</td>
<td></td>
<td>Adequate</td>
<td>50-59, n=5</td>
<td>11.71±3.97</td>
<td>8.08-18.44</td>
<td>Adequate</td>
</tr>
<tr>
<td>60-69, n=2</td>
<td>6.62±2.10</td>
<td>5.13-8.10</td>
<td>Adequate</td>
<td>70-79, n=2</td>
<td>7.22±0.89</td>
<td>6.59-7.85</td>
<td>Adequate</td>
</tr>
</tbody>
</table>

Data are presented as Mean±SD. Total energy intake was adjusted per BMI=22 for all categories. The comment on the adequacy of the intake assigns the likelihood of the adequacy in certain age group based on the comparison with proposed average requirement. PAL value set at 1.6.

MJ, Mega Joules

**Habitual Intake of Macronutrients**

a. Total Carbohydrates: Glycemic Carbohydrates and Dietary Fibers

Irrelevant of gender and age category, mean daily intake within the subjects was below proposed range of recommended daily carbohydrates intake of 45-60% TE (Table 3). Intake of carbohydrates in our group ranged between 26.46 to 56.22% TE, considered as moderate carbohydrate intake (Oh, 2019). Very-low and low carbohydrate intake of less than 14% TE, is favorably associated with weight management and glycemic control (Tay et al., 2018). Protagonists of beneficial Mediterranean diet promote larger proportion of polyunsaturated (PUFA) and monounsaturated fats (MUFA), keeping the daily carbohydrate level substantial (Martínez-González et al., 2015). Recent meta-analyses in subjects from Western countries indicated deleterious effects of long-term low-carbohydrate and low-carbohydrate/high-protein diet, in terms of cardiovascular, cerebrovascular, cancer and all-cause mortality (Mazidi, Katsiki, Mikhailidis, Sattar, & Banach, 2019). Data from Japanese subjects indicate favorably associated low-carbohydrate diet with
cardiovascular outcome and mortality over 29 years (Nakamura et al., 2014). The discrepancy might be due to the inherently higher intake of carbohydrates in Japan (over 60% of total energy), while subjects from the pooled analyses were with mean carbohydrate intake of 39% in the lowest quartile (Mazidi et al., 2019).

When discussing low-carbohydrate dietary pattern, bidirectional considerations are of vital importance: the macronutrient distribution at costs of carbohydrate decrease; and type of carbohydrate to be promoted in a balanced diet. Results from the Prospective Urban Rural Epidemiology (PURE) study conducted within 18 countries worldwide, indicate deleterious association of carbohydrate intake with total mortality (Dehghan et al., 2017). Of importance, up to 70% TE within the PURE populations was ascribed to refined carbohydrates, possibly driving the observed deleterious associations. Intake of whole grains rich in complex carbohydrates is inversely associated with 9% lower total and 15% lower cardiovascular mortality, among cc 45000 men and 75000 of American women (Wu et al., 2015).

We report mean daily intake from total carbohydrates of 41.86±7.42% TE, below previously reported intake among 88 Serbian adults of 44.25 ± 8.12% TE (14 11). In the group of 25 Serbian healthy women, previously reported daily intake of carbohydrates is 54.3± 5.5% TE (Kardum et al., 2014), while in 503 women of reproductive age residing in Serbia, the intake is 50.94±7.23% TE (Zekovic et al., 2017). However, in our study among 35 adults, intake from carbohydrates was not different among men (41.04±7.41% TE) and women (41.42±6.83% TE) (p for trend=0.877). Our group is of limited size to draw conclusion on possible gender-specific intake, and national survey should address the issue.

We conclude that the trend towards lower carbohydrate intake within the group might not emerge as hazardous, assuming pronounced consumption of complex carbohydrates.

b. Dietary fibers

Mean intake of total dietary fibers was 28.68±9.39 g/day, above AI of 25 g/day proposed by EFSA (EFSA, 2017). Individual daily fiber intake ranged from as low as 9.05 g. The latter indicates substantial proportion of individuals from the group seemingly consuming suboptimal levels of dietary fibers. Intake of non-digestible dietary fibers is with known health-promoting properties with regards to weight management, cor-
onary heart disease and diabetes Type 2 (Lee & Hase, 2014). Dietary fibers are found in whole grain cereals, pulses, potato, vegetables and fruits. Recent molecular research indicates possible interaction of dietary fibers with polyphenols in food matrices of fruits such as mango, papaya or guava (Gonzalez-Aguilar, Blancas-Benitez, & Sayago-Ayerdi, 2017). The interaction might affect bio-accessibility of dietary polyphenols, potentially corrupting its beneficial health effects (Gonzalez-Aguilar et al., 2017; Quirós-Sauceda et al., 2014). Health professionals dealing with conceptualization of individual nutritional and dietary plans of the subjects in the area should foster substantial intake of dietary fibers from available food.

Gender-specific analyses showed insignificantly higher (p=0.703) fiber intake among women (29.15±7.77 g/day) in comparison with men (27.89±11.96 g/day). Intake of women ranged from 15.37 to 43.68 g of dietary fibers daily, while in men this range was wider, from 9.05 to 46.06 g/day. Fiber consumption might be associated with particular food choices, since fibers are also abundant in starch-rich food, such as potato and cereals. Individualized dietary plans must be wise in proposing intake of fibers not associated with excessive glycemic load.

Although only 5 adolescents were present within the sample, all except one failed to reach optimal AI levels of dietary fibers proposed for the age group (EFSA, 2017). Due to the small sample size, and lack of data to yield individual requirements for the subjects, we are not able to comment on the possible implications of the insufficient fiber intake in the age group within the region. Since mean carbohydrate intake was below lower limit of the reference range proposed for adequate supply of the carbs, one of the potentially prudent individual approaches in the subjects might be replacement of the habitual carbohydrates with fiber-rich whole grain cereals, rice, potatoes, as well as adequate consumption of fruits.

