Population Studies on Serum Cholesterol and Dietary Fat in Yugoslavia

By JOSEF BROŽEK, PH.D.,* RATKO BUZINA, M.D.,† AND FEDOR MIKIČ, M.D.†
With the Technical Assistance of AGNEZA HORVAT, MAG. PHARM.,† MIA ZEBEC, MAG. PHARM.,‡ AND MALEMPATI M. RAO, M.A.§

POPULATION differences in regard to serum cholesterol are considered important from the point of view of public health.[1] While the level of serum cholesterol can not serve as a reliable criterion of blood vessel integrity in individuals[2] (cf. 2), it was found useful as an index of the so-called atherogenic potential in population studies.[3] The observed differences in cholesterol have been related to the fat content of the diet but previous population studies concentrated on the amount rather than the kind of fat.[4]

The principal aim of this study was to examine the relative effects on serum cholesterol of different dietary fats in populations sharply differentiated in their fat consumption. Opportunities for such a field study presented themselves in the fall of 1953 in Croatia, with its marked contrasts between the various areas: the fertile, corn and hog-raising Northeastern area; (the "animal fat" region); the Adriatic islands where the climate is ideally fitted for growing olive trees but little else except some vegetables, fruit (figs and grapes), and nuts (almonds); the "olive oil" region, and the barren mountain area off the Adriatic coast, which until very recently was economically underdeveloped, its agriculture unproductive and which was characterized by a low intake of all kinds of fat.

From the Laboratory of Physiological Hygiene, School of Public Health, University of Minnesota, Minneapolis, Minn.

* Professor School of Public Health, University of Minnesota, Minneapolis.

† Central Institute of Hygiene, Zagreb.

‡ School of Public Health, University of Zagreb.

§ Statistics.

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Qualitatively, the contrast between the "animal fat" region near the Hungarian border and the "olive oil" island region is very clear and definite, and attention will be focused principally on the comparison of these two areas.

DIET

The three dietary regions were selected on the basis of information available at the Division of Food and Nutrition, Central Institute of Hygiene, Zagreb, supplemented by the preliminary results of a food consumption survey organized by Ferber in 1952[5] and by contacts with the local public health authorities.

The town of Dalj, near Osijek, served as a prototype of settlements in the "animal fat" area. The villages of Boraje, Lepenica and Vrsno in the Dalmatian Hinterlands, some 20 miles East of Sibenik, are located in the "low-fat" region. Sali, on Long Island (Dugi Otok), was considered a good representative of the "olive oil" area.

Even though substantial variations are present within each region, Ferber's study[5] contains data useful for the characterization of the animal-fat and low-fat areas. In 1952 in the Osijek (animal-fat) region the average daily per capita caloric intake was 2,763 cal as contrasted with the value of 1,952 cal for Dalmatia's low-fat area. This difference is reflected in the adult body weight, as will be shown in the anthropometric description of the subjects.

The total protein intake in these two areas was 97 and 69 g per day, with a still more striking contrast in animal protein (52 vs. 19 g). The reported carbohydrate intake was

similar, with 352 and 343 g per day while consumption of milk (307 vs. 98 ml/day), fresh meat (100 vs. 20 g /day), and fat (98 vs. 26 g/day) differed markedly.

In the Osijek (animal-fat) region a substantial amount of fat is consumed in the form of smoked bacon, fat pork meat, ham, and dairy products while the use of vegetable oils is negligible. In the selected locality (Dalj) the consumption of pork, meat, and lard is very high in comparison with either the Dalmatian Hinterlands (low-fat area) or Long Island (olive-oil area). In the region of continental (low-fat) Dalmatia in which the field work was done the dietary picture was somewhat complicated by the recent opportunities for some of the peasants to work on factory construction at Ražine near the coast. This provided additional income enabling a limited number of families to raise somewhat their standard of living. Both lard and some olive oil were used in this area.

