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Changes in Major Risk Factors for Cardiovascular Diseases over 25 Years in the Serbian Cohorts of the Seven Countries Study

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The Serbian cohorts of the Seven Countries Study were examined four times during a 25-year period. Large changes were observed in average serum cholesterol, blood pressure and the prevalence of smoking in these ageing cohorts. Comparison of men of the same age strata e.g. men aged 50–59 examined at baseline and after 10 years of follow-up and of men aged 65–69 examined after 10 and 25 years of follow-up showed that serum cholesterol increased by about 45% in Zrenjanin, 35% in Velika Krsna and 15% in Belgrade over the last 25 years. Systolic blood pressure increased by 9% in Zrenjanin and 7% in Velika Krsna. No significant increase in systolic blood pressure was observed in Belgrade. There was no major secular trend in smoking and the changes observed during 25 years were mainly due to ageing. No major change was observed in body mass index. The increases in serum cholesterol and blood pressure observed in the Serbian cohorts during the last 25 years are compatibile with the increase in cardiovascular disease mortality observed in Yugoslavian men aged 30–69 during the period 1970–1984.

Recent World Health Organization statistics show a 35% increase in cardiovascular disease mortality among males aged 30–69 in the former Yugoslavia between 1970 and 1984.[1] The increase was 65% for ischaemic heart disease mortality and 19% for cerebrovascular disease. Although the age-standardized mortality from ischaemic heart disease in males aged 30–69 (154/100 000) is still among the lowest in the industrialized countries, the age-standardized mortality from cerebrovascular diseases (107/100 000) is now one of the highest in these countries.[1] It is therefore of interest to know what changes have occurred in risk factors for cardiovascular diseases during the last decades. In the 1960s five Yugoslavian cohorts were enrolled in the Seven Countries Study, one in Dalmatia, one in Slavonia and three in Serbia.[2-4] All cohorts were re-examined after 5 and 10 years of follow-up. However, only the Serbian cohorts have been re-examined after 25 years. The changes in risk factors in these three cohorts over 25 years are reported here.

METHODS

In 1962, 511 (response 97%) men aged 40–59 in the rural village of Velika Krsna, south of Belgrade, were examined for the Seven Countries Study.[4] In 1963, 516 men (response 98%) aged 40–59, working in a large cooperative in Zrenjanin, north of Belgrade, participated in the Study. In 1964, 538 (response 80%) faculty members of the University of Belgrade aged 40–59 were enrolled in the study. These cohorts were re-examined after 5, 10 and 25 years. The response rates of the survivors during the re-examinations after

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5 and 10 years were more than 90%. At the 25-year re-examinations the response rates were 94% in Velika Krsna, 80% in Zrenjanin, and 77% in Belgrade.

Total cholesterol was determined in serum from a fasting blood sample at the baseline examination and during 5 and 10 years of follow-up.[2] Aliquots of 0.1 ml serum were put on Whatman filter paper and transported to the Central Laboratory in Minneapolis, MN. Cholesterol determinations were carried out according to the Abell-Kendall method modified by Anderson and Keys.[5,6] During the 25-year follow-up surveys non-fasting blood samples were obtained. Serum was prepared and stored at −20°C during the fieldwork periods of 1 week. The sera were transported in dry ice by plane to the Netherlands and stored at −80°C until analysis. Cholesterol determinations were carried out enzymatically using the CHOD-PAP Monotestkit (Boehringer Mannheim)[7,8] in the Lipid Laboratory of the Department of Human Nutrition, Agricultural University, Wageningen. This laboratory is standardized according to the criteria of the WHO Lipid Standardization Laboratory, Centers for Disease Control, Atlanta, GA.

Blood pressure was measured with an ordinary mercury sphygmomanometer in the supine position at the end of the physical examination as described in the Seven Countries Study protocol.[2] Height and weight were measured according to the Seven Countries Study protocol[2] and body mass index (BMI) (weight/height^2) was calculated. Information about smoking habits was obtained during the physical examination by asking questions according to Seven Countries Study protocol.[2] During the 25-year follow-up survey in Velika Krsna the participant was only asked whether he smoked. No information was collected on the number of cigarettes smoked.