According to the pilot data the consumption of food rich in dietary fibers should be promoted in the South-Eastern region of Serbia.

c. Total Fat and Fatty Acid groups Intake

To evaluate fat intake in the group, we used guidelines proposed by FAO/WHO expert consortium on fat intake for health across different age categories (Elmadfa & Kornsteiner, 2009; Uauy & Dangour, 2009). According to both EFSA and FAO/WHO opinion, reference intake
range for daily total fat intake should be between 20% and 35% TE, irrespective of the age group. Subjects from the South Eastern Serbia in our study eat fat pronouncedly (>40% TE from total fat in the complete group and across gender and age categories) as presented in Table 3.

Intake of saturated fats should not exceed 10% TE (EFSA, 2017; Elmadfa & Kornsteiner, 2009; Uauy & Dangour, 2009). Our data indicate excessive intake of saturated fats across all groups, with minimum range level at 9% TE.

Proposed intake of PUFA should fall between 5% and 15% TE for subjects under 18 years for the prevention of nutrition-related chronic disease (Uauy & Dangour, 2009), encompassing acceptable macronutrient distribution range (AMDR) of 6-11% TE proposed for adults only (Elmadfa & Kornsteiner, 2009). In our study, intake range indicated adequate PUFA consumption within adolescents (4.23-14.33% TE) (Uauy & Dangour, 2009). Mean intake of PUFA within adults was 10.34% TE, falling within the AMDR for adults (Elmadfa & Kornsteiner, 2009).

For non-infant subjects under 18 no restriction limit for MUFA intake for the prevention of nutrition-related chronic disease is proposed (Uauy & Dangour, 2009). FAO/WHO guidelines set AMDR of 15-20% TE for MUFA intake in adults. Mean MUFA intake in our sample and across the categories did not exceed 15% TE and appeared the lowest within adolescent group (12.92±3.60% TE).

Our data show seemingly higher intake of fats and fat groups within the subjects residing in South Eastern Serbia. Adult subjects consumed 43.21±6.32% TE from fats, comparable to previously reported data among 87 apparently healthy middle-aged Serbian adults, with mean daily intake of 39.93±6.79% TE from total fats (Pantovic et al., 2018). On the contrary, previously reported data from our group among 503 healthy women, indicate substantially lower mean daily intake of total fats of 28.30±7.21% TE (Zekovic et al., 2017). Herein, we observed no differences (p for trend=0.750) in fat intake, among adult men (42.76±6.82% TE) and women (43.48±6.15% TE). We conclude no gender-specific premises in dietary intake related to fat in the group of South Eastern Serbs.

We conclude substantial intake of fats within the South Eastern region of Serbia that might not appear as hazardous. Public health strategies in the region should enforce replacement of saturated fats with long-chain unsaturated counterparts.
d. Protein intake

For evaluation of optimal protein intake, we used scientific opinion proposed by EFSA (EFSA, 2017) based on kg-adjusted average requirements of daily protein intake. We used method of Estimated Average Requirement Cut Point (Murphy & Poos, 2002; IOM, 2000) to evaluate the proportion of the group likely to be with (in)adequate daily intake of proteins, regardless of protein source (animal or plant). Based on the individual data, only one person tended to be with observed daily intake lower than AR for the age and gender category, allowing implication that substantial proportion of the group (seemingly 100%) is with adequate protein intake.

Mean daily intake of total protein among adults, men and women in our study was 15.51±3.31% TE, 16.20±4.20% TE and 15.10±2.69% TE, respectively, comparable to previously reported data in 87 apparently healthy Serbian subjects (Pantovic et al., 2018). In 503 Serbian women, intake of proteins was 20.22±3.50% (Zekovic et al., 2017), which was higher comparing to herein analyzed women.

Table 3 Intake of macronutrients in 40 randomly selected subjects residing in South-Eastern Serbia

<table>
<thead>
<tr>
<th>Carbohydrate, %TE</th>
<th>Mean±SD</th>
<th>Range</th>
<th>DRV</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total sample, n 40</td>
<td>41.86±7.42</td>
<td>26.46, 56.22</td>
<td>45-60%, RI</td>
<td>Suboptimal</td>
</tr>
<tr>
<td>Total Fiber Intake, g</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adolescents, n 5</td>
<td>11-14 y, n 2</td>
<td>17.34±0.21</td>
<td>17.19, 17.49</td>
<td>19 g/day, AI</td>
</tr>
<tr>
<td>Adults, n 35</td>
<td>15-17 y, n 3</td>
<td>18.92±4.79</td>
<td>14.28, 23.84</td>
<td>21 g/day, AI</td>
</tr>
<tr>
<td>Fats, %TE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adolescents, n 5</td>
<td>Total Fat</td>
<td>39.56±8.25</td>
<td>30.35, 48.95</td>
<td>30-40%, AMDR</td>
</tr>
<tr>
<td>MUFA</td>
<td>12.92±3.57</td>
<td>8.98, 17.25</td>
<td>No restriction</td>
<td>n/a</td>
</tr>
<tr>
<td>PUFA</td>
<td>8.89±4.27</td>
<td>4.23, 14.33</td>
<td>5-15%, AMDR</td>
<td>Likely Optimal/Suboptimal</td>
</tr>
<tr>
<td>SFA</td>
<td>13.13±2.54</td>
<td>10.73, 16.72</td>
<td>&lt;10%, MAL</td>
<td>Likely excessive</td>
</tr>
<tr>
<td>Total Fat</td>
<td>45.21±6.32</td>
<td>30.50, 55.97</td>
<td>20-35%, AMDR</td>
<td>Likely Optimal</td>
</tr>
<tr>
<td>Adults, n 35</td>
<td>MUFA</td>
<td>14.29±3.45</td>
<td>8.92, 23.12</td>
<td>15-20%, AMDR</td>
</tr>
<tr>
<td>PUFA</td>
<td>10.34±3.43</td>
<td>5.26, 18.91</td>
<td>6-11%, AMDR</td>
<td>Likely Optimal</td>
</tr>
<tr>
<td>SFA</td>
<td>13.66±3.05</td>
<td>8.67, 20.73</td>
<td>&lt;10%, MAL</td>
<td>Likely excessive</td>
</tr>
<tr>
<td>Protein, g/kg bw</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adolescents, n 5</td>
<td>Male, n 1</td>
<td>2.44</td>
<td>2.44, 2.44</td>
<td>0.70-0.75 g/kg bw, AR</td>
</tr>
<tr>
<td>Female, n 4</td>
<td>1.64±0.82</td>
<td>0.88-2.54</td>
<td>0.67-0.73 g/kg bw, AR</td>
<td>Likely Optimal</td>
</tr>
<tr>
<td>Adults, n 35</td>
<td>1.33±0.41</td>
<td>0.52, 2.38</td>
<td>0.66 g/kg bw</td>
<td>Likely Optimal</td>
</tr>
</tbody>
</table>
Data are presented as Mean±SD or range (minimum, maximum in the whole sample, or across age categories. Dietary Reference Values are per EFSA scientific opinion, except for bolded text denoting comparison with FAO/WHO proposed values.

