# Mortality from malignant neoplasm, ischemic heart disease and diabetes mellitus and per capita food consumption in Serbia; ecological study

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Mortality from malignant neoplasm, ischemic heart disease and diabetes mellitus and per capita food consumption in Serbia; ecological study

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ABSTRACT

Objectives: This paper investigates correlation between mortality from malignant neoplasm, ischemic heart disease and diabetes mellitus, and the consumption of food-groups in Serbia.

Design: Ecological study was applied in this work. Pearson correlation was performed to examine association between mortality rates and per capita food consumption. The Survey covered the whole territory of Serbia: from the total number of households foreseen for survey (nationally representative sample of 4800 households), 85% and over were actually surveyed.

Results: In Serbia, markedly increasing trends of malignant neoplasm, ischemic heart disease, and diabetes mellitus mortality rates were observed in the 1991-2010 period. These mortality trends were positively correlated with consumption of eggs ($r = +0.890$, $+0.544$ and $+0.680$, respectively), and negatively correlated with consumption of animal fat ($r = -0.892$, $-0.473$ and $-0.501$, respectively) and hard drinks ($r = -0.974$, $-0.519$ and $-0.691$, respectively). Beef and wine consumption were inversely correlated with cancer and ischemic heart disease mortality. Milk consumption correlated inversely only with cancer mortality, while beer intake correlated positively only with mortality from diabetes mellitus.

Conclusions: Correlations between unfavourable mortality trends from malignant neoplasm, ischemic heart disease and diabetes mellitus and food consumption were observed and should be assessed in future analytical epidemiological studies. Diet-related health promotion, an initiative that gave results in other countries, is sorely needed in Serbia.

Key words: Malignant neoplasm, ischemic heart disease, and diabetes mellitus mortality trend; Per capita food consumption; Ecological study.
BACKGROUND

Most common causes of death in developed countries are malignant neoplasm, ischemic heart disease and diabetes mellitus. The top two causes, heart disease and malignant neoplasm, accounted for 47.5% of all deaths in the United States of America in 2010. The cardiovascular diseases are the main cause of death in the European Union: they account for 42 per cent of all deaths in the total population. Diabetes was the underlying cause in 2.8% of all deaths in the United States of America in 2010. Worldwide, diabetes caused 1.4 million deaths (2.6% of all causes) in 2011, up from 1.0 million (1.9%) deaths in 2000.

In the last two decades, cardiovascular mortality declined steeply in the European Union and United States of America, due to decreases in both the ischemic heart disease and stroke mortality. Since 1990s, decreased mortality trend from all types of cancer was reported in the USA. In contrast, death rates from diabetes mellitus in the same time interval increased, yielding a net annual increase of 1.9%. Many predictions indicate a growing burden of diabetes, particularly in developing countries. In some European countries, most migrant groups (particularly migrants from North Africa, South Asia or countries with low gross domestic product) have higher diabetes mortality rates than the local-born populations. Mortality varying by place and population has always been used to generate hypotheses on causation. Many epidemiological studies have identified correlation between mortality trends from cancer, coronary heart disease and diabetes mellitus, and food consumption.

Low rates of cancer and ischemic heart disease in Southern European countries (Greece and Italy in particular) are traditionally explained by the ‘Mediterranean diet’. ‘Mediterranean diet’ means high consumption of olive oil, legumes, unrefined cereals, fruit and vegetables, moderate consumption of dairy products (mostly cheese and yogurt), fish and wine, and low consumption of meat. Moderate consumption of alcohol, together with other subtly beneficial lifestyles, was associated with 22% lower risk of death. In Latin America, the per capita supply of milk showed a strong positive correlation with incidence of childhood type 1 diabetes mellitus. Inverse associations were observed for eggs and coffee consumption in several studies, while other studies did not confirm such findings.

Since 1990s, cardiovascular disease and malignant neoplasm became the first and second most prevalent causes of death in Serbia, accounting for upward of 55% and 20% of all deaths, respectively. In the same period, diabetes mellitus represented about 3% of all deaths. These three causes of death together accounted for almost 80% of the total mortality in Serbia, with increasing tendency. There is scant information regarding the impact of diet on mortality in Serbia. The purpose of this paper is to analyse the time trend in decreases from malignant neoplasm, ischemic heart disease and diabetes mellitus in Serbia in correlation to changes in per capita consumption of foodstuffs and alcohol.
MATERIAL AND METHODS

Data source

Annual data on the number of deaths due to malignant neoplasm – codes 140-208 in the 9th and codes C00-C97 in the 10th revision of the International Classification of Diseases, ischemic heart diseases – codes 410-414 in the 9th and codes I20-I25 in the 10th revision, and diabetes mellitus – code 250 in the 9th and codes E10-E14 the 10th revision – were obtained from the mortality statistics published by the National Statistical Office of the Republic of Serbia for the 1991-2010 period (unpublished data). Data about Serbian population were extracted from demographic statistics database from the Statistical Office of the Republic of Serbia. Data for the Republic of Serbia, without the Autonomous Province of Kosovo and Metohia, for which data are unavailable since 1998, were presented in this paper. The Serbian population data were provided on the basis of official censuses in 1991 and 2002, while for inter-census years the estimates of the resident population were obtained from the state Statistical Office database.

Measures

Age-standardized mortality rates were calculated using the World standard population, by the method of direct standardisation. Rates are expressed as deaths per 100,000 persons. Linear trend model was used to examine trend of mortality from the malignant neoplasm, ischemic heart disease, and diabetes mellitus.

The data on per capita food and alcohol consumption were obtained by the Household Budget Survey. The Household Budget Survey is conducted annually, and harmonised with the international standards and recommendations, which provide for an international comparison of data. According to the sample plan, every chosen household is the survey unit. The Survey covered the whole territory of Serbia: from the total number of households foreseen for survey (nationally representative sample of 4800 households), 85% and over were actually surveyed. Diary and interview method based on questionnaires have been applied in the Survey. The data were collected for the entire observed year (1 January until 31 December, inclusive), thereby taking into account effects seasonal variations have on the diet. Each household was recorded for two weeks. Data quality assessment by phone was performed on a random sample (10% of surveyed households) in each even quarter during the year. For a few years, data were missing for one or more food items. No extrapolation was made for missing data. Nine different foods or food-groups were selected for comparison, including animal and vegetable fat, red meat, beef, pork, poultry, total meat, dried and processed meat, fish, eggs, milk, cheese, yogurt, coffee, beer, wine, hard drinks, fruit and vegetables.

Statistical analysis

Correlation coefficients were calculated for the variables of food consumption and adjusted mortality rates for the same year. Two-sided $P$ values were reported and considered to indicate
statistical significance when lesser than 0.05. All statistical analyses were conducted using the Statistical Package for Social Sciences software (SPSS Inc., version 19.0, Chicago, IL).

RESULTS

In Serbia (without the Autonomous Province of Kosovo and Metohia), the markedly increasing trends in mortality rates from malignant neoplasm \( y = 119.8x + 1.2, P < 0.000 \), ischemic heart disease \( y = 68.8x + 0.6, P = 0.025 \), and diabetes mellitus \( y = 14.1x + 0.1, P = 0.001 \) were observed during the 1991-2010 period (Figure 1).

Pearson correlation shows that mortality trend for malignant neoplasm had a statistically significant positive correlation with mortality from ischemic heart disease and diabetes mellitus (Table 1). There was a lack of correlation between ischemic heart disease and diabetes mellitus.

Pearson’s correlation coefficients between age-adjusted mortality rates and per capita food consumption are shown in Table 3. The mortality trends for malignant neoplasm, ischemic heart disease, and diabetes mellitus were positively correlated with eggs consumption \( r = +0.890, +0.544, \) and \(+0.680, \) respectively), and negatively correlated with animal fat \( r = -0.892, -0.473 \) and \(-0.501, \) respectively) and hard drinks consumption \( r = -0.974, -0.519 \) and \(-0.691, \) respectively). Consumption of beer was correlated only with diabetes mellitus mortality rates \( r = +0.600 \). Milk consumption negatively correlated only with overall cancer mortality trend \( r = -0.695 \). Per capita consumption of pork and cheese was not correlated with any of the observed mortality trends.

Additionally, in the observed period in Serbia, a positive correlation between the overall cancer mortality trend and consumption of vegetable fat, poultry, total meat, dried and processed meat, fish, yogurt, coffee, fruit and vegetables was demonstrated \( r = +0.674, +0.895, +0.461, +0.898, +0.861, +0.894, +0.558, +0.760 \) and \(+0.749, \) respectively). Further, beef, milk and wine consumption showed a highly significant negative relationship with the overall cancer mortality rates \( r = -0.740, -0.695 \) and \(-0.571, \) respectively). Also, no association was found for consumption of red meat and beer.

In addition, negative correlations between consumption of read meat \( r = -0.560 \), beef \( r = -0.565 \) and wine \( r = -0.655 \) and mortality trend from ischemic heart disease were found in Serbia. Also, no association was found for consumption of vegetable fat, poultry, total meat, dried and processed meat, fish, milk, yogurt, coffee, beer, fruit and vegetables.

Notwithstanding, consumption of vegetable fat, poultry, total meat, dried and processed meat, fish, yogurt, coffee, fruits and vegetables was positively correlated with mortality from diabetes mellitus \( r = +0.606, +0.748, +0.491, +0.701, +0.612, +0.622, +0.516, +0.737, +0.723, \) respectively).

Also, no association was found for per capita consumption of red meat, beef, milk and wine.

DISCUSSION

We assessed ecological correlations between per capita food consumption and mortality trends of malignant neoplasm, ischemic heart disease, and diabetes mellitus in Serbia in the last two
decades. Increasing trends of mortality rates from malignant neoplasm, ischemic heart disease, and diabetes mellitus were positively correlated with eggs consumption, and negatively correlated with animal fat and hard drinks intake. For all selected mortality trends, the most consistent association was inverse correlation with hard drinks consumption. Certain foods are considered associated with the selected diseases.

During the two last decades, number of deaths from cancer, ischemic heart disease and diabetes mellitus in Serbia increased. Although mortality from coronary heart diseases have continued to decline in the European Union (27 countries), the USA, Australia, Japan, and most Latin American countries, unfavourable trends are still observed in the Russian Federation and other countries of the former Soviet Union (Ukraine, Belarus, etc.). In Western Europe, cancer mortality has steadily declined since the late 1980s, but in central/eastern Europe mortality from major cancer sites has been increasing up to the late 1990s/early 2000s. Diabetes mellitus accounted for 6% of adult deaths in the African Region, and 15.7% in the North American Region, where diabetes is a major cause of premature mortality. Diabetes mellitus prevalence increases, particularly in low- and middle-income countries. These unfavourable trends are coincident with dramatic socioeconomic changes that occurred in developed countries, especially in the former communist countries. Changes in prevalence of risk factors may have played an important role in the observed increase in mortality (i.e. smoking, obesity, etc.). Transition to the market economy had a particularly pertinent role in the changes of dietary pattern, including increases in the percentage of the population eating away from home (particularly at fast-food restaurants), larger portion sizes of foods and beverages, increased consumption of sweets, etc.

A significant positive correlation between overall cancer mortality rates and mortality rates for ischemic heart disease and diabetes mellitus may indicate their reciprocal aetiology. Diet plays an important role in the occurrence of these diseases, as indicated in a number of epidemiological studies.

The correlation between per capita food consumption and rates of mortality from cancer, ischemic heart disease and diabetes mellitus has been found to vary in significance and magnitude across countries. The most consistent link, found in a number of environmental studies, was between mortality trend and animal fat consumption, but was confirmed neither in our nor in some other studies. Among eighteen European countries, total mortality was negatively, albeit non-significantly, correlated with fish and seafood (r = -0.47), and olive oil consumption (r = -0.44). Naska et al also showed the expected inverse associations between coronary mortality and consumption of olive oil (r = -0.85) and fish and seafood (r = -0.59), and inverse correlation of total cancer mortality and fish and seafood (r = -0.63) consumption. In The Nurses’ Health Study, significant inverse associations were observed between nut consumption and deaths due to cancer and heart disease, but not significantly for diabetes mellitus death. Increased consumption of palm oil
and other oil-crops is related to higher ischemic heart disease mortality rates in developing countries.\textsuperscript{15, 25} In the 10 DAFNE countries, coronary heart disease mortality was not significantly correlated with per capita consumption of read meat, fish, milk and dairy products, eggs and ethanol; for these foodstuffs, correlations with mortality from breast and colon cancer, diseases with established nutritional aetiology, have not been confirmed.\textsuperscript{30} Secular mortality trend from colorectal cancer in Spain has been positively correlated with consumption of red meat, poultry, fish, vegetables and fruit.\textsuperscript{27} In Latin America and the Caribbean countries, statistically significant positive correlation was found between fish \((r = 0.411)\) and cardiovascular mortality.\textsuperscript{15} Analysis of the 20-year coronary heart mortality in Finland, Italy and the Netherlands showed no association with total fish consumption.\textsuperscript{31} Similarly, fish consumption was not significantly associated with mortality from ischemic heart disease in Serbia; significant positive correlations were found between fish consumption and mortality from cancer and diabetes mellitus.