Changes in risk factor levels were studied. First the average risk factor levels over 25 years of men aged 40–59 at baseline were plotted. In order to separate secular trends from trends due to ageing, risk factor levels of men aged 50–59 in 1962–1964 and 1972–1974 were compared. The same was done for men aged 65–69 in 1972–1974 and 1987–1989. To obtain information about selection, the participating survivors during the 25 years of follow-up were compared with the non-participants with respect to risk factor status at the beginning of the study. Non-participants were defined as the sum of the men who died during 25 years of follow-up and the non-responders among the survivors who were invited for the 25-year follow-up survey. Groups were compared using t tests. The statistical analyses were carried out on a personal computer using SPSS/PC+ package programs.[9]

RESULTS

Average risk factor levels over 25 years of men aged 40–59 at baseline are shown in Figures 1–4. At the baseline examination the average serum total cholesterol level of the Belgrade cohort was >1 mmol/l higher than that of the men in Zrenjanin and Velika Krsna (Figure 1). After the second examination

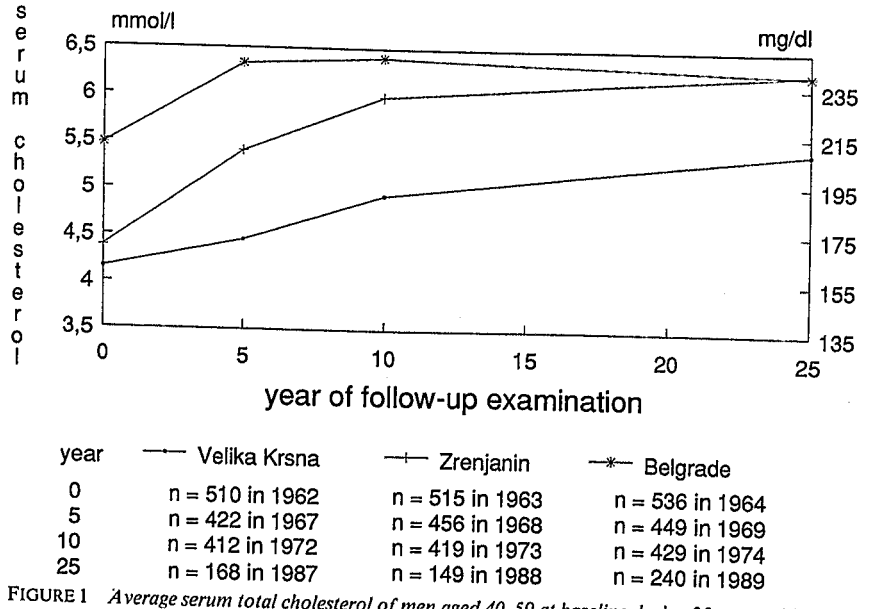

FIGURE 1 *Average serum total cholesterol of men aged 40–59 at baseline during 25 years of follow-up in the Serbian cohorts of the Seven Countries Study*

the average serum cholesterol level of the Belgrade professors stabilized at about 6.2 mmol/1. In Zrenjanin an increase of about 2 mmol/l was observed during the 25-year follow-up period. The increase was larger during the first 10 years of follow-up than in the second 15 years. In Velika Krsna a more gradual increase in serum cholesterol was observed from about 4 to about 5.5 mmol/l between 1962 and 1987.

At the baseline examination the average systolic blood pressure (SBP) was similar in all three Serbian cohorts (Figure 2). In these cohorts average SBP level increased, as expected, during follow-up. The largest increase was observed in Zrenjanin and the smallest in Belgrade. The average difference in SBP between Zrenjanin and Belgrade was >20 mm Hg at the 25-year follow-up survey. Large differences in BMI were observed between the three cohorts at the baseline examination (Figure 3). The BMI was lowest among farmers in Velika Krsna, intermediate among workers in Zrenjanin and highest among Belgrade professors. The BMI did not change much during 25 years of follow-up.