%TE, % of total daily energy intake; AI, Adequate Intake; AR, Average Requirement range, depending on the years of age; AMDR, acceptable macronutrient distribution range; BW, body weight; DRV, Dietary Reference Value; MAL, maximum level.

*aResult on the particular intake is inconclusive and Personalized Nutritional Strategy is recommended on individual level to allow for adequate intake.

Habitual Intake of Micronutrients

Minerals

Mean daily intake of microelements in 40 subjects residing in South-Eastern Serbia is presented in Table 4.

a. Sodium

Virtually all subjects in the group were with excessive level of sodium intake (above 2 g of daily sodium intake proposed safe with regards to blood pressure levels (WHO, 2012)). Inadequate sodium intake is adversely associated with elevated blood pressure and related cardiovascular events, and reduction and iodination of dietary salt is mandatory.

In a recent large-scale study conducted within 16171 US Hispanics, median usual daily intake of sodium was 2574 mg and 3747 mg in Latino women and men, respectively, indicating somewhat lower intake then in our study group (Elfassy et al., 2019).

We conclude the sodium intake to be inadequate in the group of 40 subjects dwelling in South-Eastern Serbia.

b. Potassium

Potassium intake was above proposed AI values. Only in adolescent group of 15-17 years, mean intake of 2962.57 mg/day was somewhat below proposed 3500 mg/day. Within the adolescent group there were as few as 3 subjects (1 boy and 2 girls), obviously limiting the conclusion. Potassium rich food such as vegetables, fruits, starchy roots or tubers, whole grains, dairy products and coffee is recommended in the group.

In a recent large-scale study conducted within 16171 US Hispanics, median usual daily intake of potassium was 2069 mg and 2649 mg in Latino women and men, respectively, indicating somewhat lower intake then in our study group (Elfassy et al., 2019).

We conclude the potassium intake to be likely optimal in our study group, except for the adolescent group 15-17 years.
c. Calcium

Due to the requirement for adequate bone health, recommended daily calcium intake is additionally stratified according to age limit of 24 years. In our group, 5 subjects of 18-24 years group were with mean daily intake of 1146.75±527.54 mg, while those ≥25 years consumed 1188.38±609.78 mg of calcium daily. Intake of calcium in adult subjects was comparable to previously reported intake in apparently healthy Serbian adults (904.31 ± 342.49 mg/day) (Pantovic et al., 2018).

A systematic review conducted within EURECCA network of excellence indicated lower calcium intake in countries of Central and Eastern Europe, inclusive of Serbia, in comparison with other European countries (Novaković et al., 2013).

Based on AR values, there were 22.5% of subjects with inadequate intake of the nutrient (Table 6). There were no differences in intake level with reference to age group, although only small number of subjects was available within adolescent group. Higher percentage of females inadequately consuming calcium sources was not significantly different from males (p=0.528). Sex-specific data are in line with previously published report with Serbian women being with lower calcium intake in comparison with men (Pantovic et al., 2018).

We conclude calcium intake to be likely inadequate in our study group.

d. Magnesium

Daily mean intake of magnesium was 317.62 mg and 429.90 mg for adolescent and adult group respectively, both higher than respective values for AI of 300 and 350 mg daily defined for males (respective AI for females is even lower).

Recent meta-analyses showed inverse association between dietary magnesium and hypertension (Han et al., 2017) and diabetes mellitus (Fang et al., 2016). Interestingly, the association between serum magnesium concentration and hypertension was not confirmed (Han et al., 2017)

We conclude that magnesium intake in the group is likely adequate.

e. Iron

All subjects in the group were with likely adequate intake of iron (mg/day) across respective age and gender group. This is in line with previously reviewed data (Novaković et al., 2013) indicating adequate
iron status in adults residing in Central and Eastern European countries, inclusive of Serbia, in comparison with other European countries (Novaković et al., 2013).

Mean daily intake in women and men was 28.02±53.05 mg/day and 21.13±10.45 mg/day, indicating high variability of the intake. Both mean levels were higher from respective proposed PRIs for the adult women (16 and 11 mg/day for pre- and post-menopausal women, respectively) and men (11 mg/day).

We conclude the intake of iron in the group to be likely adequate.

f. Phosphorous

Daily intake of phosphorous was above proposed AI of the mineral, across age and gender groups. This is expected since inadequate phosphorous status is seldom due to improper intake, but rather due to metabolic dysfunction. Interestingly, there was a tendency of significantly lower phosphorous intake in women (1383.37±312.62 mg/day), in comparison with men (1696.64.37±646.46 mg/day), in line with previously reported data from our group in healthy men and women from Serbia (Pantovic et al., 2018).

We conclude the intake of phosphorous to be optimal in the studied group.

g. Selenium

In our group, subjects were with mean daily intake exceeding proposed AI values across gender and age categories. Only in female adolescents of 15-17 years of age, intake of selenium was likely suboptimal, with daily average intake of 45±21.21 μg falling below proposed AI for the group (70 μg/day). Small number of subjects in the group (2 girls) once again limits us in drawing conclusions. The same 2 girls in the group of female adolescents were also with seemingly lower intake of potassium. Taken together, the data imply lower intake of fruits and vegetables rich in the minerals, such as banana or peach. Personalized approach is foreseen in resolving the issues with indicative suboptimal intake of micronutrients, to determine food and food groups of interests and the prevalence of their consumption.