A positive relationship between mortality from coronary heart disease and eggs consumption was observed in study involving 40 countries,\textsuperscript{32} in contrast to the results in some other studies.\textsuperscript{15} According to our results, eggs were the only dietary item that showed harmful effect on the heart. This probable effect has been related to specific micronutrients contained in eggs.

Our finding of a strongly inverse effect of hard drinks consumption on total cancer and diabetes mellitus mortality is similar to the results from developing countries.\textsuperscript{15} A weaker inverse correlation between ischemic heart disease and hard drinks consumption in Serbia is contrary to the results obtained from the study in 40 countries.\textsuperscript{32} But, following adjustment for cholesterol and saturated fat, alcohol was no longer correlated to coronary heart mortality. In the same study, the differences for coronary heart mortality between France and Finland suggested possible effects of other dietary factors, primarily milk consumption.

Positive correlation between consumption of fruit and mortality from cancer and ischemic heart disease, noticed in Switzerland and Spain, could only be confirmed for ischemic heart disease in our study.\textsuperscript{27, 28} In Serbia, consumption of vegetable oil, poultry, dried meat and processed meat items, coffee and yogurt had a positive correlation with mortality rates for cancer and diabetes mellitus. In similar fashion to dairy products, meat and fruit positively correlated with the incidence of common cancers in Iran,\textsuperscript{33} while in certain countries a significant positive correlation of coronary heart disease mortality with milk and its products was observed.\textsuperscript{32, 34} Some studies have produced different findings.\textsuperscript{26, 35} Dairy products may contribute to the composition of saturated fatty acids in the diet, and thus may explain the correlation. Per capita consumption of pork and cheese had weak inverse association with ischemic heart disease mortality and weak positive association with cancer and diabetes mellitus mortality in Serbia. Contrary findings were obtained in some studies,\textsuperscript{25} but not all of them.\textsuperscript{32}

**Strengths and limitations of the study**
This study is the first to document a relationship between increased mortality from malignant
neoplasm, ischemic heart disease and diabetes mellitus, and per capita food consumption in Serbia.

Ecological correlational studies, which can be done quickly and inexpensively, may be useful
in the generation of hypotheses concerning the dietary aetiology of various chronic diseases, thanks
to comparison of different populations, or within the same population between different periods. The
longitudinal nature of our national-level data enabled us to analyse the link between mortality trends
and per capita foodstuffs consumption, as well as comparison with other countries. The Serbian
mortality statistics data is considered comprehensive and reliable according to the WHO standards.\textsuperscript{36}
Also, the Household Budget Survey in Serbia was harmonised with the international standards and
recommendations, and enabled international comparison of data.

The same correlations were present in our ecological study, which had but one major
limitation: lack of directly measured data on average individual dietary consumption. Also, data on
other risk factors (including per capita consumption of cigarettes, obesity, occupation, level of
education, etc.) were not available in Serbia for the time period considered. Certainly, some of the
differences found in international comparisons may be partially attributed to differences in
methodology applied in various studies. Some measurement errors are inevitable, because food
consumption was self-reported. Unmeasured or poorly measured foodstuffs and lack of measures for
important potential confounders can be a major source of bias in an ecological research.

In conclusion, correlations between mortality trends for malignant neoplasm, ischemic heart
disease and diabetes mellitus, and dietary habits in Serbia were observed and further effort is needed
in order to investigate a possible causative association, using epidemiological analytical studies.

Acknowledgement

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Contract No. 175042.

Competing interests

All authors declare: no financial or non-financial interests that may be relevant to the submitted
work.

Contributors: MI conceived and designed the study, took part in data collection, data analysis,
interpretation of the results, and manuscript preparation, editing, and review. ZZ, DL, VP, GS and
GDJ performed data analysis and manuscript editing and review. All authors took part in editing of
the final version and gave approval for submission.
REFERENCES


Figure 1.
Trend for malignant neoplasm, ischemic heart disease and diabetes mellitus mortality rates in Serbia, excluding the Autonomous Province of Kosovo and Metohia, 1991-2010

\[ Y_{\text{Malignant neoplasm}} = 119.8x + 1.2; \ P < 0.000 \]

\[ Y_{\text{Ischemic heart disease}} = 68.8x + 0.6; \ P = 0.025 \]

\[ Y_{\text{Diabetes mellitus}} = 14.1x + 0.1; \ P = 0.001 \]

ASR (W) = Age Standardized Rate (per 100,000, using world standard population)
Table 1

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<th>Ischemic heart disease</th>
<th>Diabetes mellitus</th>
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<td>Malignant neoplasm</td>
<td>0.550*</td>
<td>0.698**</td>
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<tr>
<td>Ischemic heart disease</td>
<td></td>
<td></td>
<td>0.199</td>
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<td>Diabetes mellitus</td>
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* p<0.01; ** p<0.001.
Table 2

Pearson’s correlation coefficients between *per capita* food consumption and malignant neoplasm, ischemic heart disease, and diabetes mellitus mortality rates in Serbia (without Autonomous Province Kosovo and Metohia), 1991-2010

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<td><strong>Fat</strong></td>
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<tr>
<td>Animal</td>
<td>-0.892**</td>
<td>-0.473*</td>
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<td>Vegetable</td>
<td>0.674**</td>
<td>-0.092</td>
<td>0.606**</td>
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<td><strong>Meat</strong></td>
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<tr>
<td>Red meat</td>
<td>-0.277</td>
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<td>-0.065</td>
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<td>Beef</td>
<td>-0.740**</td>
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<td>-0.363</td>
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<td>Pork</td>
<td>0.147</td>
<td>-0.346</td>
<td>0.164</td>
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<td>Poultry</td>
<td>0.895**</td>
<td>0.314</td>
<td>0.748**</td>
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<tr>
<td>Total meat</td>
<td>0.461*</td>
<td>-0.131</td>
<td>0.491*</td>
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<td>Dried and processed</td>
<td>0.898**</td>
<td>0.241</td>
<td>0.701**</td>
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<td><strong>Fish</strong></td>
<td>0.861**</td>
<td>0.349</td>
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<td><strong>Eggs</strong></td>
<td>0.890**</td>
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<td>Milk</td>
<td>-0.695**</td>
<td>-0.168</td>
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<td>Cheese</td>
<td>0.100</td>
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<td>Yogurt</td>
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<td><strong>Coffee</strong></td>
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<td>Hard drinks</td>
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<td>-0.691**</td>
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<td><strong>Fruits</strong></td>
<td>0.760**</td>
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<tr>
<td><strong>Vegetables</strong></td>
<td>0.749**</td>
<td>0.265</td>
<td>0.723**</td>
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* 0.01<p<0.05; ** p<0.01.
**Association of the consumption of common food groups and beverages with mortality from cancer, ischemic heart disease and diabetes mellitus in Serbia, 1991-2010; ecological study**

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Association of the consumption of common food groups and beverages with mortality from cancer, ischemic heart disease and diabetes mellitus in Serbia, 1991-2010; ecological study

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ABSTRACT

Objectives: This paper reports association between mortality rates from cancer, ischemic heart disease and diabetes mellitus, and the consumption of common food groups and beverages in Serbia.

Design: In this ecological study data on both mortality and the average annual consumption of common food groups and beverages per household's member were obtained from official data-collection sources. The multivariate linear regression analysis was used to determine the strength of the associations between consumption of common food groups and beverages and mortality rates.

Results: Markedly increasing trends of cancer, ischemic heart disease, and diabetes mellitus mortality rates were observed in Serbia in the 1991-2010 period. Mortality rates from cancer were negatively associated with consumption of vegetable oil ($P = 0.005$) and grains ($0.001$) and same was found for ischemic heart disease ($P = 0.002$ and $0.021$, respectively), while consumption of other dairy products showed a significant positive association ($P = 0.000$ and $0.032$, respectively). Both in men and women, mortality rates from diabetes mellitus showed a significant positive association with consumption of poultry ($P = 0.014$ and $0.004$, respectively). Consumption of beef and grains showed a significant negative association with cancer mortality rates in both genders ($P = 0.002$ and $0.000$ in men and $P = 0.000$ and $0.014$ in women, respectively), while consumption of cheese was negatively associated only in men ($P = 0.000$). Mortality rates of diabetes mellitus showed a significant positive association with consumption of animal fat and other dairy products only in women ($P = 0.003$ and $0.046$, respectively).

Conclusions: Association between unfavourable mortality rates from cancer, ischemic heart disease and diabetes mellitus and common food groups and beverages consumption was observed and should be assessed in future analytical epidemiological studies. Promotion of healthy diet is sorely needed in Serbia.

Key words: Cancer; Ischemic heart disease; Diabetes mellitus; Mortality trend; Food groups and beverages consumption; Ecological study.
Strengths and limitations of this study

- To the best of our knowledge, this population-based ecological study is the first to document a relationship between mortality from cancer, ischemic heart disease and diabetes mellitus, and consumption of food and beverages in Serbia.

- Mortality rates from cancer, ischemic heart disease and diabetes mellitus in Serbia have increased during the last two decades, and Serbia tops the list of European countries with highest mortality rates.

- We found that the consumption of beef and grains was negatively associated with mortality rates from cancer in both genders, while consumption of other dairy products showed a significant positive association. Consumption of vegetable oil showed a significant negative association with mortality rates from ischemic heart disease in both genders, while consumption of beer was positively associated. Association between consumption of poultry and mortality rates from diabetes mellitus was significantly positive both in men and women.

- The inability to fully control confounding factors in assessing relationships between mortality and consumption of food and beverages in Serbia is the limitation of this study.

- Findings have important implications for promotion of healthy diet in Serbia.
BACKGROUND

Most common causes of death in developed countries are cancer, ischemic heart disease and diabetes mellitus. The top two causes, heart disease and cancer, accounted for 47.5% of all deaths in the United States of America in 2010. The cardiovascular diseases are the main cause of death in the European Union: they account for 42 per cent of all deaths in the total population. Diabetes was the underlying cause in 2.8% of all deaths in the United States of America in 2010. Worldwide, diabetes caused 1.4 million deaths (2.6% of all causes) in 2011, up from 1.0 million (1.9%) deaths in 2000.

In the last two decades, cardiovascular mortality declined steeply in the European Union and United States of America, due to decreases in both the ischemic heart disease and stroke mortality. Since 1990s, decreased mortality trend from all types of cancer was reported in the USA. In contrast, death rates from diabetes mellitus in the same time interval increased, yielding a net annual increase of 1.9%. Many predictions indicate a growing burden of diabetes, particularly in developing countries. In some European countries, most migrant groups (particularly migrants from North Africa, South Asia or countries with low gross domestic product) have higher diabetes mortality rates than the local-born populations. Mortality varying by place and population has always been used to generate hypotheses on causation. Many epidemiological studies have identified correlation between mortality trends from cancer, coronary heart disease and diabetes mellitus, and food consumption.

Low rates of cancer and ischemic heart disease in Southern European countries (Greece and Italy in particular) are traditionally explained by the ‘Mediterranean diet’. ‘Mediterranean diet’ means high consumption of olive oil, legumes, unrefined cereals, fruit and vegetables, moderate consumption of dairy products (mostly cheese and yogurt), fish and wine, and low consumption of meat. Moderate consumption of alcohol, together with other subtly beneficial lifestyles, was associated with 22% lower risk of death. In Latin America, the per capita supply of milk showed a strong positive correlation with incidence of childhood type 1 diabetes mellitus. Inverse associations were observed for eggs and coffee consumption in several studies, while other studies did not confirm such findings.