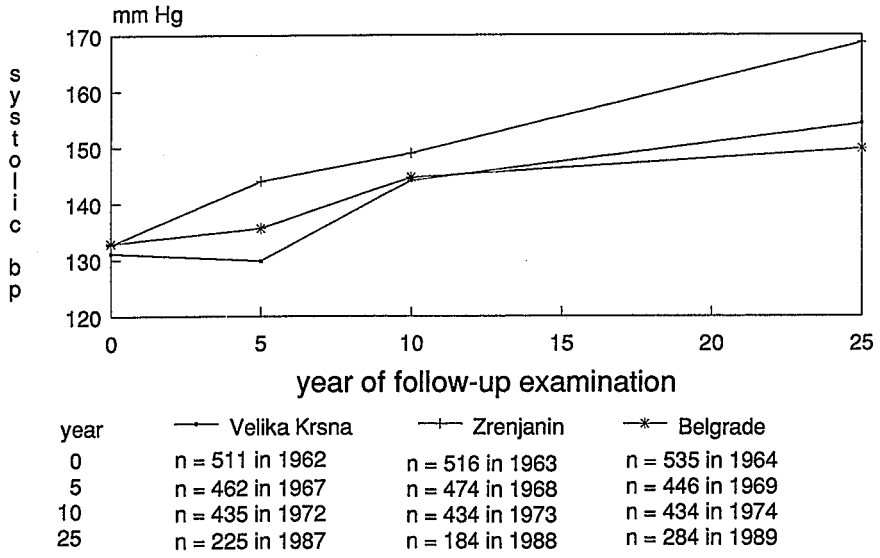

FIGURE 2 *Average systolic blood pressure of men aged 40–59 at baseline during 25 years of follow-up in the Serbian cohorts of the Seven Countries Study*

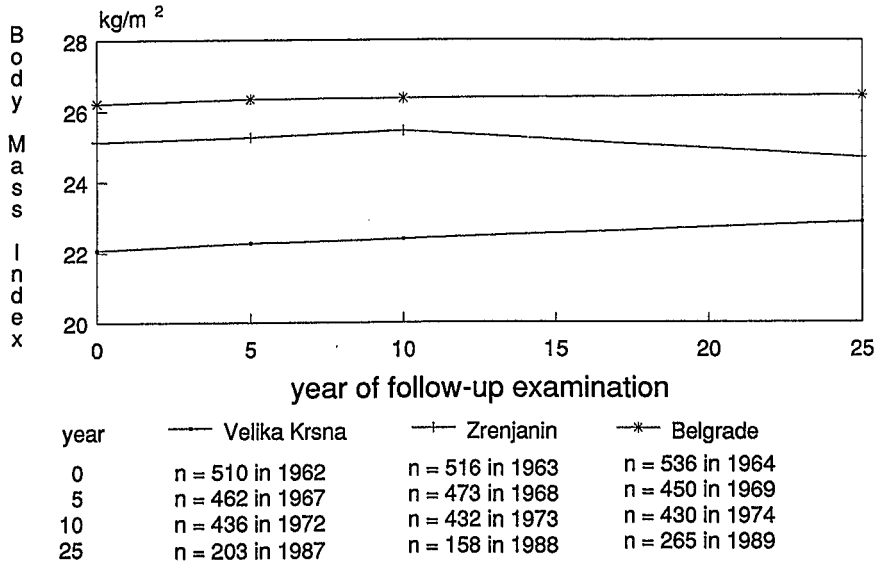

FIGURE 3 *Average body mass index of men aged 40–59 at baseline during 25 years of follow-up in the Serbian cohorts of the Seven Countries Study*

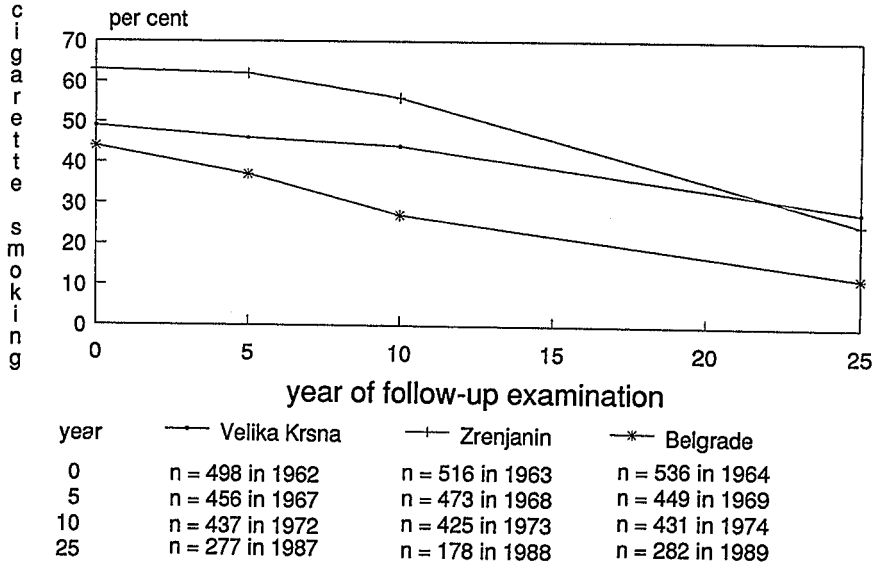

FIGURE 4 *Percentage cigarette smokers among men aged 40–59 at baseline during 25 years of follow-up in the Serbian cohorts of the Seven Countries Study*