Previous data from our group confirm the improvement in selenium plasma status in last 20 years within 142 subjects residing in Eastern Serbia and Belgrade. The improvement is likely associated with imple-
mentation of policies towards enrichment of livestock and poultry feed with selenium (Pavlovic, Miletic, Zekovic, Nikolic, & Glibetic, 2018).

We conclude the intake of selenium to be likely optimal within the group, except for the category of adolescents of 15-17 years of age.

h. Zinc

Assuming mean level of phytate intake of 600 mg/day, mean zinc intake in women and men was 12.12 and 13.86 mg/day, respectively, above respective sex-specific PRIs of 9.3 and 11.7 mg/day. Adolescents of 11-14 years of age, as well as boys of 15-17 years of age were with likely sufficient zinc intake. Once again, only girls in the category of 15-17 years of age were with mean intake of 6.76±0.83 mg of zinc daily, falling below proposed AR of 9.9 mg/day for the group. Previous data indicate level of zinc intake in Serbia as part of Central and Eastern Europe countries, comparable to intake in other European countries (Novaković et al., 2013). The review exploited data from Serbian YUSAD study (Gurinovic, Kadvan, Vukotic, & Nedeljkovic, 2011), reporting mean intake of zinc within men, women, boys and girls, of 15±9, 12±8, 12±8 and 10±7 mg/day, respectively. Our data are comparable to YUSAD data for adult category, however our data indicate slightly lower intake of zinc within both adolescent groups. A comprehensive systematic review, meta-analyses and meta-regression, demonstrated significantly lower intake of zinc among diabetic patients, only if the condition is with associated co-morbidities (Fernández-Cao et al., 2018). However, blood zinc status was lower in diabetic subjects in comparison with healthy controls, regardless of the complications, indicating necessity for adequate biomarkers of the nutrient intake (Fernández-Cao et al., 2018).

Once again, for the two girls, inadequate mineral intake is indicative of improper dietary habits. Assuming mean level of phytate intake of 600 mg/day among adults, estimated inadequacy regarding Zn intake is 15%, with 14.3% among male and 15.4% of inadequate intake among female (p=0.967). The results must be interpreted cautiously, taking into account absorption of the mineral dependent on particular phytate intake, distribution of zinc in the body, and bioavailability from diverse food groups.

We conclude zinc intake to be likely adequate within adults, and likely inadequate within the adolescent category of 15-17 years of age.
Table 4 Intake of microelements in 40 subjects residing in South Eastern Serbia
Таблица 4 Унос микроелемената код 40 испитаника из југоисточне Србије

<table>
<thead>
<tr>
<th></th>
<th>11-14</th>
<th>15-17</th>
<th>≥18</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±SD</td>
<td>Range</td>
<td>Mean±SD</td>
</tr>
<tr>
<td>Sodium, g</td>
<td>3.94±1.84</td>
<td>2.63, 5.24</td>
<td>3.12±0.53</td>
</tr>
<tr>
<td>Potassium, mg</td>
<td>3037.75±581.31</td>
<td>2626.70, 3448.79</td>
<td>2962.57±129.98</td>
</tr>
<tr>
<td>Calcium, mg</td>
<td>1134.59±143.71</td>
<td>1032.97, 1236.21</td>
<td>1049.41±361.54</td>
</tr>
<tr>
<td>Magnesium, mg</td>
<td>339.81±12.37</td>
<td>331.06, 348.55</td>
<td>302.83±54.07</td>
</tr>
<tr>
<td>Iron, mg</td>
<td>11.22±0.95</td>
<td>12.55, 13.89</td>
<td>11.05±2.13</td>
</tr>
<tr>
<td>Phosphorus, mg</td>
<td>1377.91±76.13</td>
<td>1324.07, 1431.74</td>
<td>1287.64±167.91</td>
</tr>
<tr>
<td>Selenium, μg</td>
<td>75.00±21.21</td>
<td>60.00, 90.00</td>
<td>53.33±20.82</td>
</tr>
<tr>
<td>Zinc, mg</td>
<td>11.09±2.72</td>
<td>9.17, 13.01</td>
<td>9.93±5.51</td>
</tr>
</tbody>
</table>

Data are presented as Mean±SD and Range (Minimum, Maximum).

Vitamins

Distribution of mean daily vitamin intake within 40 subjects residing in South-Eastern Serbia is presented in Table 5.

a. Vitamin B1 – Thiamine

Mean intake of thiamine among adult subjects of 0.20±0.11 mg/MJ was comparable to average thiamine intake calculated from 13 dietary surveys of 9 EU countries among adults of 0.11–0.24 mg/MJ (EFSA, 2017).

Apparently, all subjects in the sample were with adequate B1 intake (100%), the latter important for prevention of beri-beri with known cardiovascular and neurological symptoms.

We conclude intake of vitamin B1 to be adequate within the group.

b. Vitamin B2 – Riboflavin

Mean daily riboflavin intake was 1.56±0.43 and 2.20±0.97 mg among adolescents (11-18 years) and adult subjects, respectively. Estimates from 13 surveys within 9 EU countries indicate mean riboflavin intake within following ranges within adolescents and adults: 1.2-2.2 mg/day and 1.4-2.2 mg/day (EFSA, 2017), respectively and comparable to data we obtained. In our study, 15% of subjects were likely to be with inadequate intake of riboflavin, with 14.3% and 15.4% of male and female subjects, respectively (p=0.967).

We conclude intake of riboflavin to be borderline adequate within the group.

c. Vitamin B3 – Niacin

Mean niacin intake within the complete sample (n=40) was 31.21±10.14 mg/day. Our result compares to reported intake among rep-
A representative sample of Spanish subjects (n=2009), with mean daily intake of niacin equivalents of 29.1±0.2 mg/day (Mielgo-Ayuso et al., 2018).

Intake of vitamin B3, calculated as intake of niacin equivalents per mega-joules of total daily intake was seemingly adequate (100%) in the population.

We conclude intake of niacin to be adequate within the group.

d. Vitamin B6 – Pyridoxine

Mean daily intake of vitamin B6 was 2.33±1.13, 2.04±0.75 and 2.49±1.27 mg/day, within complete group (n=40), male and female participants, respectively. Previous data from the representative Spanish sample indicated lower intake of B6 within the Spanish adults in comparison with our group (Mielgo-Ayuso et al., 2018).