Serbia (officially the Republic of Serbia) is a small country in southeast Europe: according to World Bank estimates, Serbia today is an upper-middle income country with dominant service sector, followed by the industry and agriculture. But, Serbia has experienced dramatic changes over the past decades: in addition to civil wars and the dissolution of the former Yugoslavia in the 1990s, the United Nations sanctions and trade embargo in 1992, the political isolation and the collapse of the economy in 1993, NATO bombing in the 1999, and democratic socio-economic changes in 2000s, the Kosovo’s unilateral declaration of independence in 2008, and transition to market economy and financial crisis marked the beginning of the century. Since the 1990s, Serbia has the largest
refugee population in Europe - about half a million refugees and internally displaced persons sought refuge in the country following the series of Yugoslav wars. As of 2002 census, Serbs are the largest ethnic group in Serbia (representing 83% of the total population), but another 37 ethnicities also live on its territory (including Hungarians that are the largest ethnic minority in Serbia and representing 3.9% of the country's population, followed by Bosniaks and Roma with a similar share of around 2%, etc). Serbian cuisine is largely heterogeneous and includes characteristics of the Balkans, the Mediterranean, Turkish and Hungarian cuisines. Slivovitz, a highly alcoholic drink, is a famous rakia which is considered the national drink of Serbia.

The prominent immediate effect of wars and disintegration of the Serbia was significantly increased mortality from cancer and ischemic heart diseases during the last two decades.\textsuperscript{18-20} Serbia tops the list of European countries with highest cancer mortality rates.\textsuperscript{18, 19} Since 1990s, cardiovascular disease and cancer became the first and second most prevalent causes of death in Serbia, accounting for upward of 55% and 20% of all deaths, respectively.\textsuperscript{21, 22} In the same period, diabetes mellitus represented the cause for 3\% of all deaths. These three causes of death together accounted for almost 80\% of the total mortality in Serbia, with increasing tendency. There is scant information regarding the impact of diet on mortality in Serbia. This paper aimed to assess the time trend in mortality from most common diseases in Serbia, and to analyse the relationship between consumption of common food groups and beverages and mortality rates.

**MATERIAL AND METHODS**

**Data source**

In this ecological study data on both health outcome (mortality from cancer, ischemic heart disease and diabetes mellitus) and the exposure (the average annual consumption of common food groups and beverages per household's member) were obtained from official data-collection sources - the National Statistical Office of the Republic of Serbia.\textsuperscript{21}

Data on persons who died of cancer (site codes 140-208 revision 9 and codes C00-C97 revision 10 of the International Classification of Diseases - ICD), ischemic heart diseases (ICD-9 codes 410-414 and ICD-10 codes I20-I25) and diabetes mellitus (ICD-9 code 250 and ICD-10 codes E10-E14) were obtained from the National Statistical Office of the Republic of Serbia (unpublished data). Causes of death in Serbia are available from 1997 according to the ICD-10, and from the previous period according to ICD-9.

Data on causes of death are collected through the Death Certificate Form (DEM-2) (Official Gazette SR Serbia No. 8/2005). In Serbia, determination of death and cause of death are the responsibility of the physician. Death data have been collected and submitted by registrars. Data on the deceased and on the cause of death are collected routinely for the entire territory of Serbia. All public hospitals are obliged by law to send individual hospitalization reports to regional institutes of public health which aggregate data and send a report to Institute of Public Health of Serbia. Mortality
statistics includes all (100%) death occurrences in Serbia. The World Health Organization assessed the quality of the official mortality statistics in Serbia as medium-quality (criterion used: completeness reporting is >90% and ill-defined causes and injury deaths with undetermined intent appear on <10% of registrations).23

The study comprised the entire population of the Republic of Serbia, during the period 1991-2010. Data for 1998-2010 are not available for the Autonomous Province of Kosovo and Metohia, which declared itself independent in 2008. Data on the number and composition of the Serbian population by gender and age were presented according to 1991 and 2002 censuses, while for inter-census years the estimates of the resident population were obtained from the state Statistical Office database. The analysis was conducted on the entire Serbian population (approximately 7.5 million inhabitants).21 Since 1990s, Serbia had the largest internally displaced persons and refugee population in Europe (formed between 7% and 7.5% of its population) for which data were included in the Serbian population and could not be set aside as a special contingent.

The study was conducted between June 2013 and September 2014. The cancer, ischemic heart disease and diabetes mellitus mortality was estimated for the period 1991-2010.

The National Statistical Office of the Republic of Serbia has conducted survey of household and individual food and beverages consumption in Serbia - the Household Budget Survey.24 The Household Budget Survey in Serbia is conducted annually since 1960s, and harmonised with the international standards and recommendations (Eurostat, International Labour Organization and the United Nations) from 2003, which provides grounds for an international comparison of data. The Household Budget Survey covered the whole territory of the Republic of Serbia. The household, either it is multi-member (a community of persons whose members live together in the same dwelling unit and spend realized income jointly) or one-member (single person, living alone and spending its own income), chosen according to the sample plan was a survey unit. Each year the survey comprises a nationally representative sample of non-institutionalized persons residing in Serbia. The sample is a two-stage, stratified sample. The first stage units are enumeration districts and the second stage units are households. Sample selection was carried out by selecting the first stage units (enumeration districts) in proportion to the number of households, and the second stage units (households) were selected with equal probability (simple random selection). According to the EUROSTAT recommendations, total number of households predicted for surveying is 4800 for the Republic of Serbia. Also, as recommended by EUROSTAT, out of the total number of households envisaged for the survey, the lowest percentage of households that must be surveyed is 85% (which was achieved for each year). Given that a household in Serbia consists an average of 3 members, the household budget survey includes about 13500-14500 inhabitants. Data are collected for the whole observed year (from January 1 to December 31). Every fifteen days, 200 households have been chosen to keep a diary on personal consumption of food and beverages (thereby taking into account
effects seasonal variations have on the diet). Diary method has been applied in this survey: each surveyed household keeps a diary (statistical form D-1) for the reference period. The survey collects data on household consumption, i.e. data on basic elements of individual consumption. The substitution of households is not predicted. Diary of the consumed quantities of food and beverages in every household was kept by a chosen person (it was the person responsible for food in the house, most often it was a housewife). Specially trained surveyors visit the households at least three times in purpose of helping with diary.

**Measures**

Three types of death rates were calculated: crude, specific (age- and sex-specific) and age-standardized. Foremost, number of deaths from cancer, ischemic heart disease and diabetes mellitus were included in the numerator for mortality rates, while Serbian population data was used as the denominator for each year. Then, the standardization was performed by direct method (Segi's World standard population was used as standard population, stratified by 5-year age strata). Age-standardized mortality rates are expressed as deaths per 100 000 persons. Linear trend model was used to examine trend of mortality rates from cancer, ischemic heart disease, and diabetes mellitus.

There are about 80 food items listed in the Household Budget Survey. For ease of presentation of the consumption, list of consumed quantities of food and beverages is aggregated into several groups: meat (beef - fresh and frozen, pork - fresh and frozen, poultry - fresh and frozen, fish – fresh and frozen and canned, other kind of meat - fresh and frozen mutton and goat meat, and dried and processed meat – including dried bacon, salami and sausages, hot dogs and debreziner, and other sausage products, canned and manufactured meat), eggs, dairy (milk, cheese, and other dairy products (including yogurts, butter, cream and sour cream), fats (animal fat: including pork fat, suet, lard, raw bacon) and oils (sunflower oil, and other vegetable oils), fruits (citrus fruits, bananas, apples, grapes, other kind of fruits and manufactured fruits), vegetables (potatoes, cabbage, tomatoes, peppers, bean, carrots, parsley, celery and similar, onion, garlic and leek, other kind of vegetables and manufactured vegetables), coffee, alcohol beverages (beer, wine, hard drinks), sweets and sweeteners (biscuits, chocolate, honey, sugar) and grains (bread and cereals, flour and products from flour, pastas and other, rice). Food groups and beverages were represented by following units: meats (kg), eggs (piece), milk (l), cheese (kg), other dairy products (l), animal fat (kg), vegetable oil (l), fruits (kg), vegetables (kg), coffee (kg), beverages (l). Data quality assessment by phone was performed on a random sample (10% of surveyed households).

**Statistical analysis**

We examined the relationship between mortality rates and consumption of the common food groups and beverages by calculating a number of bivariate correlation coefficients - the *Pearson's correlation coefficients* (*r*). In addition, the explanatory variables might be correlated among themselves (possible confounders). The aim of multivariate linear regression analysis was to assess
the strength of the relationship between independent variables (consumption of common food groups and beverages) on variation in the dependent variable (mortality rates). Here we used the Stepwise (backward deletion) multivariate linear regression method. In trying to disentangle the relationships involved in a set of the independent variables, we checked collinearity diagnostics which resulted in omitting variables that showed significant mutual correlation ($r > 0.9$). Due to the control of the influence of gender on the association between consumption of food and beverages and cancer, ischemic heart disease and diabetes mellitus mortality rates, we conducted the multivariate linear regression analysis for men and women separately. Multivariate linear regression analysis was used to determine $B$ coefficient (with 95 percent confidence intervals - CI). $B$ - unstandardized coefficient indicates how much the dependent variable varies with one independent variable when all other independent variables are held constant. Two-sided $P$ values were reported and considered to indicate statistical significance when lesser than 0.05. All statistical analyses were conducted using the Statistical Package for Social Sciences software (SPSS Inc., version 20.0, Chicago, IL).

**Ethical approval**

This study is part of a larger research approved by the Ethics Committee of the Faculty of Medical Sciences, University of Kragujevac (Ref. No.: 01-4806), entitled "The survival of patients suffering from the most common chronic diseases (malignant tumors, cardiovascular and cerebrovascular diseases)".

**RESULTS**

In Serbia (without the Autonomous Province of Kosovo and Metohia), the markedly increasing trends in mortality rates from cancer ($y = 119.8x + 1.2, P = 0.000$), ischemic heart disease ($y = 68.8x + 0.6, P = 0.025$), and diabetes mellitus ($y = 14.1x + 0.1, P = 0.001$) were observed during the 1991-2010 period (Figure 1). The significant increase in mortality rates was found in both sexes, with the exception of insignificant increase in mortality rates from ischemic heart disease in men only (data not shown).

Pearson’s correlation coefficients between age-standardized mortality rates and per capita food and beverages consumption are shown in Table 1. The mortality rates for all three selected chronic diseases in Serbia were positively correlated with eggs consumption ($r = +0.890$, +0.544, and +0.680, respectively), and negatively correlated with animal fat ($r = -0.892$, -0.473 and -0.501, respectively) and hard drinks consumption ($r = -0.974$, -0.519 and -0.691, respectively). In contrast to that, per capita consumption of pork and cheese was not correlated with any of the observed mortality rates.

A multivariate linear regression analysis revealed positive association between cancer mortality rates and consumption of poultry ($B = +1.13$, 95% CI 0.62 to 1.65, $P = 0.000$), milk ($B = +0.10$, 95% CI 0.02 to 0.17, $P = 0.016$) and other dairy products ($B = +0.45$, 95% CI 0.30 to 0.60, $P = 0.000$), and negative association with consumption of beef ($B = -1.42$, 95% CI -1.93 to -0.92, $P = 0.000$).
vegetable oil (B = -1.34, 95% CI -2.21 to -0.48, P = 0.005) and grains (B = -0.13, 95% CI -0.21 to -0.06, P = 0.002) (Table 2). Also, a multivariate linear regression analysis showed positive association between ischemic heart disease mortality rates and consumption of beer (B = +1.36, 95% CI 0.72 to 2.00, P = 0.000) and other dairy products (B = +0.49, 95% CI 0.05 to 0.92, P = 0.032), and negative association with consumption of vegetable oil (B = -4.82, 95% CI -7.20 to -2.45, P = 0.001), cheese (B = -2.50, 95% CI -4.41 to -0.59, P = 0.014) and grains (B = -0.25, 95% CI -0.47 to -0.04, P = 0.021). Between the consumption of poultry and mortality rates from diabetes mellitus a positive association was observed (B = +0.61, 95% CI 0.33 to 0.90, P = 0.000).

The animal fat, eggs and other dairy products consumption was significantly positively related with cancer mortality rates in men (P = 0.000) (Table 3). Beef and grains consumption showed a significant negative association with cancer mortality rates in both genders (P = 0.002 and 0.000 in men, respectively; P = 0.000 and 0.014 in women, respectively), while consumption of cheese was negatively associated in men only (P = 0.000). For both genders, the consumption of vegetable oils was negatively (P = 0.000 in men, and P = 0.001 in women) and beer consumption was positively (P = 0.018 in men, and P = 0.000 in women) associated with mortality rates from ischemic heart disease. Association between poultry consumption and mortality rates from diabetes mellitus was significantly positive both in men and women (P = 0.014 and 0.004, respectively). Consumption of animal fat and other dairy products showed significant positive association with mortality rates of diabetes mellitus only in women.