The percentage of cigarette smokers at the baseline examination varied between about 60% in Zrenjanin and about 40% in Belgrade (Figure 4). During 25 years of follow-up the percentage of smokers decreased among the survivors of all three cohorts. However, the decrease among the Belgrade professors was much larger than among the survivors of the other cohorts. At the 25-years follow-up only about 15% of the surviving Belgrade professors smoked cigarettes compared with about 30% of survivors of the cohorts in Velika Krsna and Zrenjanin.

Comparisons of risk factor levels of men aged 50–59 examined at baseline and 10 years later showed, in all three cohorts, a significant increase in serum total cholesterol and in Velika Krsna a significant increase in blood pressure and in BMI (Table 1). Significant changes in the prevalence of cigarette smoking were not observed.

In men aged 65–69 examined 15 years apart a significant increase in serum total cholesterol was observed in Velika Krsna and Zrenjanin but not in Belgrade (Table 2). Systolic blood pressure increased signifi-

TABLE 1 *Risk factor levels of men aged 50–59 in the Serbian cohorts of the Seven Countries Study between 1962–1964 and 1972–1974*

	No.	Mean	SD	No.	Mean	SD	t value
Velika Krsna		1962			1972		
Total cholesterol (mmol/l)	287	4.17	0.83	192	4.94	0.91	9.55***
Systolic blood pressure (mm Hg)	288	135.0	19.9	203	139.6	22.3	2.39*
Body mass index (kg/m^2)	288	21.8	2.6	203	22.5	2.7	2.89**
Cigarette smokers (%)	279	49.5	50.1	203	47.3	50.1	-0.48
Zrenjanin		1963			1973		
Total cholesterol (mmol/l)	267	4.4	0.8	219	5.95	1.15	17.43***
Systolic blood pressure (mm Hg)	268	138.2	20.9	224	141.0	21.5	1.46
Body mass index (kg/m^2)	268	25.0	3.5	223	25.4	3.8	1.21
Cigarette smokers (%)	268	63.8	48.2	220	57.7	49.5	-1.37
Belgrade		1964			1974		
Total cholesterol (mmol/l)	227	5.59	1.15	253	6.35	1.04	7.59***
Systolic blood pressure (mm Hg)	227	137.5	19.4	255	140.9	20.2	1.88
Body mass index (kg/m^2)	227	26.4	3.2	252	26.2	2.7	-0.74
Cigarette smokers (%)	227	35.7	48.0	253	32.0	46.8	-0.85

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

cantly in Zrenjanin, however BMI did not change in the elderly men in the three cohorts over 15 years. The prevalence of cigarette smoking decreased significantly in Zrenjanin but not in Velika Krsna and Belgrade.

Comparisons between risk factor levels at the baseline examination of participating survivors and non-participants at 25 years of follow-up showed the expected differences (Table 3). Initial age, serum cholesterol, SBP and cigarette smoking were lower in participating survivors compared with non-participants. These differences were statistically significant with the exception of serum cholesterol in Velika Krsna. Body mass index did not differ between participating survivors and non-participants at the baseline examination.

TABLE 2 *Risk factor levels of men aged 65–69 in the Serbian cohorts of the Seven Countries Study between 1972–1974 and 1987–1989*

	No.	Mean	SD	No.	Mean	SD	t value
Velika Krsna		1972			1987		
Total cholesterol (mmol/l)	114	4.87	0.97	74	5.69	1.19	5.12***
Systolic blood pressure (mm Hg)	119	148.9	21.7	97	153.7	25.3	1.47
Body mass index (kg/m^2)	119	22.3	2.7	89	22.6	2.4	0.82
Cigarette smokers (%)	119	39.5	49.1	98	36.7	48.5	−0.27
Zrenjanin		1973			1988		
Total cholesterol (mmol/l)	72	5.91	1.27	72	6.58	1.32	2.98**
Systolic blood pressure (mm Hg)	75	158.1	25.0	74	169.8	29.6	2.61**
Body mass index (kg/m^2)	74	25.4	4.3	66	25.3	3.3	−0.22
Cigarette smokers (%)	73	48.0	50.3	70	24.3	43.2	−3.02**
Belgrade		1974			1989		
Total cholesterol (mmol/l)	63	6.2	1.19	130	6.36	1.03	0.93
Systolic blood pressure (mm Hg)	65	149.8	19.4	154	147.6	19.5	−0.74
Body mass index (kg/m^2)	65	26.9	3.3	146	26.4	2.7	−1.18
Cigarette smokers (%)	65	18.5	39.1	154	16.9	37.6	−0.28