Vitamin B6, essential for amino-acid and gluco-lipid metabolism, was likely inadequately consumed in 17.5% of all subjects, out of which 14.3% of males and 19.2% of females.

We conclude intake of pyridoxine to be borderline adequate within the group.

e. Vitamin B9 – Folate

Intake of folate within the total sample was 651.8±1673.36 μg/day, with mean daily intake of 350.54±164.38 and 903.27±2243.69 μg, within men and women, respectively. Mean folate intake previously reported in 503 Serbian women of reproductive age was 211.00±81.06 μg/day (Zekovic et al., 2017), substantially lower then reported herein. Of note, in our study folate intake within women ranged from 136-10908 μg/day, implying possible intake of vitamin B9 in individual women as part of maternity strategies.

According to EAR Cut Point method, 25% of subjects were with likely inadequate folate intake, out of which 42.9% and 15.4% of men and women, respectively (p=0.162). Similar trends were observed across age categories.

We conclude folate intake to be likely inadequate within men and women, except for individual cases, pertaining the necessity of personalized strategies.

f. Vitamin B12 – Cobalamin

Cumulative mean daily cobalamin intake calculated across age groups was high above proposed AI values for the groups. Interestingly, when we calculated the intake across sexes within age category, male
subjects presented with higher cobalamin intake, which tended to be significant in adult subjects (p=0.077). Only intake within girls 15-17 years (mean daily intake=2.36 μg) was below proposed AI level. The same two girls were with likely low intake of potassium, selenium and zinc, underlining the necessity of personalized approach to determine food intake, and potentially inadequate intake of fruits and vegetables, indicative through lower intake of minerals and vitamin.

Higher intake of vitamin B12 is associated with lower coronary heart disease risk (Jayedi & Zargar, 2018), as well as healthy lifestyle in subjects at risk of colorectal cancer (Banjari & Kožić, 2018).

We conclude intake of cobalamin to be likely optimal within the group, except for the adolescent girls.

g. Vitamin C

Daily intake of vitamin C in adults subjects within our group, ranged from 39.53 to 883.49 mg (mean±SD=248.32±192.06 mg/day). Previous work from 13000 Spanish subjects (>40 years) reported mean intake ranging from 148 to 445 mg/day (Martín-Calvo & Martínez-González, 2017). The analysis in Spanish adults reported decreased cardiovascular disease risk with increasing vitamin C intake, which was however confounded by dietary fiber intake for cardiovascular mortality (Martín-Calvo & Martínez-González, 2017). The latter finding indicates importance of discussing food matrices and interactions of containing nutrients, with respect to its healthcare implications. In our study, intake of vitamin C tended to be higher among female subjects, irrespective of age (272.46±184.032 in females vs. 156.62±171.015 in males, p=0.059), with observed high inter-individual variability in the intake. The percentage of inadequate intake was higher in males then females, with 57.1% and 0% of respective subjects with seemingly inadequate intake of the vitamin. In a previous study among 1588 Swedish subjects (≥25 years of age), mean daily vitamin C intake ranged from 33.1-158 mg/day in women, and 27.6-140 mg/day in men, across arbitrary levels of low to high daily intake (Raposo et al., 2017). The results are in line with our data, indicating misbalance in habitual vitamin C intake in favor of women.

We conclude vitamin C to be adequate in female subjects and likely inadequate in male subjects.
h. Vitamin A
In our group, mean intake of vitamin A retinol equivalents was 504.80±172.39 μg/day in adolescents and 814.75±368.748 μg/day in adults. Based on dietary surveys conducted in 9 EU countries, summarized mean intake of vitamins from A group, ranging from 597-1078 μg RE/day in adolescents of 10-18 years, and between 816-1498 μg RE/day in adults (EFSA, 2017), comparable to our results.

Overall, 22.5% of subjects were with seemingly inadequate intake of vitamin A, out of which 28.6% and 19.2% of males and females, respectively (p=0.644).

We conclude the vitamin A to be likely inadequate within the group.

i. Vitamin D
All subjects (range: 0.71-13.99 μg/d) were with vitamin D intake lower to proposed daily adequate intake of 15 μg, irrespective of gender and age. Mean daily intake in women (4.39±3.00 μg) and men (4.18±3.00 μg) was not significantly different (p for trend=0.846). In our previous report, intake of 53 apparently healthy women and 34 men was 3.63 ± 4.90 4.20 ± 4.43 μ/day, comparable to our results (Pantovic et al., 2018). It is unadvisable to comment adequacy of the vitamin D intake, since AR and PRI are not established, limiting the interpretability of empirically derived AI. In addition, vitamin D is extensively synthesized upon UVB radiation, leaving requirement of dietary vitamin D as low as zero with appropriate sun exposure. We are not in the possession of sun exposure data for the study subjects. Necessity of biomarker evaluation together with dietary intake data is obvious for evaluation of nutritional status.

We conclude vitamin D intake to be likely inadequate in study subjects, however direct health implications might not be of concern due to environmentally driven synthesis of the vitamin.

j. Vitamin E
Mean daily intake of vitamin E was 27.61±16.63 mg/day in men and 31.59±14.89 mg/day in women, outweighing proposed AI of 11 and 13 mg/day, indicating likely optimal intake of the anti-oxidant vitamin. Previous work from Swedish cohort, reported lower levels of dietary vitamin E, with mean intake levels ranging from 5.6-11.5 in women, and 5.2-10.2 mg/day in men (Raposo et al., 2017).