DISCUSSION

Mortality rates from cancer, ischemic heart disease and diabetes mellitus in Serbia have increased during the last two decades. We found that the consumption of beef and grains was negatively associated with mortality rates from cancer in both genders, while consumption of other dairy products showed a significant positive association. Consumption of vegetable oil showed a significant negative association with mortality rates from ischemic heart disease in both genders, while consumption of beer was positively associated. Association between consumption of poultry and mortality rates from diabetes mellitus was significantly positive both in men and women.

Although mortality from coronary heart diseases has continued to decline in the European Union (27 countries), the United States of America, Australia, Japan and most Latin American countries, unfavourable trends are still observed in the Russian Federation and other countries of the former Soviet Union (Ukraine, Belarus, etc.). In Western Europe, cancer mortality has steadily declined since the late 1980s, but in central/eastern Europe mortality from major cancer sites has been increasing up to the late 1990s/early 2000s. Diabetes mellitus accounted for 6% of adult deceased in the African Region, and 15.7% in the North American Region, where diabetes is a major cause of premature mortality. Diabetes mellitus prevalence increases, particularly in low- and middle-income countries. These unfavourable trends are coincident with dramatic socioeconomic
changes that occurred in developing countries, especially in the former communist countries. Thus, these countries have undergone “westernization”, including the adoption of the new dietary habits (such as increased animal fat and red meat consumption and low vegetable intake), obesity and physical inactivity, cigarette smoking and alcohol use, which characterized newly economically developed countries.29 The devastating economic crisis, which was exacerbated by the sanctions and the wars in former Yugoslavia, hundreds of thousands of refugees, unemployment, the collapse of the national currency and the inability to purchase needed medications, the deterioration of public health, and then bombing of Serbia, further affected the general circumstances in Serbia.30 Diet plays an important role in the occurrence of these diseases, as indicated in a number of epidemiological studies.23, 31-33

The association between consumption of food and beverages and mortality rates from cancer, ischemic heart disease and diabetes mellitus has been found to vary in significance and magnitude across countries.6, 11, 15, 34 Numerous ecological studies showed association between cancer mortality rates and consumption of some animal products (meat, milk, fish and eggs), sweeteners, some fats, grains and vegetables.35, 36 The most consistent association was between mortality rates and animal fat consumption,37, 38 but was not confirmed in some other studies.15 In our study, consumption of animal fat was associated with cancer mortality in men and with mortality rates of diabetes mellitus in women only.

Among eighteen European countries,33 coronary mortality was negatively correlated with olive oil consumption.26 Increased consumption of palm oil and other oil-crops is related to higher ischemic heart disease mortality rates in developing countries.15, 32 Our study revealed that vegetable oil consumption was negatively associated with cancer mortality and mortality rates from ischemic heart disease.

According to the Data Food Networking (DAFNE) project, coronary heart disease mortality was not significantly correlated with per capita consumption of meat, fish, milk and dairy products, eggs and ethanol; for these foods, correlations with mortality from breast and colon cancer, diseases with established nutritional aetiology, have not been confirmed.39 Secular mortality trend from colorectal cancer in Spain has been positively correlated with consumption of poultry, vegetables and fruit.34 Similarly, poultry consumption was positively associated with cancer mortality and mortality rates from diabetes mellitus in Serbia. Also, our finding of a strongly inverse effect of beef and grains consumption on total cancer and ischemic heart disease mortality is similar to the results from developing countries.15

A positive association between mortality from coronary heart disease and eggs consumption was observed in study involving 40 countries,40 in contrast to the results in some other studies.15 According to our results, eggs showed effect on cancer mortality rates only in men. This probable effect has been related to specific micronutrients (cholesterol, fatty acids, etc) contained in eggs.41
Positive association between consumption of milk and other dairy products and mortality from cancer and ischemic heart disease noticed in Serbia, is in contrast to the results in some other studies\(^{42}\) but not in all.\(^{43,44}\) Per capita consumption of cheese had inverse association with ischemic heart disease mortality in Serbia. Contrary findings were obtained in some studies, but not all of them.\(^{40}\)

In Serbia, consumption of beer had a positive association with mortality rates from ischemic heart disease. Some studies have produced different findings.\(^{45,46}\) This positive association between coronary mortality and beer consumption in Serbia in both genders, may be confounded by genetic, psychosocial, or lifestyle-related factors (e.g. cigarette smoking, obesity, physical inactivity, etc).

**Strengths and limitations of the study**

This study is the first to document a relationship between increased mortality from cancer, ischemic heart disease and diabetes mellitus, and consumption of food and beverages in Serbia.

The longitudinal nature of our national-level data enabled us to analyse the link between mortality trends and food and beverages consumption. The Serbian mortality statistics data is considered comprehensive and reliable according to the World Health Organization standards.\(^{23}\) Also, the proportion of deaths assigned to ill-defined cause-of-death codes (revision 9 codes 780-799 and revision 10 codes R00-R99) in the observed period in Serbia was on average 6.8%, with a non-significant decreasing trend\(^ {21}\) \((P = 0.137)\). Consequently, changes in the ICD classification over the time period studied could not have a substantial effect on changes in the mortality rates of cancer, ischemic heart disease and diabetes mellitus in Serbia. Also, the Household Budget Survey in Serbia was harmonised with the international standards and recommendations.\(^{21}\) Assessment of the quality of data on consumption of foods and beverages was performed quarterly in each of the households by phone on a random sample (10% of surveyed households). Finally, our study may be useful in the generation of new hypotheses concerning the dietary aetiology of these most common chronic diseases in Serbia, which should be evaluated by further analytical epidemiological researches.

But, this ecological study has some limitations. Firstly, this ecological study has examined relationship between outcome and exposure at the population level whereas this association may not exist at the individual level. Thus, use of average exposure levels may mask more complicated relationships with the disease. Also, very important problems in this study refer to the temporal ambiguity, collinearity and confounding. Multivariate analysis has helped in the control of confounding and multicollinearity in our study, but we cannot adjust well for most of relevant exposures (including consumption of cigarettes, obesity, physical activity, occupation, level of education, reproductive characteristics, etc) for which data were not available for the entire Serbian population in the studied period. Lack of the incidence data for these chronic diseases in Serbia in the observed period may be a source of selection bias in our study, because we did not have data on how long did people have the disease.\(^ {21}\) For example, this ecological study can not answer whether the
men who died from cancer in Serbia were those who actually had diet a high animal fat diet in studied period. In this study some measurement errors were inevitable, because food consumption was self-reported. Finally, unmeasured or poorly measured food and beverages consumption as well as lack of measures for important potential confounders can be a major source of bias in our ecological study. Additionally, consumption of any specific food-group may actually just be a marker for a whole range of other socio-economic, biological and psychological factors (such as poverty, traumatism, stress, comorbidity, etc) that characterized period of the fall of communism and the civil wars in the former Yugoslavia since 1990s, for which no data is available for Serbia during the whole studied period. Further, inability to control for bias and confounding factors may be responsible for some inconsistency in our findings in comparison to other studies.

In conclusion, consumption of food and beverages is of potential importance in mortality trends for cancer, ischemic heart disease and diabetes mellitus in Serbia. Further effort is needed in order to investigate a possible causative association, using epidemiological analytical studies.

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Competing interests
All authors declare: no financial or non-financial interests that may be relevant to the submitted work.

Contributors: MI conceived and designed the study, took part in data collection, data analysis, interpretation of the results, and manuscript preparation, editing, and review. II, GS and IZM performed data analysis and manuscript editing and review. All authors took part in editing of the final version and gave approval for submission.

REFERENCES


Table 1

Pearson’s correlation coefficients (r) between per capita food and beverages consumption with mortality from cancer, ischemic heart disease, and diabetes mellitus (age-standardized mortality rates, per 100 000 persons, using direct method, by Segi’s World Standard Population) in Serbia, 1991-2010

<table>
<thead>
<tr>
<th>Food groups and beverages (unit)</th>
<th>Cancer mortality</th>
<th>Ischemic heart disease mortality</th>
<th>Diabetes mellitus mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meats</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Beef (kg)</td>
<td>-0.740**</td>
<td>-0.565**</td>
<td>-0.363</td>
</tr>
<tr>
<td>- Pork (kg)</td>
<td>+0.147</td>
<td>-0.346</td>
<td>+0.164</td>
</tr>
<tr>
<td>- Poultry (kg)</td>
<td>+0.895**</td>
<td>+0.314</td>
<td>+0.748**</td>
</tr>
<tr>
<td>- Fish (all kinds) (kg)</td>
<td>+0.861**</td>
<td>0.349</td>
<td>+0.612**</td>
</tr>
<tr>
<td>- Other meat (kg)</td>
<td>-0.577*</td>
<td>-0.541*</td>
<td>-0.265</td>
</tr>
<tr>
<td>- Dried meat (kg)</td>
<td>+0.878**</td>
<td>+0.235</td>
<td>+0.692**</td>
</tr>
<tr>
<td>Eggs (piece)</td>
<td>+0.890**</td>
<td>+0.544*</td>
<td>+0.680**</td>
</tr>
<tr>
<td>Dairy</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>- Milk (l)</td>
<td>-0.695**</td>
<td>-0.168</td>
<td>-0.301</td>
</tr>
<tr>
<td>- Cheese (kg)</td>
<td>+0.100</td>
<td>-0.298</td>
<td>+0.131</td>
</tr>
<tr>
<td>- Other dairy products (l)</td>
<td>+0.880**</td>
<td>+0.357</td>
<td>+0.625**</td>
</tr>
<tr>
<td>Fats and oils</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>- Animal fat (kg)</td>
<td>-0.892**</td>
<td>-0.473*</td>
<td>-0.501*</td>
</tr>
<tr>
<td>- Vegetable oil (l)</td>
<td>+0.674**</td>
<td>-0.092</td>
<td>+0.606**</td>
</tr>
<tr>
<td>Fruits (all kinds) (kg)</td>
<td>+0.760**</td>
<td>+0.300</td>
<td>+0.737**</td>
</tr>
<tr>
<td>Vegetables (all kinds) (kg)</td>
<td>+0.749**</td>
<td>+0.265</td>
<td>+0.723**</td>
</tr>
<tr>
<td>Coffee (kg)</td>
<td>+0.558*</td>
<td>+0.015</td>
<td>+0.516*</td>
</tr>
<tr>
<td>Alcohol beverages</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Beer (l)</td>
<td>+0.492</td>
<td>+0.379</td>
<td>+0.600*</td>
</tr>
<tr>
<td>- Wine (l)</td>
<td>-0.571*</td>
<td>-0.655**</td>
<td>-0.349</td>
</tr>
<tr>
<td>- Hard drinks (l)</td>
<td>-0.974**</td>
<td>-0.519*</td>
<td>-0.691**</td>
</tr>
<tr>
<td>Sweets and sweeteners (kg)</td>
<td>+0.536*</td>
<td>-0.151</td>
<td>+0.464*</td>
</tr>
<tr>
<td>Grains (kg)</td>
<td>-0.843**</td>
<td>-0.346</td>
<td>-0.397</td>
</tr>
</tbody>
</table>

* 0.01<p<0.05; ** p<0.01.
### Table 2

Association of the consumption of food and beverages with mortality from cancer, ischemic heart disease and diabetes mellitus in Serbia, 1991-2010; multivariate linear regression analysis

<table>
<thead>
<tr>
<th>Mortality</th>
<th>Food and beverages (items)</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Positive</td>
<td>Negative</td>
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<tr>
<td></td>
<td>Items</td>
<td>B</td>
<td>95% CI</td>
<td>P</td>
<td>Items</td>
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<tr>
<td></td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>Cancer</td>
<td>Poultry</td>
<td>+1.13</td>
<td>(0.62 to 1.65)</td>
<td>0.000</td>
<td>Beef</td>
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<td></td>
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</tr>
<tr>
<td></td>
<td>Milk</td>
<td>+0.10</td>
<td>(0.02 to 0.17)</td>
<td>0.016</td>
<td>Vegetable oil</td>
</tr>
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<td></td>
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<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Other dairy products</td>
<td>+0.45</td>
<td>(0.30 to 0.60)</td>
<td>0.000</td>
<td>Grains</td>
</tr>
<tr>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Ischemic heart disease</td>
<td>Beer</td>
<td>+1.36</td>
<td>(0.72 to 2.00)</td>
<td>0.000</td>
<td>Vegetable oil</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Other dairy products</td>
<td>+0.49</td>
<td>(0.05 to 0.92)</td>
<td>0.032</td>
<td>Cheese</td>
</tr>
<tr>
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<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>Poultry</td>
<td>+0.61</td>
<td>(0.33 to 0.90)</td>
<td>0.000</td>
<td></td>
</tr>
</tbody>
</table>

B – Unstandardized Coefficient; CI – Confidence Interval; P – Probability.
### Table 3
Association of the consumption of food and beverages with mortality from cancer, ischemic heart disease and diabetes mellitus in Serbia, by gender, 1991-2010; multivariate linear regression analysis

<table>
<thead>
<tr>
<th>Mortality</th>
<th>Food and beverages (items)</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>P</td>
<td>Negative</td>
</tr>
<tr>
<td>Cancer</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Animal fat</td>
<td>0.000</td>
<td></td>
<td>Beef</td>
</tr>
<tr>
<td>Eggs</td>
<td>0.000</td>
<td></td>
<td>Cheese</td>
</tr>
<tr>
<td>Other dairy products</td>
<td>0.000</td>
<td></td>
<td>Grains</td>
</tr>
<tr>
<td>Ischemic heart disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beer</td>
<td>0.018</td>
<td></td>
<td>Vegetable oil</td>
</tr>
<tr>
<td>Other dairy products</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poultry</td>
<td>0.014</td>
<td></td>
<td>Animal fat</td>
</tr>
<tr>
<td>Other dairy products</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
**Figure 1.** Trends from cancer, ischemic heart disease and diabetes mellitus mortality rates in Serbia, excluding the Autonomous Province of Kosovo and Metohija, 1991-2019

- **Cancer:** $y = 19.8x + 12, P<0.001$
- **Ischemic heart disease:** $y = 68.8x + 0.6, P=0.025$
- **Diabetes mellitus:** $y = 14.1x + 0.1, P=0.001$

**ASR (W)** - Age Standardized Rate of mortality (per 100,000 persons, using direct method, by Segi’s World Standard Population)

209x148mm (300 x 300 DPI)
The RECORD statement – checklist of items, extended from the STROBE statement, that should be reported in observational studies using routinely collected health data.