** $P < 0.01$; *** $P < 0.001$.

TABLE 3 *Risk factor levels of participants and non-participants at the baseline survey of men aged 40–59 in the Serbian cohorts of the Seven Countries Study*

	Participants			Non-participants[a]			
	No.	Mean	SD	No.	Mean	SD	t value
Velika Krsna							
Age	227	47.0	5.7	284	52.2	5.5	10.34***
Total cholesterol (mmol/l)	226	4.07	0.77	284	4.19	0.85	1.64
Systolic blood pressure (mm Hg)	227	127.5	13.8	284	134.0	20.6	4.19***
Body mass index (kg/m^2)	227	22.3	2.6	283	21.9	2.7	−1.78
Cigarette smokers (%)	221	38.0	48.7	277	58.1	49.4	4.55***
Zrenjanin							
Age	184	47.2	4.9	332	50.3	5.3	6.70***
Total cholesterol (mmol/l)	184	4.22	0.78	331	4.44	0.87	2.97**
Systolic blood pressure (mm Hg)	184	127.0	13.0	332	135.8	20.6	5.95***
Body mass index (kg/m^2)	184	24.9	3.4	332	25.3	3.8	1.20
Cigarette smokers (%)	184	49.5	50.1	332	70.8	45.5	4.78***
Belgrade							
Age	287	46.6	5.3	249	49.1	5.8	5.31***
Total cholesterol (mmol/l)	287	5.31	1.00	249	5.58	1.11	2.89**
Systolic blood pressure (mm Hg)	286	129.2	13.7	249	137.0	20.9	5.00***
Body mass index (kg/m^2)	287	26.3	2.7	249	26.2	3.2	−0.41
Cigarette smokers (%)	287	35.9	48.1	249	52.6	50.0	3.93***

[a] Non-participants = deceased + non-respondents.
** $P < 0.01$; *** $P < 0.001$.

DISCUSSION

The purpose of the present study was to document changes in major risk factors of cardiovascular diseases in the Serbian cohorts of the Seven Countries Study over a 25-year period. This is of interest because of the dramatic increase in cardiovascular disease mortality observed in the former Yugoslavia during the period 1970–1984.[1] In this study we investigated whether changes in major risk factors in population samples are consistent with changes in cardiovascular disease mortality in the population at large.

Studying long-term trends in risk factors in ageing cohorts has several limitations. Changes in risk factor level, due to secular trends and to ageing itself, can only be separated out when sufficiently large age ranges are studied and when the original sample remains representative for the total population over time. Also quality control of the measured risk factors is of paramount importance. Only when good quality control of the measurements over time is guaranteed can observed changes be called real and not ascribed to changes in methodology.

The largest changes in the Serbian cohorts were observed in serum total cholesterol (Figure 1). In the 1960s and early 1970s increases in serum cholesterol of between 14 and 35% were observed in men aged 50–59 (Table 1). Increases of between 3 and 17% were noted in men aged 65–69 in the 1970s and 1980s (Table 2). Taken together these results suggest that serum cholesterol levels increased by about 15% in Belgrade, about 35% in Velika Krsna and about 45% in Zrenjanin over the 25 years. These changes are not due to ageing because they were calculated by comparing the data of men aged 50–59 examined at baseline, with those of men who had reached this age 10 years apart and of men aged 65–69 examined 15 years after each other. It is unlikely that these increases can be ascribed to changes in methodology. In the 1960s and early 1970s the determinations were carried out in one laboratory in Minneapolis using a modification of the reference Abell-Kendall method. The determinations for the 25-year follow-up examinations were also carried out in one standardized laboratory producing cholesterol values equivalent to Abell-Kendall.[10]

Systolic blood pressure increased with age (Figure 2). In men aged 50–59 an increase in SBP of 2–3% was observed in all three cohorts between 1962–1964 and 1972–1974. This increase reached statistical significance in Velika Krsna only. In men aged 65–69 SBP increased by 7% in Zrenjanin between 1973 and 1988. During this period significant differences were not observed in Velika Krsna and Belgrade. During the 25 years blood pressure was always measured according to the Seven Countries Study protocol. It is therefore unlikely that the increase in blood pressure can be ascribed to methodological changes. With the exception of a small increase in BMI during the first 10 years in Velika Krsna no changes in BMI were observed during the 25-year period.