We conclude the intake of vitamin E to be likely optimal in the group.
Table 5  Intake of vitamins in 40 subjects residing in South Eastern Serbia

<table>
<thead>
<tr>
<th></th>
<th>11-14 n 2</th>
<th>15-17 n 3</th>
<th>≥18 n 35</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin E, mg&lt;sup&gt;d&lt;/sup&gt;</td>
<td>26.6±21.17</td>
<td>22.0±8.92</td>
<td>30.1±15.44</td>
</tr>
<tr>
<td>Vitamin D, µg</td>
<td>3.7±3.26</td>
<td>3.1±3.09</td>
<td>4.3±2.96</td>
</tr>
<tr>
<td>Vitamin A, µg&lt;sup&gt;b&lt;/sup&gt;</td>
<td>416.6±21.01</td>
<td>348.0±10.0</td>
<td>814.7±36.7</td>
</tr>
<tr>
<td>Vitamin C, mg</td>
<td>74.9±10.15</td>
<td>74.6±23.55</td>
<td>248.3±192.0</td>
</tr>
<tr>
<td>Vitamin B12, µg</td>
<td>16.6±18.30</td>
<td>6.3±7.03</td>
<td>6.0±6.60</td>
</tr>
<tr>
<td>Folate, µg</td>
<td>213.5±9.19</td>
<td>405.3±114.13</td>
<td>697.9±1786.7</td>
</tr>
<tr>
<td>Vitamin B6, mg</td>
<td>2.17±1.03</td>
<td>2.60±1.38</td>
<td>2.32±1.14</td>
</tr>
<tr>
<td>Niacin, mg/MP&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.5±1.40</td>
<td>1.1±1.31</td>
<td>3.0±1.07</td>
</tr>
<tr>
<td>Riboflavin, mg</td>
<td>1.4±0.00</td>
<td>1.6±0.60</td>
<td>2.2±0.97</td>
</tr>
<tr>
<td>Thiamine, mg/MP&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.16±0.06</td>
<td>0.20±0.09</td>
<td>0.20±0.11</td>
</tr>
</tbody>
</table>

Data are presented as Mean±SD and Range (Minimum, Maximum).
<sup>a</sup>Intake is calculated per daily energy intake in Mega Joules.
<sup>b</sup>Intake is expressed as retinol equivalents.
<sup>c</sup>Intake is expressed as niacin equivalents.
<sup>d</sup>Intake is expressed as α-Tocopherol.

Table 6 presents distribution of inadequate intake across genders, based on available DRVs for ARs and indicative gender-specific cut-offs – for calcium, iron, zinc, folate, niacin, riboflavin, thiamine, vitamin A, vitamin B6 and vitamin C.

Table 6  Distribution of inadequate intake of micronutrients across sex and age categories according to established Average Requirements

<table>
<thead>
<tr>
<th></th>
<th>Total, n 40</th>
<th>Sex</th>
<th>p&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Age categories, years</th>
<th>p&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Female, n 26</td>
<td>Male, n 14</td>
<td>11-14, n 2</td>
<td>15-17, n 3</td>
<td>≥18, n 35</td>
</tr>
<tr>
<td>Calcium</td>
<td>22.5 (9)</td>
<td>26.9 (7)</td>
<td>14.3 (2)</td>
<td>0.528</td>
<td>66.7 (2)</td>
</tr>
<tr>
<td>Zinc</td>
<td>15 (6)</td>
<td>15.4 (4)</td>
<td>14.3 (2)</td>
<td>0.967</td>
<td>66.7 (2)</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>15 (6)</td>
<td>15.4 (4)</td>
<td>14.3 (2)</td>
<td>0.967</td>
<td>33.3 (1)</td>
</tr>
<tr>
<td>Vitamin B6</td>
<td>17.5 (5)</td>
<td>19.2 (5)</td>
<td>14.3 (2)</td>
<td>0.812</td>
<td>33.3 (1)</td>
</tr>
<tr>
<td>Folate</td>
<td>25 (10)</td>
<td>15.4 (4)</td>
<td>42.9 (6)</td>
<td>0.162</td>
<td>50.1 (1)</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>20 (8)</td>
<td>0</td>
<td>57.1 (8)</td>
<td>0.003</td>
<td>33.3 (1)</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>22.5 (9)</td>
<td>19.2 (5)</td>
<td>28.6 (4)</td>
<td>0.644</td>
<td>33.3 (1)</td>
</tr>
<tr>
<td>Iron</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>n/a</td>
<td>0</td>
</tr>
<tr>
<td>Thiamine</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>n/a</td>
<td>0</td>
</tr>
<tr>
<td>Niacin</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>n/a</td>
<td>0</td>
</tr>
</tbody>
</table>

Data are presented as % of total number in the group, with number of subjects in the group indicated within the brackets.
<sup>a</sup>Mann-Whitney test was applied to analyze if there is different distribution across sex categories.
<sup>b</sup>Kruskal-Wallis test was applied to analyze if there is different distribution across age categories.
Table 7 presents a summary of the estimated adequacy of micronutrient intake in South-Eastern Serbia, based on consumption data for the 40 subjects residing in the area.

Table 7  Estimated adequacy of micronutrient intake in South-Eastern Serbia, based on consumption data for 40 subjects residing in the area

<table>
<thead>
<tr>
<th></th>
<th>Sex</th>
<th>Age categories, years</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Female, n 26</td>
<td>Male, n 14</td>
</tr>
<tr>
<td>Sodium</td>
<td>Inadequate</td>
<td>Inadequate</td>
</tr>
<tr>
<td>Potassium</td>
<td>Optimal</td>
<td>Optimal</td>
</tr>
<tr>
<td>Calcium</td>
<td>Inadequate</td>
<td>Adequate</td>
</tr>
<tr>
<td>Magnesium</td>
<td>Adequate</td>
<td>Adequate</td>
</tr>
<tr>
<td>Iron</td>
<td>Adequate</td>
<td>Adequate</td>
</tr>
<tr>
<td>Phosphorous</td>
<td>Optimal</td>
<td>Optimal</td>
</tr>
<tr>
<td>Selenium</td>
<td>Optimal</td>
<td>Optimal</td>
</tr>
<tr>
<td>Zinc</td>
<td>Adequate</td>
<td>Adequate</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>Optimal</td>
<td>Optimal</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>Inadequate</td>
<td>Inadequate</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>Inadequate</td>
<td>Inadequate</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>Adequate</td>
<td>Inadequate</td>
</tr>
<tr>
<td>Vitamin B12</td>
<td>Optimal</td>
<td>Optimal</td>
</tr>
<tr>
<td>Folate</td>
<td>Inadequate</td>
<td>Inadequate</td>
</tr>
<tr>
<td>Vitamin B6*</td>
<td>Adequate</td>
<td>Adequate</td>
</tr>
<tr>
<td>Niacin</td>
<td>Adequate</td>
<td>Adequate</td>
</tr>
<tr>
<td>Riboflavin*</td>
<td>Adequate</td>
<td>Adequate</td>
</tr>
<tr>
<td>Thiamine</td>
<td>Adequate</td>
<td>Adequate</td>
</tr>
</tbody>
</table>