<table>
<thead>
<tr>
<th>Item No.</th>
<th>STROBE items</th>
<th>Location in manuscript where items are reported</th>
<th>RECORD items</th>
<th>Location in manuscript where items are reported</th>
</tr>
</thead>
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<td><strong>Title and abstract</strong></td>
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<tr>
<td>1</td>
<td>(a) Indicate the study’s design with a commonly used term in the title or the abstract (b) Provide in the abstract an informative and balanced summary of what was done and what was found</td>
<td>RECORD 1.1: The type of data used should be specified in the title or abstract. When possible, the name of the databases used should be included. RECORD 1.2: If applicable, the geographic region and timeframe within which the study took place should be reported in the title or abstract. RECORD 1.3: If linkage between databases was conducted for the study, this should be clearly stated in the title or abstract.</td>
<td></td>
<td>Pages 1 and 2.</td>
</tr>
<tr>
<td><strong>Introduction</strong></td>
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<tr>
<td>Background rationale</td>
<td>2</td>
<td>Explain the scientific background and rationale for the investigation being reported</td>
<td></td>
<td>Page 4.</td>
</tr>
<tr>
<td>Objectives</td>
<td>3</td>
<td>State specific objectives, including any prespecified hypotheses</td>
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<td>Page 5.</td>
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<tr>
<td><strong>Methods</strong></td>
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<tr>
<td>Study Design</td>
<td>4</td>
<td>Present key elements of study design early in the paper</td>
<td></td>
<td>Page 5.</td>
</tr>
<tr>
<td>Setting</td>
<td>5</td>
<td>Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection</td>
<td></td>
<td>Pages 5-7.</td>
</tr>
<tr>
<td>Participants</td>
<td>6</td>
<td><em>(a) Cohort study</em> - Give the eligibility criteria, and the</td>
<td>RECORD 6.1: The methods of study population selection (such as codes or</td>
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</tr>
<tr>
<td>Section</td>
<td>Page Numbers</td>
<td>Details</td>
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<tr>
<td>Sources and methods of selection of participants. Describe methods of follow-up</td>
<td>6-7</td>
<td>RECORD 6.2: Any validation studies of the codes or algorithms used to select the population should be referenced. If validation was conducted for this study and not published elsewhere, detailed methods and results should be provided.</td>
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<tr>
<td>Case-control study - Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls</td>
<td>6-7</td>
<td>RECORD 6.3: If the study involved linkage of databases, consider use of a flow diagram or other graphical display to demonstrate the data linkage process, including the number of individuals with linked data at each stage.</td>
<td></td>
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<tr>
<td>Cross-sectional study - Give the eligibility criteria, and the sources and methods of selection of participants</td>
<td>6-7</td>
<td>Linkage displayed using tables (Table 1-3).</td>
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<tr>
<td>(b) Cohort study - For matched studies, give matching criteria and number of exposed and unexposed</td>
<td>6-7</td>
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<tr>
<td>Case-control study - For matched studies, give matching criteria and the number of controls per case</td>
<td>6-7</td>
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<tr>
<td>Variables</td>
<td>7</td>
<td>CLEARLY DEFINE ALL OUTCOMES, EXPOSURES, PREDICTORS, POTENTIAL CONFOUNDERS, AND EFFECT MODIFIERS. GIVE DIAGNOSTIC CRITERIA, IF APPLICABLE.</td>
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<tr>
<td>Data sources/measurement</td>
<td>8</td>
<td>RECORD 7.1: A COMPLETE LIST OF CODES AND ALGORITHMS USED TO CLASSIFY EXPOSURES, OUTCOMES, CONFOUNDERS, AND EFFECT MODIFIERS SHOULD BE PROVIDED. IF THESE CANNOT BE REPORTED, AN EXPLANATION SHOULD BE PROVIDED.</td>
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<tr>
<td>Bias</td>
<td>9</td>
<td>Pages 7 and 8.</td>
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<td>Study size</td>
<td>10</td>
<td>Pages 5-7.</td>
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<tr>
<td>1.1</td>
<td>Quantitative variables</td>
<td>11 Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why</td>
<td></td>
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<td>2.1</td>
<td>Statistical methods</td>
<td>12 (a) Describe all statistical methods, including those used to control for confounding. (b) Describe any methods used to examine subgroups and interactions. (c) Explain how missing data were addressed. (d) Cohort study - If applicable, explain how loss to follow-up was addressed. Case-control study - If applicable, explain how matching of cases and controls was addressed. Cross-sectional study - If applicable, describe analytical methods taking account of sampling strategy. (e) Describe any sensitivity analyses.</td>
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<td>3.1</td>
<td>Data access and cleaning methods</td>
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</tbody>
</table>

**Page 6.**

population-based ecological study that comprised the entire population of Serbia.

**Page 7.**

**Page 7 and 8.**

**Page 5.**

RECORD 12.1: Authors should describe the extent to which the investigators had access to the database population used to create the study population.
<table>
<thead>
<tr>
<th>RECORD 12.2</th>
<th>Authors should provide information on the data cleaning methods used in the study.</th>
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<td>..</td>
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<tr>
<td>RECORD 12.3</td>
<td>State whether the study included person-level, institutional-level, or other data linkage across two or more databases. The methods of linkage and methods of linkage quality evaluation should be provided.</td>
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<td></td>
<td>This is a population-based ecological study that comprised the entire population of Serbia. Page 6.</td>
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</tbody>
</table>

### Results

<table>
<thead>
<tr>
<th>Participants</th>
<th>13</th>
<th>(a) Report the numbers of individuals at each stage of the study (e.g., numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed) (b) Give reasons for non-participation at each stage. (c) Consider use of a flow diagram</th>
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</thead>
<tbody>
<tr>
<td>Descriptive data</td>
<td>14</td>
<td>(a) Give characteristics of study participants (e.g., demographic, clinical, social) and information on exposures and potential confounders (b) Indicate the number of participants with missing data for each variable of interest (c) Cohort study - summarise follow-up time (e.g., average and total amount)</td>
</tr>
<tr>
<td>Outcome data</td>
<td>15</td>
<td>Cohort study - Report numbers of outcome events or summary measures over time Case-control study - Report numbers in each exposure</td>
</tr>
</tbody>
</table>

This is a population-based ecological study that comprised the entire population of Serbia. Page 6.
<table>
<thead>
<tr>
<th>Category</th>
<th>Page(s)</th>
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<tr>
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</tbody>
</table>

**Main results**

| 16                                           | Page 8 and 9.      | (a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (e.g., 95% confidence interval). Make clear which confounders were adjusted for and why they were included.
| (b) Report category boundaries when continuous variables were categorized.
| (c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period. |

**Other analyses**

| 17                                           | Page 8 and 9.      | Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses.                                                                                                       |

**Discussion**

| 18                                           | Page 9.            | Summarise key results with reference to study objectives.                                                                                                                                              |
| 19                                           | Pages 9 and 10.    | Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias. RECORD 19.1: Discuss the implications of using data that were not created or collected to answer the specific research question(s). Include discussion of misclassification bias, unmeasured confounding, missing data, and changing eligibility over time, as they pertain to the study being reported. |
| 20                                           | Pages 9-11.        | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of                                                                                                      |
analyses, results from similar studies, and other relevant evidence

| Generalisability | 21 | Discuss the generalisability (external validity) of the study results | Pagers 11 and 12. |

**Other Information**

| Funding | 22 | Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based | Page 12. |

| Accessibility of protocol, raw data, and programming code | .. | RECORD 22.1: Authors should provide information on how to access any supplemental information such as the study protocol, raw data, or programming code. | No data available. |


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**Association of the consumption of common food groups and beverages with mortality from cancer, ischemic heart disease and diabetes mellitus in Serbia, 1991-2010; ecological study**

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<th>BMJ Open</th>
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| Complete List of Authors: | Ilic, Milena; Faculty of Medical Sciences, Department of Epidemiology  
                            | Ilic, Irena; Faculty of Medical Sciences, University of Kragujevac, Kragujevac  
                            | Sojanovic, Goran; Medical College for Professional Studies, University of Belgrade, Belgrade,  
                            | Zivanovic-Macuzic, Ivana; Faculty of Medical Sciences, University of Kragujevac, Kragujevac, Department of Anatomy  |
| <b>Primary Subject Heading</b>: | Epidemiology                                 |
| Secondary Subject Heading: | Public health                                |
| Keywords: | Cancer;, Ischemic heart disease;, Diabetes mellitus;, Mortality trend;, Food groups and beverages consumption;, Ecological study. |
Association of the consumption of common food groups and beverages with mortality from cancer, ischemic heart disease and diabetes mellitus in Serbia, 1991-2010; ecological study

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³ Medical College for Professional Studies, University of Belgrade, Belgrade;
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ABSTRACT

Objectives: This paper reports association between mortality rates from cancer, ischemic heart disease and diabetes mellitus, and the consumption of common food groups and beverages in Serbia.

Design: In this ecological study data on both mortality and the average annual consumption of common food groups and beverages per household's member were obtained from official data-collection sources. The multivariate linear regression analysis was used to determine the strength of the associations between consumption of common food groups and beverages and mortality rates.

Results: Markedly increasing trends of cancer, ischemic heart disease, and diabetes mellitus mortality rates were observed in Serbia in the 1991-2010 period. Mortality rates from cancer were negatively associated with consumption of vegetable oil ($p = 0.005$) and grains ($p = 0.001$) and same was found for ischemic heart disease ($p = 0.002$ and $0.021$, respectively), while consumption of other dairy products showed a significant positive association ($p < 0.001$ and $p = 0.032$, respectively). Both in men and women, mortality rates from diabetes mellitus showed a significant positive association with consumption of poultry ($p = 0.014$ and $0.004$, respectively). Consumption of beef and grains showed a significant negative association with cancer mortality rates in both genders ($p = 0.002$ and $p < 0.001$ in men and $p < 0.001$ and $p = 0.014$ in women, respectively), while consumption of cheese was negatively associated only in men ($p < 0.001$). Mortality from diabetes mellitus showed a significant positive association with consumption of animal fat and other dairy products only in women ($p = 0.003$ and $0.046$, respectively).

Conclusions: Association between unfavourable mortality trends from cancer, ischemic heart disease and diabetes mellitus and common food groups and beverages consumption was observed and should be assessed in future analytical epidemiological studies. Promotion of healthy diet is sorely needed in Serbia.

Key words: Cancer; Ischemic heart disease; Diabetes mellitus; Mortality trend; Food groups and beverages consumption; Ecological study.
Strengths and limitations of this study

- To the best of our knowledge, this population-based ecological study is the first to document a relationship between mortality from cancer, ischemic heart disease and diabetes mellitus, and consumption of food and beverages in Serbia.

- Mortality rates from cancer, ischemic heart disease and diabetes mellitus in Serbia have increased during the last two decades, and Serbia tops the list of European countries with highest mortality rates.