The percentage of smokers decreased with age (Figure 4). No change in the percentage of cigarette smokers was observed between 1962–1964 and 1972–1974 among men aged 50–59 in the three cohorts. Also no change was observed in the percentage of cigarette smokers in men aged 65–69 in Velika Krsna and Belgrade between 1972–1974 and 1987–1989. The prevalence of smokers was reduced by 50% in men aged 65–69 in Zrenjanin between 1973 and 1988. These results suggest that the reduction in the prevalence of smokers in the three cohorts over 25 years is mainly due to ageing and not to a secular trend.

Comparison of risk factor levels of participating survivors and non-participants in the 25-year follow-up study showed that, with the exception of BMI, risk factor levels at the baseline examination were more favourable in survivors. This leads to questions about the representativeness of the elderly survivors. They are probably representative for the elderly men in the populations they represent e.g. farmers in rural Serbia (Velika Krsna), workers at a large cooperative (Zrenjanin) and faculty members of the University in Belgrade. Between these three cohorts large differences in mortality from cardiovascular diseases became apparent during the course of the follow-up. After 5 and 10 years of follow-up cardiovascular disease mortality was relatively low in the three Serbian cohorts compared with the other cohorts of the Seven Countries Study.[3,4] After 25 years of follow-up cardiovascular disease mortality was high in Zrenjanin, intermediate in Velika Krsna and low in Belgrade (Menotti A, personal communication). These results are consistent with the changes in major risk factors observed during the 25 years.

In the early 1960s the serum total cholesterol levels of the middle-aged men in Zrenjanin and Velika Krsna were among the lowest in the Seven Countries Study.[2–4] The results of the MONICA study showed that in the 1980s the average total cholesterol levels of middle-aged men in the Eastern European Countries, e.g. Czechoslovakia, Hungary and Poland, varied between 5.4 and 6.4 mmol/l.[11] In these countries, as in Yugoslavia, mortality from cardiovascular diseases increased in the period 1970–1984.[1] In the 1980s the cholesterol levels in the Eastern European countries were 1.5–2.0 mmol/l higher than those observed in middle-aged men examined in Velika Krsna and Zren-

janin 25 years earlier[2-4] and similar to those of recently examined elderly men in these Serbian cohorts. It is therefore very probable that in rural Serbia large changes in serum cholesterol occurred in the last 25 years. These changes probably occurred earlier in the highest social class. The 1 mmol/l higher average serum cholesterol levels of the faculty members of Belgrade University in the early 1960s support this hypothesis.

The average SBP levels of the middle-aged men in the three Serbian cohorts at the baseline examination were comparable with those of middle-aged men in Novi Sad, examined in the MONICA study in the 1980s.[11] Novi Sad is a city close to Belgrade and Zrenjanin. The average BMI of the men in Novi Sad was similar to that of the Belgrade professors but higher than that of the men in Zrenjanin and Velika Krsna. These results suggest that BMI probably increased over the last 25 years in middle-aged men from the middle and lower social classes. The percentage of smokers in Novi Sad was 49%. This is comparable with the percentage of cigarette smokers 25 years ago in Velika Krsna and Belgrade but lower than in Zrenjanin.

The results of the present study showed that the largest change in risk factors occurred in serum total cholesterol. The increase varied between 15% in Belgrade and 45% in Zrenjanin. Intervention studies have shown that a change of 1% in serum cholesterol is associated with a 2% change in coronary heart disease incidence.[12] This means that changes of between 30 and 90% in coronary heart disease incidence are compatible with the changes in serum cholesterol in the Serbian cohorts. The 9% increase in SBP in Zrenjanin is compatible with a 25% increase in coronary heart disease incidence and a 75% increase in the incidence of stroke.[13] No major secular trends were observed in the prevalence of cigarette smoking and in average BMI.

The results of the present study show that within Serbia, large differences in trends in risk factors exist in different socioeconomic strata. It is therefore of interest to study the dynamics of risk factors within different socioeconomic groups within one country. However, the changes observed in serum cholesterol and SBP in the Serbian cohorts of the Seven Countries Study during the last 25 years are compatible with the large increase in cardiovascular disease mortality noted between 1970 and 1984 in Yugoslavia.

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