* Borderline adequate

**Habitual Intake of Food Groups**

Figure 2 represents intake of total calories and macronutrient-specific calories from food groups among 40 analyzed subjects residing in South-Eastern Serbia. According to Figure 2a, the largest proportion of total calories is consumed from grains (26%), dairy and meat (13%) and fat and oil (12%). Proportion of calories consumed from vegetables, fruits and nuts is 7%, 5% and 5%, respectively. The largest proportion of
protein calculated as a percentage of total protein calories is consumed from meat (29%), grains and dairy (18% each), nuts (6%) and eggs (5%) (Fig 2b). The largest proportion of fat calculated as percentage of total fat calories is consumed from fat and oil (30%), meat (22%), dairy (18%) and nuts (8%) (Fig 2c). The largest proportion of carbohydrates calculated as percentage of total carbohydrate calories is consumed from grains (49%), vegetables (14%), fruits (12%) and added sugar (9%) (Fig 2d). When we analyzed the data for 29 adults over 25 years of age, the distributions remained virtually the same (data not shown).

Figure 2  Socio-economic indicators in the three counties of South-Eastern Serbia, relative to the global country indicators (dotted line)

Слика 2  Социоекономски индикатори у три округа југоисточне Србије у односу на свеукупне индикаторе у Републици (тачкаста линија)
Recent data from the Global Burden of Diseases, Injuries, and Risk Factors Study (GBD) 2017, conducted among adults over 25 years of age from 195 countries, indicate substantial fraction of chronic disease mortality and disability-adjusted life years attributable to diet and diet-related risk factors (Afshin et al., 2019). The GBD study indicates low intakes of fruit and whole grains and the highest consumption of sodium as the greatest risk factor related to the mortality and morbidity across the countries. The study indicated mean daily intake of 250 g of fruits, 360 g of vegetables, 125 g of whole grains, 21 g of nuts and seeds and 435 g of dairy as optimal with regards to disease risk and mortality (Afshin et al., 2019). Our subjects consumed mean daily levels of 236.18 g of fruits, 473.24 g of vegetables, 33.38 g of nuts, seeds or kernels and 296.80 g of dairy products, which except for dairy products were all comparable to the optimal levels. Mean daily intake of grains in our subjects is 259.89 g, exceeding proposed optimal levels of whole grain intake at 125 g (Afshin et al., 2019). We are not in the possession of the data regarding intake of whole grains and refined counterparts, limiting the conclusion on the adequacy of grain intake within the 40 subjects. In our study, daily intake of meat within the 40 subjects was 174.04 g, inclusive of all types of meats. According to GBD study, mean daily intake of red and processed meat should not exceed 23 g and 2 g respectively (Afshin et al., 2019), which is substantially below the average intake within the subjects herein analyzed. It is less likely to assume that the remaining mean daily consumed 150 g of meat products could be attributed to poultry solely, implying excessive amounts of red meat consumed within the subjects, with potentially hazardous health implications.

Limitations of the study

Few limitations of the study are of consideration. A cross-sectional approach outcasts causal relationship, and the small sample size limits the generalizability of the results. However, the comprehensive approach and EU Menu Methodology applied in the assessment of the nutritional and dietary status allows for insight into the diet quality within the subjects residing in this area of low socio-economic status.

For some nutrients, only reference intake range corresponding to population-wise intake needs is proposed by regulatory bodies, and the application of the criterion might not be the most appropriate for the
group, due to individual variation in the intake needs. Thus, in the cases where (estimated) average requirement is not proposed, the results were discussed in context of corresponding population-based parameter for optimal intake, proposed by the regulatory body and/or scientific committee.

CONCLUSION

Our report confers the role for initiatives for raising the quality of nutrition in the region of South Eastern Serbia. Due to the low socio-economic status, national measures dedicated to raising education levels on nutrition habits but also agricultural practice might be of detrimental value for sustainable health and healthy aging within the region. Current findings on the suboptimal intake of fibers in the adolescent group, as well as suboptimal intake of micronutrients within girls, indicates the necessity for nutritional education during early ages. School curricula should be upgraded to include sufficient information and educational service on healthy and sustainable food and dietary choices, for long-age living. Our result should further be confirmed in large cohorts and nutritional epidemiology studies. Further prospective research is also needed to establish the involvement of dietary habits and quality in clinical outcomes.

Our data indicate that subjects in the South-Eastern Serbia presumably adhere to the low-carbohydrate-high-fat dietary pattern-like style. Aside from energetic density, fats facilitate absorption of other liposoluble nutrients such as ADKE vitamins and have vital structural, hormonal and regulatory function as well as role in immunological homeostasis. Fat intake below 25% TE is associated with lower levels of certain vitamins in young children (EFSA, 2017). Beneficial effects of low-carbohydrate-high-fat diet are related to weight management strategies, glycemic control and subsequently diabetes management (Brouns, 2018; Noakes & Windt, 2017). Of importance, a cardio-metabolic benefit of pronounced fat intake is proposed if followed by continuing physical activity, and energy restriction from other nutrients, specifically carbs. Protagonists of low-carbohydrate-high-fat diet emphasize the need for prudent food choices, especially type of dietary fat. Higher intake of saturated fats is in our group might be due to unwise intake of red and processed meat as well as dairy products. Controversies remain related to clinical data indicating lower insulin sensitivity and increased cardiovascular risk in subjects on
high-fat diet (Lu, Wan, Yang, Huggins, & Li, 2018). Our group was with seemingly higher intake of saturated fats, and somewhat borderline lower intake of PUFA. Overall, raising awareness on the controversial aspects of differential fats from food, outweighing the stigma on deleterious effects of total fats, together with education on the proper and healthy food choices and patterns, is supported in this region, with observably higher saturated fats to PUFA intake ratio.