- We found that the consumption of beef and grains was negatively associated with mortality rates from cancer in both genders, while consumption of other dairy products showed a significant positive association. Consumption of vegetable oil showed a significant negative association with mortality rates from ischemic heart disease in both genders, while consumption of beer was positively associated. Association between consumption of poultry and mortality rates from diabetes mellitus was significantly positive both in men and women.

- The inability to fully control confounding factors in assessing relationships between mortality and consumption of food and beverages in Serbia is the limitation of this study.

- Findings have important implications for promotion of healthy diet in Serbia.
BACKGROUND

Cancer, ischemic heart disease and diabetes mellitus are most common causes of death in
developed countries. The top two causes, ischemic heart disease and cancer, accounted for 47.5%
of all deaths in the United States of America in 2010.2,3 The cardiovascular diseases are the main
cause of death in the European Union: they account for 42% of all deaths in the total population.1
Diabetes was the underlying cause in 2.8% of all deaths in the United States of America in 2010.3
Worldwide, diabetes caused 1.4 million deaths (2.6% of all causes) in 2011, up from 1.0 million
(1.9%) deaths in 2000.4

In the last two decades, cardiovascular mortality declined steeply in the European Union and
United States of America, due to decrease in both the ischemic heart disease and stroke mortality.2,5
Since 1990s, decreased mortality trend from all types of cancer was reported in the USA.2 In contrast,
death rates from diabetes mellitus in the same time interval increased, yielding a net annual increase
of 1.9%. Many predictions indicate a growing burden of diabetes, particularly in developing
countries.6 In some European countries, most migrant groups (particularly migrants from North
Africa, South Asia or countries with low gross domestic product) have higher diabetes mortality
rates than the local-born populations.7 Mortality varying by place and population has always been
used to generate hypotheses on causation. Many epidemiological studies have identified correlation
between mortality trends from cancer, coronary heart disease and diabetes mellitus, and food
consumption.8-10

Low rates of cancer and ischemic heart disease in Southern European countries (Greece and
Italy in particular) are traditionally explained by the ‘Mediterranean diet’.11 ‘Mediterranean diet’
means high consumption of olive oil, legumes, unrefined cereals, fruit and vegetables, moderate
consumption of dairy products (mostly cheese and yogurt), fish and wine, and low consumption of
meat. Moderate consumption of alcohol, together with other subtly beneficial lifestyles, was
associated with 22% lower risk of death.12 In Latin America, the per capita supply of milk showed a
strong positive correlation with incidence of childhood type 1 diabetes mellitus.13 Inverse
associations were observed for eggs and coffee consumption in several studies, while other studies
did not confirm such findings.14-16

Serbia (officially the Republic of Serbia) is a small country in southeast Europe: according to
World Bank estimates, Serbia today is an upper-middle income country with dominant service sector,
followed by the industry and agriculture.17 But, Serbia has experienced dramatic changes over the
past decades: in addition to civil wars and the dissolution of the former Yugoslavia in the 1990s, the
United Nations sanctions and trade embargo in 1992, the political isolation and the collapse of the
economy in 1993, NATO bombing in the 1999, and democratic socio-economic changes in 2000s,
the Kosovo’s unilateral declaration of independence in 2008, and transition to market economy
and financial crisis marked the beginning of the century. Since the 1990s, Serbia has the largest
refugee population in Europe - about half a million refugees and internally displaced persons sought refuge in the country following the series of Yugoslav wars. As of 2002 census, Serbs are the largest ethnic group in Serbia (representing 83% of the total population), but another 37 ethnicities also live on its territory (including Hungarians that are the largest ethnic minority in Serbia and represent 3.9% of the country's population, followed by Bosniaks and Roma with a similar share of around 2%, etc). Serbian cuisine is largely heterogeneous and includes characteristics of the Balkans, the Mediterranean, Turkish and Hungarian cuisines. Slivovitz, a highly alcoholic drink, is a famous rakia which is considered the national drink of Serbia.

The prominent immediate effect of wars and disintegration of Serbia was significantly increased mortality from cancer and ischemic heart disease during the last two decades.\textsuperscript{18-20} Serbia tops the list of European countries with highest cancer mortality rates.\textsuperscript{18,19} Since 1990s, cardiovascular disease and cancer became the first and second most prevalent causes of death in Serbia.\textsuperscript{21,22} In the same period, diabetes mellitus represented the cause for 3\% of all deaths. These three causes of death together accounted for almost 80\% of the total mortality in Serbia, with increasing tendency. There is scant information regarding the impact of diet on mortality in Serbia. This paper aimed to assess the time trend in mortality from most common diseases in Serbia, and to analyse the relationship between consumption of common food groups and beverages and mortality rates.

MATERIAL AND METHODS

Data source

In this ecological study data on both health outcome (mortality from cancer, ischemic heart disease and diabetes mellitus) and the exposure (the average annual consumption of common food groups and beverages per household's member) were obtained from official data-collection sources - the National Statistical Office of the Republic of Serbia.\textsuperscript{21}

Data on persons who died of cancer (site codes 140-208 revision 9 and codes C00-C97 revision 10 of the International Classification of Diseases - ICD), ischemic heart diseases (ICD-9 codes 410-414 and ICD-10 codes I20-I25) and diabetes mellitus (ICD-9 code 250 and ICD-10 codes E10-E14) were obtained from the National Statistical Office of the Republic of Serbia (unpublished data). Causes of death in Serbia are available from 1997 according to the ICD-10, and from the previous period according to ICD-9.

Data on causes of death are collected through the Death Certificate Form (DEM-2) (Official Gazette SR Serbia No. 8/2005). In Serbia, determination of death and cause of death are the responsibility of the physician. Death data are collected and submitted by registrars. Data on the deceased and on the cause of death are collected routinely for the entire territory of Serbia. All public hospitals are obliged by law to send individual hospitalization reports to regional institutes of public health which aggregate data and send a report to the Institute of Public Health of Serbia. Mortality
statistics includes all (100%) death occurrences in Serbia. The World Health Organization assessed the quality of the official mortality statistics in Serbia as medium-quality (criterion used: completeness reporting is >90% and ill-defined causes and injury deaths with undetermined intent appear on <10% of registrations).23

The study comprised the entire population of the Republic of Serbia, during the period 1991-2010. Data for 1998-2010 are not available for the Autonomous Province of Kosovo and Metohia, which declared itself independent in 2008. Data on the number and composition of the Serbian population by gender and age were presented according to 1991 and 2002 censuses, while for inter-census years the estimates of the resident population were obtained from the national Statistical Office database. The analysis was conducted on the entire Serbian population (approximately 7.5 million inhabitants).21 Since 1990s, Serbia had the largest internally displaced persons and refugee population in Europe (formed between 7% and 7.5% of its population) for which data were included in the Serbian population and could not be set aside as a special contingent.

The study was conducted between June 2013 and September 2014. The cancer, ischemic heart disease and diabetes mellitus mortality was estimated for the period 1991-2010.

The National Statistical Office of the Republic of Serbia has conducted a survey of household and individual food and beverages consumption in Serbia - the Household Budget Survey.24 The Household Budget Survey in Serbia is conducted annually since 1960s, and harmonised with the international standards and recommendations (Eurostat, International Labour Organization and the United Nations) from 2003, which provides grounds for an international comparison of data. The Household Budget Survey covered the whole territory of the Republic of Serbia. The household, either multi-member (a community of persons whose members live together in the same dwelling unit and spend realized income jointly) or one-member (single person, living alone and spending its own income), chosen according to the sample plan, was a survey unit. Each year the survey comprises a nationally representative sample of non-institutionalized persons residing in Serbia. The sample is a two-stage, stratified sample. The first stage units are enumeration districts and the second stage units are households. Sample selection was carried out by selecting the first stage units (enumeration districts) in proportion to the number of households, and the second stage units (households) were selected with equal probability (simple random selection). According to the EUROSTAT recommendations, total number of households predicted for surveying is 4800 for the Republic of Serbia. Also, as recommended by EUROSTAT, out of the total number of households envisaged for the survey, the lowest percentage of households that must be surveyed is 85% (which was achieved for each year). Given that a household in Serbia consists on an average of 3 members, the household budget survey includes about 13500-14500 inhabitants. Data were collected for the whole observed year (from January 1 to December 31). Every fifteen days, 200 households have been chosen to keep a diary on personal consumption of food and beverages (thereby taking into
account effects seasonal variations have on the diet). Diary method has been applied in this survey: each surveyed household keeps a diary (statistical form D-1) for the reference period. The survey collects data on household consumption, i.e. data on basic elements of individual consumption. The substitution of households is not predicted. Diary of the consumed quantities of food and beverages in every household was kept by a chosen person (it was the person responsible for food in the house, most often it was a housewife). Specially trained surveyors visit the households at least three times in purpose of helping with diary.

Measures

Three types of death rates were calculated: crude, specific (age- and sex-specific) and age-standardized. Foremost, number of deaths from cancer, ischemic heart disease and diabetes mellitus were included in the numerator for mortality rates, while Serbian population data was used as the denominator for each year. Then, the standardization was performed by direct method (Segi’s World standard population was used as standard population, stratified by 5-year age strata). Age-standardized mortality rates are expressed as deaths per 100,000 persons. Linear trend model was used to examine trend of mortality rates from cancer, ischemic heart disease, and diabetes mellitus.

There are about 80 food items listed in the Household Budget Survey. For ease of presentation of the consumption, list of consumed quantities of food and beverages is aggregated into several groups: meat (beef - fresh and frozen, pork - fresh and frozen, poultry - fresh and frozen, fish – fresh and frozen and canned, other kind of meat - fresh and frozen mutton and goat meat, and dried and processed meat – including dried bacon, salami and sausages, hot dogs and debreziner, and other sausage products, canned and manufactured meat), eggs, dairy (milk, cheese, and other dairy products (including yogurts, butter, cream and sour cream), fats (animal fat: including pork fat, suet, lard, raw bacon) and oils (sunflower oil, and other vegetable oils), fruits (citrus fruits, bananas, apples, grapes, other kind of fruits and manufactured fruits), vegetables (potatoes, cabbage, tomatoes, peppers, bean, carrots, parsley, celery and similar, onion, garlic and leek, other kind of vegetables and manufactured vegetables), coffee, alcohol beverages (beer, wine, hard drinks), sweets and sweeteners (biscuits, chocolate, honey, sugar) and grains (bread and cereals, flour and products from flour, pastas and other, rice). Food groups and beverages were represented by following units: meats (kg), eggs (piece), milk (l), cheese (kg), other dairy products (l), animal fat (kg), vegetable oil (l), fruits (kg), vegetables (kg), coffee (kg), beverages (l). Data quality assessment by phone was performed on a random sample (10% of surveyed households).

Statistical analysis

We examined the relationship between mortality rates and consumption of the common food groups and beverages by calculating a number of bivariate correlation coefficients - the Pearson's correlation coefficients ($r$). In addition, the explanatory variables might be correlated among themselves (possible confounders). The aim of multivariate linear regression analysis was to assess
the strength of the relationship between independent variables (consumption of common food groups and beverages) on variation in the dependent variable (mortality rates). Here we used the Stepwise (backward deletion) multivariate linear regression method. In trying to disentangle the relationships involved in a set of independent variables, we checked collinearity diagnostics which resulted in omitting variables that showed significant mutual correlation ($r > 0.9$). In order to control the influence of gender on the association between consumption of food and beverages and cancer, ischemic heart disease and diabetes mellitus mortality rates, we conducted the multivariate linear regression analysis for men and women separately. Multivariate linear regression analysis was used to determine the $B$ coefficient (with 95% confidence intervals - CI). $B$ - unstandardized coefficient indicates how much the dependent variable varies with one independent variable when all other independent variables are held constant. Two-sided $p$ values were reported and considered to indicate statistical significance when lesser than 0.05. All statistical analyses were conducted using the Statistical Package for Social Sciences software (SPSS Inc., version 20.0, Chicago, IL).

**Ethical approval**

This study is part of a larger research approved by the Ethics Committee of the Faculty of Medical Sciences, University of Kragujevac (Ref. No.: 01-4806), entitled "The survival of patients suffering from the most common chronic diseases (malignant tumors, cardiovascular and cerebrovascular diseases)".

**RESULTS**

In Serbia (without the Autonomous Province of Kosovo and Metohia), the markedly increasing trends in mortality rates from cancer ($y = 119.8x + 1.2$, $p < 0.001$), ischemic heart disease ($y = 68.8x + 0.6$, $p = 0.025$), and diabetes mellitus ($y = 14.1x + 0.1$, $p = 0.001$) were observed during the 1991-2010 period (Figure 1). The significant increase in mortality rates was found in both sexes, with the exception of insignificant increase in mortality rates from ischemic heart disease in men only (data not shown).