Sali, on Long Island, representative of the "olive-oil" region, is a farming-fishing community. "Farming" involves growing grapes and vegetables, (including a spinach-like plant called *blitva*, available in abundance late into the fall), and the care of extensive groves of olive trees. Fish is an important source of fresh meat. According to information obtained in interviews with the men in our sample group, olive oil represented about 90 to 95 per cent of all fat that is used as such in the household. On the basis of a limited survey of 14 households, selected at random, the average per capita intake of olive oil was estimated at about 85 g/day.

In the majority of households olive oil is the only visible fat used. It is highly appreciated and is plentiful, except following olive-crop failures. In some households animal fat is used for a short time around Christmas when a lean hog is butchered. Our field work was carried on during October.

The concrete information available for the specific localities in which the investigations were made was, in itself, limited. However, the differences in the agricultural practices and the dietary aspects of the mode of life in the "animal-fat" and "olive-oil" areas are very striking and are probably as large as can be found between any two regions inhabited by peasants of the same national stock. The total fat intake in the "animal-fat" and "olive-oil" regions was very similar, representing about 30 per cent of the total calories in both regions. The fat in the "low-fat" region represented about one third to maximally one half of that consumed in the other two areas.

SELECTION OF SUBJECTS

An effort was made to obtain random samples of the subjects within each decade and area. In Sali (olive-oil area) and Boraje with its neighboring villages (low-fat area) the population census of 1953, in Dalj (animal-fat area) the list of voters was used as the basis. Names were then obtained taking every second, third, fourth man, according to the number of individuals engaged in agricultural work in each decade, until 25 to 30 names were obtained. These men received an invitation, using local administrative channels, to avail themselves of a special medical examination. The yield varied somewhat but approximated about 90 per cent, on the average. It was highest on the island ("olive-oil" area), where the ground work was prepared most adequately by prior correspondence and meetings with the town administration and invited influential citizens. It was lowest in the mountainous "low-fat" area. Unfortunately, hemolysis of some of the samples reduced the number of individuals for whom the cholesterol content of the blood could be determined.

For the sake of good public relations a few additional men not qualified either on account of age or occupational activity were examined. Their data were not considered here. A few individuals with infectious disease (tuberculosis) or fever were examined clinically but were not measured and were excluded from the sample.

METHODS

Body Measurements

The body measurements included in this study serve to characterize (1) size and bulk

(weight) of the body, (2) bony framework, (3) relative underweight-overweight and (4) leanness-fatness. Height, weight, bicristal diameter (as a measure of the bony width of the pelvic girdle), upper arm circumference, and the skinfolds at the back of the upper arm (over the triceps) and below the right scapula were measured in accordance with the preliminary recommendations of the Committee on Nutritional Anthropometry.[6] In addition, the width of the limb bones was characterized in terms of the epicondylar diameters of the humerus and femur. Chest circumference was measured at the level of the mammae, at the end of normal expiration. The level of the "waist" circumference was defined as midway between the top of the iliac crest and the ribs, along the midaxillary line. The "waist" skinfold was measured at the intersection of the waist circumference and the midaxillary line. Ventral skinfold was measured on the upper arm (over the biceps) at the level of the upper arm circumference.

Blood pressure was determined with a pressure cuff, calibrated against standard pressures.

Serum Cholesterol Determinations

Determination of cholesterol in blood was performed by a modification of the method of Bloor.[7] In this method there is no saponification and the results are somewhat higher than those obtained by the method of Abell *et al.*,[8] which is currently used in this Laboratory. The samples taken at the place of investigation were centrifuged and sent to the chemical laboratory in Zagreb. During transportation the samples were kept on ice. The maximum time before the analysis was eight days. Lipids were extracted from the serum using Bloor's mixture. The solvent was evaporated and the color was developed using Liebermann-Burchard reagent at 22° C and read at 625 mμ in the Beckman spectrophotometer.