The results indicate borderline higher intake of fats at costs of carbohydrates within the region. Although dietary pattern does not appear as hazardous, advancing nutritional habits is desirable. Education and national/regional policies for advancement of dietary quality in South Eastern Serbia should promote complex carbohydrates and relevant food choices such as whole grains, legumes, fruits and vegetables; as well as optimize omega-3 balance through increased intake of fish, seafood, nuts and green vegetables.

Finally, our results underline needs for individualized concept in tailoring dietary advice, as “one size fits all” concept stands out emerging trends in personalized nutrition and health service.

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РЕЗИМЕ

У југоисточној Србији дијетарни обрасци неретко су базирани на калоричној, зачињеној и пикантној храни, као важан фактор прехране али и социјалне интеракције у региону. Додавање соли, пржење и печење на уљу, само су неке од уобичајених кулинарских приступа у овом делу земље. Месо и месне прерађевине су на свакодневном јеловнику. С обзиром на неодвојиву везу социоекономског профилта и навика у исхрани, стратегије јавног здравља и нутритивних политика у југоисточној Србији морају бити усаглашене са специфичним економским контекстом. У оквиру овог рада приказали смо социоекономски статус три округа југоисточне Србије: јабланички, нишки и пиротски, а на основу података и аналитичког сервиса Републичког секретаријата за јавне политике.

Главни циљ ове студије био је анализира нутритивног статуса, у смислу уноса макронутријената, минерала и витамина, у оквиру случајно изабраног узорка 40 људи (10-74 година) са сталним местим пребивалишта у три поменута округа. Даље смо анализирали калоријски допринос различитих извора хране укупном дневном енергетском уносу, као и у односу на појединачне макронутријенте. Користили смо софтверску платформу DIET ASSESS & PLAN, као и националну базу података композиције и конзумације намирница Serbian Food Composition Database, хармонизовану према ЕвроФИР стандардима. Регулатива Европске агенције за безбедност хране, Светске здравствене организације и других релевантних тела коришћена је за евалуацију уноса појединачних нутријената.

У оквиру јабланичког, нишавског и пиротског региона, 5 од 6, 6 од 12, односно 3 од 4 јединица самоуправе биле су нивоа развијености који је испод 60% републичког просека, указујући на значајно низак ниво економске развијености у региону, потенцијално повезане са малнутрицијом и неадекватним дијетарним навикама.

Ради анализе нутритивног статуса, укључено је 40 испитаника, од којих 14 мушког и 26 женског пола, затим 5 адолесцената (11-18 година) и 35 одраслих (старијих од 18 година). Међу адолесценти-
ма био је један дечак и 4 девојчице, док је међу одраслима било 13 мушкираца и 22 жене. У целој групи било је 16 испитаника са присуством хроничне болести или стања, а њих 13 је било на хроничној фармаколошкој медикацији.

Анализа нутритивног статуса на групном нивоу указала је на дијетарни образац који тежи нижем уносу угљених хидрата (опсег уноса 26.46-56.22% укупног дневног енергетског уноса) и значајнијем уносу масти (средња вредност укупног дневног енергетског уноса више од 40%, независно од пола и старосних група). Наши подаци указују и на могућ субоптималан унос дијетних влакана у региону. Наши резултати остављају простор за подизање нивоа знања међу становницима региона, а везано за нутритивне навике, специфично значај диференцијалних група масти и повећања конзумације дуголанчаних незасићених масти на рачун засићених масти из хране, ради бенефитних здравствених импликација. Процењивање хране богате омега-3 масним киселинама као што је риба, коштуњаво воће или лиснато зелено поврће, као и унос сложених угљених хидрата првенство дијетних влакана, основа су за побољшање концепта регионалних нутритивних политика.

Даља анализа показала је да стратегије побољшања квалитета исхрane међу испитаницима који живе у југоисточној Србији треба усмерити на оптимизацију уноса следећих микронутријената: натријума, калијума, затим витамина B2 (рибофлавин), B6 (пиридоксин), B9 (фолата), витамина D, као и витамина C специфично у популацији женског пола. У групи адолесцената 15-17 година индикован је неодговарајући унос цинка, калијума, селена и витамина B12 (кобаламина). Персонализоване политике морају узети у обзир и детаљну клиничку слику и историју болести, а ради финале концептуализације индивидуалног нутритивног, дијетарног плана и плана оброка. Унос фосфора, магнезијума, гвожђа, витамина B1 и B3 (ниацин), цинка међу одраслима, затим витамина Е, показао се као одговарајући међу испитаницима из југоисточне Србије.

Резултати студије Global Burden of Diseases, Injuries, and Risk Factors Study међу испитаницима старијим од 25 година у 195 земаља указују на следеће дневне количине конзумације као оптималне за здравље: 250g воћа, 360g поврћа, 125g житарица целог зрна, 21g коштуњавих плодова, 435g млечних производа. Поредивши са претходним, у нашој студији испитаници су конзумирали оптимал-
не нивое, изузев млечних производа који су у количини од 296.80g дневно били испод препорученог нивоа. Наши испитаници су дневно конзумирали 259.89g укупних житарица, што далеко превазилази препоручени ниво житарица целог зрна, вероватно указујући на висок ниво уноса рафинисаних угљених хидрата. Слично је и са уносом меса који је у нашој групи износио 174.04g дневно, укључујући све врсте меса и месних производа, што далеко превазилази препоручених 23g белог меса и 2g месних прерађевина дневно. Индикованих просечно 150g повишених дневних уноса меса и месних прерађевина, треба да се узму у обзир приликом креирања дијетарних препорука у региону.

Наши резултати указују на потребу за иницијативама ка побољшању квалитета исхране у региону југоисточне Србије. С обзиром на низак социоекономски статус, националне мере у смеру едукације о нутритивним навикама али и агропривредне праксе могу бити од кључне вредности за одрживи развој и очување здравља у региону. Индиковани неадекватан унос минерала у групи адолесценткиња указује и на потребу ревидирања школских програма и подизања нивоа основног образовања о значају квалитета исхране и савесних избора хране за здравље и дуг живот.

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