Pearson’s correlation coefficients between age-standardized mortality rates and *per capita* food and beverages consumption are shown in Table 1. The mortality rates for all three selected chronic diseases in Serbia were positively correlated with consumption of eggs ($r = +0.890$, $+0.544$, and $+0.680$, respectively), and negatively correlated with animal fat ($r = -0.892$, $-0.473$ and $-0.501$, respectively) and hard drinks consumption ($r = -0.974$, $-0.519$ and $-0.691$, respectively). In contrast to that, *per capita* consumption of pork and cheese was not correlated with any of the observed mortality rates.

A multivariate linear regression analysis revealed positive association between cancer mortality rates and consumption of poultry ($B = +1.13$, 95% CI 0.62 to 1.65, $p < 0.001$), milk ($B = +0.10$, 95% CI 0.02 to 0.17, $p = 0.016$) and other dairy products ($B = +0.45$, 95% CI 0.30 to 0.60, $p < 0.001$), and negative association with consumption of beef ($B = -1.42$, 95% CI -1.93 to -0.92, $p <$
0.001), vegetable oil (B = -1.34, 95% CI -2.21 to -0.48, p = 0.005) and grains (B = -0.13, 95% CI -0.21 to -0.06, p = 0.002) (Table 2). Also, the multivariate linear regression analysis showed positive association between ischemic heart disease mortality rates and consumption of beer (B = +1.36, 95% CI 0.72 to 2.00, p < 0.001) and other dairy products (B = +0.49, 95% CI 0.05 to 0.92, p = 0.032), and negative association with consumption of vegetable oil (B = -4.82, 95% CI -7.20 to -2.45, p = 0.001), cheese (B = -2.50, 95% CI -4.41 to -0.59, p = 0.014) and grains (B = -0.25, 95% CI -0.47 to -0.04, p = 0.021). Between the consumption of poultry and mortality rates from diabetes mellitus a positive association was observed (B = +0.61, 95% CI 0.33 to 0.90, p < 0.001).

The animal fat, eggs and other dairy products consumption was significantly positively related with cancer mortality rates in men (p < 0.001) (Table 3). Beef and grains consumption showed a significant negative association with cancer mortality rates in both genders (p = 0.002 and p < 0.001 in men, respectively; p < 0.001 and p = 0.014 in women, respectively), while consumption of cheese was negatively associated in men only (p < 0.001). For both genders, the consumption of vegetable oils was negatively (p < 0.001 in men, and p = 0.001 in women) and beer consumption was positively (p = 0.018 in men, and p < 0.001 in women) associated with mortality rates from ischemic heart disease. Association between poultry consumption and mortality rates from diabetes mellitus was significantly positive both in men and women (p = 0.014 and 0.004, respectively). Consumption of animal fat and other dairy products showed significant positive association with mortality from diabetes mellitus only in women.

DISCUSSION

One of the main findings in this study was the significant increase of mortality from cancer, ischemic heart disease and diabetes mellitus within the observed period in Serbia. According to the World Health Organization estimates, in the recent years Serbia has been the country with the highest mortality due to cancer and diabetes mellitus in Europe, while mortality of ischemic heart disease was lower in comparison to the rest of European Region countries.1,22 Although mortality from coronary heart diseases has continued to decline in the European Union (27 countries), the United States of America, Australia, Japan and most Latin American countries, unfavourable trends are still observed in the Russian Federation and other countries of the former Soviet Union (Ukraine, Belarus, etc.).26 In Western Europe, cancer mortality has steadily declined since the late 1980s, but in central/eastern Europe mortality from major cancer sites has been increasing up to the late 1990s/early 2000s.27 Diabetes mellitus accounted for 6% of deceased adults in the African Region, and 15.7% in the North American Region, where diabetes is a major cause of premature mortality.28 Diabetes mellitus prevalence and mortality is increasing, particularly in low- and middle-income countries.6,22 These unfavourable trends are coincident with dramatic socioeconomic changes that occurred in developing countries, especially in the former communist countries. Thus, these countries have undergone “westernization”, including the adoption of the new dietary habits (such as
increased animal fat and red meat consumption, increased consumption of processed food, and low vegetable intake), obesity and physical inactivity, cigarette smoking and alcohol use, which characterized newly economically developed countries.\textsuperscript{29} Between the two time periods over 43 years (1961/1965–2000/2004), Serbia, like other countries in the Mediterranean area who were a part of the Seven countries study, has also undergone this ‘westernization’ of food habits and experienced a convergence in terms of non-Mediterranean food groups.\textsuperscript{30} The devastating economic crisis, which was exacerbated by the sanctions and the wars in former Yugoslavia, hundreds of thousands of refugees, unemployment, the collapse of the national currency and the inability to purchase needed medications, the deterioration of public health, and then bombing of Serbia, further affected the general circumstances in Serbia.\textsuperscript{31} Diet plays an important role in the occurrence of these diseases, as indicated in a number of epidemiological studies.\textsuperscript{23,32-34}

The association between consumption of food and beverages and mortality rates from cancer, ischemic heart disease and diabetes mellitus has been found to vary in significance and magnitude across countries.\textsuperscript{6,11,15,35}

Numerous ecological studies showed association between cancer mortality rates and consumption of some animal products (meat, milk, fish and eggs), sweeteners, some fats, grains and vegetables.\textsuperscript{36,37} The most consistent association was between cancer mortality and animal fat consumption,\textsuperscript{38,39} but was not confirmed in some other studies.\textsuperscript{15,40} In our study, consumption of animal fat and eggs was associated with cancer mortality in men only. This probable effect has been related to specific micronutrients (cholesterol, fatty acids, etc) contained in egg yolk and fats.\textsuperscript{41} Secular mortality trend from colorectal cancer in Spain has been positively correlated with consumption of poultry, vegetables and fruit.\textsuperscript{35} Northern Europe seems to have adopted a healthier dietary pattern during recent decades, possibly due to both the improvements in availability of food groups (such as olive oil, fruits, vegetables, fish and seafood) and the implementation of nutrition education.\textsuperscript{30} We found that the consumption of beef and grains was negatively associated with mortality from cancer in both genders, while consumption of other dairy products showed a significant positive association. These results could be explained with the intake of nutrients which contain grains, beef and dairy products (such as high quality proteins, fibers, vitamins and minerals) and that may have anticarcinogenic properties.\textsuperscript{42} Besides, substantial differences according to gender may be the result of different exposure to lifestyle-related risk factors, such as smoking habits, sedentary, obesity and diabetes.

According to the Data Food Networking (DAFNE) project, coronary heart disease mortality was not significantly correlated with per capita consumption of meat, fish, milk and dairy products, eggs and ethanol.\textsuperscript{40} In eighteen European countries, coronary mortality was negatively correlated with olive oil consumption.\textsuperscript{26,34} Increased consumption of palm oil and other oil-crops is related to higher ischemic heart disease mortality rates in developing countries.\textsuperscript{15,33} Our study revealed that
vegetable oil consumption was negatively associated with mortality rates from ischemic heart disease in both genders, while consumption of beer was positively associated. Some studies have produced different findings.\textsuperscript{43,44} Beer consumption has been associated with an increased coronary mortality probably due to a decrease in antioxidant capacity and changes in lipid profiles and inflammatory markers (eg. C reactive protein, interleukin 6, tumour necrosis factor α).\textsuperscript{45} Decreased mortality has been associated with the consumption of vegetable oils, which are rich with monounsaturated and polyunsaturated fatty acids with well-known cardioprotective effects\textsuperscript{46} A positive correlation between mortality from coronary heart disease and eggs consumption was observed in a study involving 40 countries,\textsuperscript{47} in contrast to the results in some other studies.\textsuperscript{15} Our study failed to demonstrate association between eggs consumption and mortality from ischemic heart disease, which could be explained with the hidden intercorrelations among some components of food and beverages which were aggregated into several groups or with confounding effects of some lifestyle factors (age, smoking, physical inactivity, hypertension, diabetes mellitus).

Consumption of poultry had a positive association with mortality from diabetes mellitus in both genders in Serbia. Although the saturated fat load in poultry may be lower than in typical red meats, poultry provides no fibers or complex carbohydrates and that could be the possible explanation for this association. Positive association between consumption of animal fat and other dairy products and mortality from diabetes mellitus was noticed in women in Serbia. The detrimental effects reported for animal fat and dairy products may be due to the effects of saturated fatty acids in these food sources.\textsuperscript{48} Studies inquiring the role of other dairy products as a heterogeneous food group (including specific low-fat and high-fat dairy foods, butter) in the diabetes mellitus mortality, have not yielded consistent results.\textsuperscript{49} This positive association may be confounded by genetic, psychosocial, or lifestyle-related factors (e.g. cigarette smoking, obesity, physical inactivity, etc).

**Strengths and limitations of the study**

This study is the first to document a relationship between increased mortality from cancer, ischemic heart disease and diabetes mellitus, and consumption of food and beverages in Serbia.

The longitudinal nature of our national-level data enabled us to analyse the link between mortality trends and food and beverages consumption. The Serbian mortality statistics data is considered comprehensive and reliable according to the World Health Organization standards.\textsuperscript{23} Also, the proportion of deaths assigned to ill-defined cause-of-death codes (revision 9 codes 780-799 and revision 10 codes R00-R99) in the observed period in Serbia was on average 6.8%, with a non-significant decreasing trend ($P = 0.137$).\textsuperscript{21} Consequently, changes in the ICD classification over the time period studied could not have a substantial effect on changes in the mortality rates from cancer, ischemic heart disease and diabetes mellitus in Serbia. Also, the Household Budget Survey in Serbia was harmonised with the international standards and recommendations.\textsuperscript{21} Assessment of the quality of data on consumption of foods and beverages was performed quarterly in each of the households.
by phone on a random sample (10% of surveyed households). Finally, our study may be useful in the

generation of new hypotheses concerning the dietary aetiology of these most common chronic
diseases in Serbia, which should be evaluated by further analytical epidemiological researches.

Admittedly, this ecological study has some limitations. Firstly, this ecological study has
examined relationship between outcome and exposure at the population level whereas this
association may not exist at the individual level. Thus, the use of average exposure levels may mask
more complicated relationships with the disease. Also, very important problems in this study refer to
the temporal ambiguity, collinearity and confounding. Multivariate analysis has helped in the control
of confounding and multicollinearity in our study, but we cannot adjust well for most of relevant
exposures (including consumption of cigarettes, obesity, physical activity, occupation, level of
education, reproductive characteristics, etc) for which data were not available for the entire Serbian
population in the studied period. Lack of the incidence data for these chronic diseases in Serbia in the
observed period may be a source of selection bias in our study, because we did not have data on how
long did people have the disease. For example, this ecological study can not answer whether the
men who died from cancer in Serbia were those who actually had a high animal fat diet in the studied
period. In this study some measurement errors were inevitable, because food consumption was self-
reported and maybe incorporated subtle errors due to misunderstandings, misreporting or
nonresponse from the randomly selected households. Also, this ecological study evaluated both the
mortality rates and the food consumption referring to the same period (one and the same year). The
appropriate time-lag period was not incorporated into the analysis, because data on past exposures
were not available. In the event that exposures are changed markedly over time, this may lead to a
spurious association between food consumption and mortality. Finally, unmeasured or poorly
measured food and beverages consumption as well as lack of measures for important potential
confounders can be a major source of bias in our ecological study. Of course, these calculations do
not take into account the effects of other dietary intake (eg. salt, spices, additives) that could be of
influence on mortality because they are not included in the Household Budget Survey. Additionally,
consumption of any specific food-group may actually just be a marker for a whole range of other
socio-economic, biological and psychological factors (such as poverty, traumatism, stress,
comorbidity, etc) that characterized period of the fall of communism and the civil wars in the former
Yugoslavia since 1990s, for which no data is available for Serbia during the whole studied period.

In conclusion, consumption of food and beverages is of potential importance in mortality
trends for cancer, ischemic heart disease and diabetes mellitus in Serbia. Further effort is needed in
order to investigate a possible causative association, using epidemiological analytical studies.
Acknowledgement

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Competing interests

All authors declare: no financial or non-financial interests that may be relevant to the submitted work.

Contributors: MI conceived and designed the study, took part in data collection, data analysis, interpretation of the results, and manuscript preparation, editing, and review. II, GS and IZM performed data analysis and manuscript editing and review. All authors took part in editing of the final version and gave approval for submission.

Data sharing: No additional data available.