Statistical Analysis

Standard methods of analysis were utilized for examining the relationship between age and cholesterol in each dietary region, and the differences between the regions.[10,11] The tests, in the form of appropriate F ratios, concern (1) the presence of significant linear age trends; (2) significance of deviations from linearity; (3) difference between slopes obtained for cholesterol in the three regions; (4) significance of the distance between the regression lines, provided the slopes are not significantly different.

CHARACTERISTICS OF THE SUBJECTS

The number of subjects in each decade and dietary region, for whom valid cholesterol determinations are available, is given in Table 1. The mean ages within each decade are very similar and would allow a direct comparison of mean values of serum cholesterol.

TABLE I

Number and Mean Age of Subjects for Each Decade and Region

Diet	Age, decades: Third	Fourth	Fifth	Sixth	Total
	Size of the sample (N)				
Animal fat	21	17	25	16	79
Low fat	9	14	23	18	64
Olive oil	17	21	18	10	66
	Mean age				
Animal fat	24.7	33.7	43.7	53.6	39.0
Low fat	24.8	33.9	43.4	54.2	41.7
Olive oil	24.5	33.3	43.7	53.1	36.9

In respect to height, all three regions showed the usual trend toward lower values at higher ages but the decrements became marked only in the sixth decade. More interesting is the absence of substantial increments with age in body weight. As a consequence, the American weight standards appear to provide valid comparison for young men but not for the subsequent decades. Under these conditions it may be useful to use as a reference the ratio of weight to height based on grand means for the combined samples (N = 209) and compute the relative values of the weight-height index for each decade within the three regions. This was done in Table II.

Men from the "low-fat" area are clearly lighter no matter which criterion is used. The

TABLE II

Anthropometric Characteristics: Height (cm), Weight (kg), Relative Weight (Actual as Percentage of U. S. 1912 Standards), and Relative Weight/Height Index.

Diet	Age, decades Third	Fourth	Fifth	Sixth	Total
	Height, cm.				
Animal fat	168.7	172.1	168.3	166.8	168.9
Low fat	173.3	169.3	171.9	166.0	169.9
Olive oil	173.4	172.3	173.7	169.5	172.5
	Weight, kg				
Animal fat	66.1	67.8	67.4	65.2	66.7
Low fat	64.0	64.1	63.5	60.7	62.9
Olive oil	68.1	70.0	70.0	69.5	69.5
	Relative weight (% of U. S. 1912 standards)				
Animal fat	102.2	97.4	97.8	94.8	98.1
Low fat	94.3	95.1	88.8	89.0	90.5
Olive oil	100.4	100.5	94.8	98.2	98.6
	Relative weight/height index				
Animal fat	99.1	99.8	101.5	99.0	100.0
Low fat	95.0	95.9	93.6	92.6	94.3
Olive oil	99.5	102.9	102.1	103.9	102.1

TABLE III

Serum cholesterol, mg/100 ml, means (M) and standard deviations (SD).

Diet		Age, decades Third	Fourth	Fifth	Sixth	Total
Animal fat	M	223.6	240.6	235.7	258.1	238.1
	SD	40.4	50.5	45.6	56.9	
Low fat	M	197.4	211.2	211.0	227.5	213.8
	SD	27.9	32.5	37.4	28.4	
Olive oil	M	170.5	207.7	212.3	198.4	198.0
	SD	24.0	35.0	56.4	30.2	

TABLE IV

Age trends in serum cholesterol. Statistical tests of the presence of a significant linear trend and of the departure from linearity.