REFERENCES


Table 1
Pearson’s correlation coefficients (r) between *per capita* food and beverages consumption with mortality from cancer, ischemic heart disease, and diabetes mellitus (age-standardized mortality rates, per 100,000 persons, using direct method, by Segi’s World Standard Population) in Serbia, 1991-2010

<table>
<thead>
<tr>
<th>Food groups and beverages (unit)</th>
<th>Cancer mortality</th>
<th>Ischemic heart disease mortality</th>
<th>Diabetes mellitus mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Meats</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Beef (kg)</td>
<td>-0.740**</td>
<td>-0.565**</td>
<td>-0.363</td>
</tr>
<tr>
<td>- Pork (kg)</td>
<td>+0.147</td>
<td>-0.346</td>
<td>+0.164</td>
</tr>
<tr>
<td>- Poultry (kg)</td>
<td>+0.895**</td>
<td>+0.314</td>
<td>+0.748**</td>
</tr>
<tr>
<td>- Fish (all kinds) (kg)</td>
<td>+0.861**</td>
<td>0.349</td>
<td>+0.612**</td>
</tr>
<tr>
<td>- Other meat (kg)</td>
<td>-0.577*</td>
<td>-0.541*</td>
<td>-0.265</td>
</tr>
<tr>
<td>- Dried meat (kg)</td>
<td>+0.878**</td>
<td>+0.235</td>
<td>+0.692**</td>
</tr>
<tr>
<td><strong>Eggs (piece)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Milk (l)</td>
<td>-0.695**</td>
<td>-0.168</td>
<td>-0.301</td>
</tr>
<tr>
<td>- Cheese (kg)</td>
<td>+0.100</td>
<td>-0.298</td>
<td>+0.131</td>
</tr>
<tr>
<td>- Other dairy products (l)</td>
<td>+0.880**</td>
<td>+0.357</td>
<td>+0.625**</td>
</tr>
<tr>
<td><strong>Fats and oils</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Animal fat (kg)</td>
<td>-0.892**</td>
<td>-0.473*</td>
<td>-0.501*</td>
</tr>
<tr>
<td>- Vegetable oil (l)</td>
<td>+0.674**</td>
<td>-0.092</td>
<td>+0.606**</td>
</tr>
<tr>
<td><strong>Fruits (all kinds) (kg)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Vegetables (all kinds) (kg)</strong></td>
<td>+0.749**</td>
<td>+0.265</td>
<td>+0.723**</td>
</tr>
<tr>
<td><strong>Coffee (kg)</strong></td>
<td>+0.558*</td>
<td>+0.015</td>
<td>+0.516*</td>
</tr>
<tr>
<td><strong>Alcohol beverages</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Beer (l)</td>
<td>+0.492</td>
<td>+0.379</td>
<td>+0.600*</td>
</tr>
<tr>
<td>- Wine (l)</td>
<td>-0.571*</td>
<td>-0.655**</td>
<td>-0.349</td>
</tr>
<tr>
<td>- Hard drinks (l)</td>
<td>-0.974**</td>
<td>-0.519*</td>
<td>-0.691**</td>
</tr>
<tr>
<td><strong>Sweets and sweeteners (kg)</strong></td>
<td>+0.536*</td>
<td>-0.151</td>
<td>+0.464*</td>
</tr>
<tr>
<td><strong>Grains (kg)</strong></td>
<td>-0.843**</td>
<td>-0.346</td>
<td>-0.397</td>
</tr>
</tbody>
</table>

* 0.01 < p < 0.05; ** p < 0.01.
Table 2
Association of the consumption of food and beverages with mortality from cancer, ischemic heart disease and diabetes mellitus in Serbia, 1991-2010; multivariate linear regression analysis

| Mortality                  | Food and beverages (items) |  |  |  |  |  |  |
|----------------------------|-----------------------------|---|---|---|---|---|
|                            | Positive                    | B  | 95% CI       | p  | Negative | B  | 95% CI       | p  |
|                            | Items                       |     |   |   |   | Items                       |     |   |   |   |
|                            | Cancer                      |     |   |   |   | Ischemic heart disease      |     |   |   |   | Diabetes mellitus |     |   |   |   |
|                            | Poultry                     | 1.13 | (0.62 to 1.65) | < 0.001 | Beef                  | -1.42 | (-1.93 to -0.92) | < 0.001 |
|                            | Milk                        | 0.10 | (0.02 to 0.17) | 0.016 | Vegetable oil          | -1.34 | (-2.21 to -0.48) | 0.005 |
|                            | Other dairy products        | 0.45 | (0.30 to 0.60) | < 0.001 | Grains                | -0.13 | (-0.21 to -0.06) | 0.002 |
|                            | Beer                        | 1.36 | (0.72 to 2.00) | < 0.001 | Vegetable oil          | -4.82 | (-7.20 to -2.45) | 0.001 |
|                            | Other dairy products        | 0.49 | (0.05 to 0.92) | 0.032 | Cheese                | -2.50 | (-4.41 to -0.59) | 0.014 |
|                            |                             |     |   |   |   | Grains                | -0.25 | (-0.47 to -0.04) | 0.021 |

B – Unstandardized Coefficient; CI – Confidence Interval; p – Probability.
**Table 3**

Association of the consumption of food and beverages with mortality from cancer, ischemic heart disease and diabetes mellitus in Serbia, by gender, 1991-2010; multivariate linear regression analysis

<table>
<thead>
<tr>
<th>Mortality</th>
<th>Food and beverages (items)</th>
<th></th>
<th></th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Men</td>
<td></td>
<td>p</td>
<td>Negative</td>
<td>p</td>
</tr>
<tr>
<td>Cancer</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Animal fat</td>
<td>&lt; 0.001</td>
<td>Beef</td>
<td>0.002</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eggs</td>
<td>&lt; 0.001</td>
<td>Cheese</td>
<td>&lt; 0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other dairy products</td>
<td>&lt; 0.001</td>
<td>Grains</td>
<td>&lt; 0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ischemic heart disease</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beer</td>
<td>0.018</td>
<td>Vegetable oil</td>
<td>&lt; 0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poultry</td>
<td>0.014</td>
<td>Animal fat</td>
<td>0.003</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Poultry</td>
<td>0.004</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Other dairy products</td>
<td>0.046</td>
<td></td>
<td></td>
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FIGURE 1 Trends in male cancer, ischemic heart disease and diabetes mortality rates in Serbia, excluding the Autonomous Province of Kosovo and Metohia, 1991-2019

$\gamma_{\text{(Cancer)}} = 119.8x + 1.2; P < 0.001$

$\gamma_{\text{(Ischemic heart disease)}} = 68.8x + 0.6; P = 0.025$

$\gamma_{\text{(Diabetes mellitus)}} = 14.1x + 0.1; P = 0.001$

ASR (W) = Age Standardized Rate of mortality (per 100 000 persons, using direct method, by Segi’s World Standard Population)

209x148mm (300 x 300 DPI)
The RECORD statement – checklist of items, extended from the STROBE statement, that should be reported in observational studies using routinely collected health data.

<table>
<thead>
<tr>
<th>Item No.</th>
<th>STROBE items</th>
<th>Location in manuscript where items are reported</th>
<th>RECORD items</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Title and abstract</strong></td>
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</tr>
<tr>
<td>1</td>
<td>(a) Indicate the study’s design with a commonly used term in the title or the abstract (b) Provide in the abstract an informative and balanced summary of what was done and what was found</td>
<td>RECORD 1.1: The type of data used should be specified in the title or abstract. When possible, the name of the databases used should be included. RECORD 1.2: If applicable, the geographic region and timeframe within which the study took place should be reported in the title or abstract. RECORD 1.3: If linkage between databases was conducted for the study, this should be clearly stated in the title or abstract.</td>
<td>Pages 1 and 2.</td>
</tr>
<tr>
<td><strong>Introduction</strong></td>
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<td>2</td>
<td>Explain the scientific background and rationale for the investigation being reported</td>
<td>Page 4.</td>
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<tr>
<td>3</td>
<td>State specific objectives, including any prespecified hypotheses</td>
<td>Page 5.</td>
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<td><strong>Methods</strong></td>
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<td>4</td>
<td>Present key elements of study design early in the paper</td>
<td>Page 5.</td>
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<tr>
<td>5</td>
<td>Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection</td>
<td>Pages 5-7.</td>
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</table>
| 6 | *(a) Cohort study* - Give the eligibility criteria, and the | RECORD 6.1: The methods of study population selection (such as codes or
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sources and methods of selection of participants. Describe methods of follow-up

Case-control study - Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls

Cross-sectional study - Give the eligibility criteria, and the sources and methods of selection of participants

(b) Cohort study - For matched studies, give matching criteria and number of exposed and unexposed

Case-control study - For matched studies, give matching criteria and the number of controls per case

Variables 7 Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable.

Data sources/measurement 8 For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group

Bias 9 Describe any efforts to address potential sources of bias

Study size 10 Explain how the study size was determined

algorithms used to identify subjects) should be listed in detail. If this is not possible, an explanation should be provided.

RECORD 6.2: Any validation studies of the codes or algorithms used to select the population should be referenced. If validation was conducted for this study and not published elsewhere, detailed methods and results should be provided.

RECORD 6.3: If the study involved linkage of databases, consider use of a flow diagram or other graphical display to demonstrate the data linkage process, including the number of individuals with linked data at each stage.

Linkage displayed using tables (Table 1-3).

Variables 7

Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable.

RECORD 7.1: A complete list of codes and algorithms used to classify exposures, outcomes, confounders, and effect modifiers should be provided. If these cannot be reported, an explanation should be provided.

Data sources/measurement 8

For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group

Bias 9

Describe any efforts to address potential sources of bias

Study size 10

Explain how the study size was determined

This is a
<table>
<thead>
<tr>
<th>Quantitative variables</th>
<th>11</th>
<th>Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why</th>
</tr>
</thead>
<tbody>
<tr>
<td>Statistical methods</td>
<td>12</td>
<td>(a) Describe all statistical methods, including those used to control for confounding (b) Describe any methods used to examine subgroups and interactions (c) Explain how missing data were addressed (d) Cohort study - If applicable, explain how loss to follow-up was addressed Case-control study - If applicable, explain how matching of cases and controls was addressed Cross-sectional study - If applicable, describe analytical methods taking account of sampling strategy (e) Describe any sensitivity analyses</td>
</tr>
<tr>
<td>Data access and cleaning methods</td>
<td>..</td>
<td>RECORD 12.1: Authors should describe the extent to which the investigators had access to the database population used to create the study population.</td>
</tr>
</tbody>
</table>
| Results | 13 | Participants | (a) Report the numbers of individuals at each stage of the study (e.g., numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed)  
(b) Give reasons for non-participation at each stage.  
(c) Consider use of a flow diagram | RECORD 13.1: Describe in detail the selection of the persons included in the study (i.e., study population selection) including filtering based on data quality, data availability and linkage. The selection of included persons can be described in the text and/or by means of the study flow diagram. This is a population-based ecological study that comprised the entire population of Serbia. Page 6. |
| --- | --- | --- | --- |
| Descriptive data | 14 | (a) Give characteristics of study participants (e.g., demographic, clinical, social) and information on exposures and potential confounders  
(b) Indicate the number of participants with missing data for each variable of interest  
(c) Cohort study - summarise follow-up time (e.g., average and total amount) |  
Page 6. |
| Outcome data | 15 | Cohort study - Report numbers of outcome events or summary measures over time  
Case-control study - Report numbers in each exposure | This is a population-based ecological study that comprised the entire population }
<table>
<thead>
<tr>
<th>Page 6</th>
<th>Cross-sectional study</th>
<th>Report numbers of outcome events or summary measures of exposure.</th>
</tr>
</thead>
</table>

**Main results**

- 16 (a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (e.g., 95% confidence interval). Make clear which confounders were adjusted for and why they were included.
- (b) Report category boundaries when continuous variables were categorized.
- (c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period.

**Other analyses**

- 17 Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses.

**Discussion**

- **Key results**
  - 18 Summarise key results with reference to study objectives.
  
- **Limitations**
  - 19 Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias.
  
- **Interpretation**
  - 20 Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of
<table>
<thead>
<tr>
<th>Generalisability</th>
<th>21</th>
<th>Discuss the generalisability (external validity) of the study results</th>
<th>Pagers 11 and 12.</th>
</tr>
</thead>
</table>

**Other Information**

<table>
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<tr>
<th>Funding</th>
<th>22</th>
<th>Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based</th>
<th>Page 12.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accessibility of protocol, raw data, and programming code</td>
<td>..</td>
<td>RECORD 22.1: Authors should provide information on how to access any supplemental information such as the study protocol, raw data, or programming code.</td>
<td>No data available.</td>
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</table>


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