Diet	F-test Linear trend	F-test Departure from linearity
Animal fat	3.814†	0.533
Low fat	3.997*	0.836
Olive oil	6.370††	2.609

* Significant at 5% level.
† Approaches the 5% level of significance.
†† Significant at 1% level.

other two regions are similar to each other in weight, the "olive-oil" region exhibiting slightly higher mean values. Skinfolds, as more direct measures of leanness-fatness, are thickest in the "animal-fat" area but the difference between this and the "olive-oil" region are small. The subjects from the "low-fat" area are thinnest and the values of their circumferences and bony dimensions suggest a less robust body build. In terms of the bicristal diameter/height index subjects from the "animal-fat" region are somewhat more "stocky", with a mean of 17.6 as compared with 17.2 for the other two regions. This mean difference is small but it is statistically significant.* Regional differences in blood pressure are also small, with a suggestion of higher values in the "animal-fat" area.

RESULTS

Means and standard deviations of serum cholesterol for each decade and dietary region are given in Table III. Throughout, the means are highest for the "animal-fat" and lowest for the "olive oil" region, with the low-fat region being intermediary. The only case of overlap is at the fifth decade where the mean of the "olive-oil" group is somewhat higher than that of the "low-fat" group. While within each decade the mean ages are very similar (see Table I), the number of individuals in each decade varies. As a consequence, the mean ages for the three regions differ and the grand means for cholesterol (Table III) are not directly comparable.

In order to combine the data for all the age groups and to test statistically the significance between regions, a combination of regression analysis and analysis of variance was used. In all three regions a linear age trend is present which closely approaches or exceeds the five per cent level of statistical significance (left column in Table IV). Further analysis is substantially simplified by the fact that the age trends do not depart significantly from linearity (right column in Table IV).

Regression equations for cholesterol and age

* $F = 14.62$ (for 1 and 143 degrees of freedom, $F_{0.01} = 6.82$).

are given in Table V (left column). At a constant age of 40 years, which is close to the mean age of the three regional samples, there is a difference of 38 mg/100 ml between the results obtained from the "animal-fat" and "olive-oil" regions.

No significant differences were observed between the rates of increase in cholesterol with age (Table VI, left column). Consequently it is legitimate to use a common slope (Table VII). The difference between the cholesterol levels adjusted for age is about the same as when separate slopes for the two areas were used, i.e., 38 mg/100 ml. Statistically the differences between the levels of the regression lines are significant in the comparisons of "animal-fat" vs. "olive-oil" and "animal-fat" vs. "low-fat" areas (Table VI, right column).

TABLE V

Regression equations for serum cholesterol ($\hat{Y}$) and age (X, in years)

Diet	$\hat{Y} = a + bX$	$\hat{Y}$ for X = 40
Animal fat	200.489 + 0.972X	239.4
Low fat	180.581 + 0.795X	212.4
Olive oil	152.518 + 1.233X	201.8

TABLE VI

Differences between dietary regions in regard to the slope (b), and the level of regression lines (intercept, a, with a single common slope).

Diet	Difference between slopes, F_b	Difference between levels, F_a
Animal fat vs. Olive oil	0.135	26.435*
Animal fat vs. Low fat	0.070	14.730*
Low fat vs. Olive oil	0.410	2.287

* Significant at 1% level.

TABLE VII

Regression equations for serum cholesterol ($\hat{Y}$) and age (X, in years), using a common slope (b_c)

Diet	$\hat{Y} = a + b_cX$	$\hat{Y}$ for X = 40
Animal fat	198.221 + 1.030X	239.4
Low fat	170.751 + 1.030X	212.0
Olive oil	159.974 + 1.030X	201.2

DISCUSSION

It is now well established that variations in the proportion of calories contributed by fats tend to be reflected directly in the mean serum cholesterol concentration of many populations: peoples whose diets are low in fats tend to have lower serum cholesterol values than do peoples whose diets are high in fats. Similarly, when persons in a "high-fat" country such as the United States go on a low-fat diet, the serum cholesterol tends to fall and to stay low if the low-fat diet is continued. But these facts have to do, in general, with differences in the amounts and not in the kinds of fats in the diet. On the whole, populations consuming high-fat diets are very prosperous and their diets are high in fat because they can indulge in the more expensive foods of animal origin, and the "richest" parts of those foods (e.g., cream, butter, well-fattened meats). When such people are forced to go on a low-fat diet, either because of economic change or on medical advice, they reduce the fat in the diet by cutting down the obviously fat, "luxury" foods. Conversely, when a poor population subsisting on a cheap low-fat diet becomes more prosperous, one of the first results is a change to a diet containing more of the luxury foods, i.e., more fat meats and dairy products.

In all of these circumstances a proportionality between total fats and serum cholesterol tends to prevail. A high-fat diet in which animal fats represent only a small part of the total fats is a rare situation among populations though it is readily contrived in experiments. Experiments on man with such diets have shown considerable differences between the cholesterogenic effects of different dietary fats. With almost equal amounts of fats in the experimental diets, Groen and others[12] observed lower serum cholesterol values when their subjects were on vegetarian diets than on ordinary mixed diets. Kinsell and others reported a lowering of the serum cholesterol when hospitalized patients were fed on a synthetic diet containing large amounts of cottonseed and soy oil[13,14–16]. In this Laboratory, Keys and Anderson carried out two six-month experiments on 23 and 26 men respectively and found significantly lower cholesterol values

with cottonseed oil than with butterfat in the diet, even though calories, proteins, total fats and dietary cholesterol were constant. At 36 to 37 per cent of total calories from all fats and three-fourths of the fat being either butterfat or cottonseed oil, the mean serum cholesterol value for 26 men was 240.7 (±8.0) mg per 100 ml on butterfat and 195.9 (± 6.7) on cottonseed oil. Finally, it was observed that lower serum cholesterol averages were found for men in Naples, whose diets averaged 20 per cent of total calories, than for men in dietary experiments in Minnesota on diets equivalent in total fats.[17]

This was the background for the present study. Since it was completed there have been several reports indicating that the proportion of saturated to unsaturated fatty acids in the dietary fats may be a major determinant of the cholesterogenic effect.[18–21] Many animal fats, including butterfat and probably the fats of all common meat animals (but not of fish), are relatively saturated. Vegetable fats run all the way from very saturated (coconut oil) to relatively unsaturated. Olive oil is moderately unsaturated, with an iodine number of 79–88.

The present results are, of course, compatible with the theory that the relative saturation of the fatty acids is important in fixing the serum cholesterol level. Undoubtedly, the mean saturation of the fatty acids in the diet of the people in the "animal fat" region was considerably greater than fats in the diet in the "low-fat" region, but uncertainties on this point, as well as the complications introduced by different calorie intakes, make deductions from the "low-fat" region hazardous.

SUMMARY

Serum cholesterol was determined in clinically healthy subjects in three localities of rural Croatia, markedly differing in the fat content of the diet. Two of the areas were closely similar in the total amount of fat, estimated at 85 to 100 g/day, but differed sharply in the kind of fats used.

In the "animal-fat" area (N = 79), over 90 per cent of the fat intake was of animal origin. It was consumed in the form of lard, fat pork meat, smoked bacon and ham, and dairy products.

In the "olive-oil" area (N = 66) this vegetable oil, containing a substantially higher proportion of unsaturated fatty acid, covered a similarly high proportion of the total fat consumption. There was very little of dairy products and sea fish served as a principal of fresh meat.

In the third, "low-fat" area (N = 64) both animal and vegetable oil were used, in a total amount estimated to be below 50 per cent of that consumed in the other two regions.

Statistical analysis indicated that (1) significant linear age trends in serum cholesterol were present, (2) within the given age limits (19 to 59 years), there was no significant departure from linearity in the age trends of serum cholesterol for any area, (3) differences between linear slopes were not significant, but (4) the level of regression lines was significantly higher in the "animal-fat" than in the "low-fat" or the "olive-oil" areas. The corresponding age-adjusted values of serum cholesterol for age 40 years were 239, 211 and 201 mg/100 ml, respectively.

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