dietary studies and epidemiology of heart diseases

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INTRODUCTION

by A. KEYS

Dietary surveys are made for many different purposes, most commonly to reveal the extent and frequency of nutrient inadequacies in the diets of the populations surveyed. Dietary deficiencies were not the focal point of interest in the surveys reported in this volume, and, in fact, dietary deficiencies did not prominently appear in any of the samples studied. The major purpose of the currently reported surveys was to characterize the diets of the men in terms of nutrient items that may be relevant to atherogenesis and its complications.

One of the most intensely debated and potentially important theories of medical science is that the amount and kind of lipids in the habitual diet has a powerful influence on atherogenesis and its eventual clinical complications, notably coronary heart disease. The original idea of such a relationship came from experiments on animals and all over the world much information is being provided from dietary experiments on a wide variety of animal species but the actual relevance to men of such experiments is uncertain. The facts in regard to the human disease and its relationship to the diet of man must come from studies on man himself.

According to the theory as commonly stated, the serum cholesterol concentration is a major but not necessarily the only intermediary link between the diet and susceptibility to atherosclerosis and its complications. Several facts are now abundantly clear. The risk of future heart attacks is strongly related to the serum cholesterol level (1, 2, 4, 5, 9, 10, 11). And the serum cholesterol concentration of man is readily altered by changes in the lipids in the diet. Under controlled conditions the average serum cholesterol response to a change in the diet can be predicted rather well from the fatty acid and cholesterol composition of the diets exchanged (3, 6, 8). But to what extent, if any, do these facts find reflections in differences in average serum cholesterol levels and in the frequency of coronary heart disease in contrasting populations?

In an effort to answer these and many other questions, an international cooperative research program on cardiovascular epidemiology was started in 1958. The general idea was to examine essentially all men aged 40-59 in geographically defined areas selected as probably affording large contrasts in the frequency of coronary heart disease and/or in the diet, and to follow the subsequent disease experience of these men. The eventual goal was to discover the relationship between the incidence of disease and the pre-disease characteristics of the men, including such items of the mode of life as the habitual diet. The organization, methods, and initial examination findings in the samples in seven countries have been reported recently (7).

An essential part of this research program was the quantitative ascertainment of nutrients in the usual diets characteristic of the men in the several samples. Because it would be impracticable to study in detail the diets of all men in the samples — which ranged from over 500 to almost 1000 men each — recourse was had to subsampling. Accordingly, the program involved the collection of dietary data on statistical sub-samples of the men so as to be able to characterize them in terms of averages and standard deviations for calories, proteins, total fats and the fatty acids in the habitual diets. The results of these studies, which originally appeared separately in various issues of VOEDING, are brought together in this volume.

The basic method was the same for all of the samples. As noted above, sub-samples were drawn for detailed dietary studies from the men who had been examined for follow-up in regard to the development of disease and death. Because of the sampling procedure used, it is believed that the statistics for the dietary intakes found for the sub-samples should be reasonably applicable to describe the whole samples.

Seven consecutive days of household weighings of all foods consumed by each individual provided the basic data. Such seven-days surveys were repeated at various times of the year so as to cover the seasons of the year. As will be seen, seasonal variations in the nutrients considered proved to be surprisingly small. Though there were seasonal differences in individual food items and in total calories, the proportions of calories provided by proteins, fats and carbohydrates varied little.

These repetitions of the surveys provided data for the analysis of the intra- as well as inter-individual variations, in regard to diet, of the men. Intra-individual variation was found to account for most of the variance, within each of the samples, of the nutrients considered. In fact, within any one sample it was not possible reliably to distinguish individuals as being relatively high (or low) in protein or fat proportions in the diet. This fact, together with the observed, and expected, rather substantial intra-individual variation in serum cholesterol levels, also means that it is nearly useless to attempt an analysis of the relationship, within any one sample, between dietary factors and serum cholesterol concentration. On the other hand, when the averages of the samples are compared, differences between the averages of serum cholesterol concentration appear to conform reasonably well to expectations derived from controlled dietary experiments. In general, the average serum cholesterol concentration proved to be directly related to the average percentage of total calories provided by saturated fatty acids.

These findings on dietary lipids and serum cholesterol have important implications for the analysis of epidemiological data on the diet and the frequency of coronary heart disease. Within populations in which dietary customs are relatively homogeneous, serum cholesterol differences observed between individuals are largely unexplained by differences between the contemporary diets of those individuals. But when groups which differ substantially in the average diet are compared, the group average serum cholesterol values are explained, at least in considerable part, by the average diets.

When the program for these dietary studies was started it was felt that seven-day surveys would be essential to provide acceptable data to indicate the habitual diet. However, as will be seen, three-day surveys may do as well with populations such as considered here.

Because of doubts about the reliability of tables of food composition for application to widely differing populations, arrangements were made to collect and analyse chemically replicates or equivalent composites of the actual foods eaten by each man. At the same time tables of food composition were applied to the dietary data so that comparison could be made between the results of chemical analysis and those from calculation. It was found that calculations from suitable tables of food composition provide good estimates of the average calorie, protein and total fat intakes. Perhaps intakes of individual fatty acids can also be estimated acceptably from tabular values applied to the food records but comparative trials of methods in these studies were too few to allow final conclusions on this point.

In the report on the dietary studies in the Netherlands, below, it will be seen that careful use of a questionnaire-interview method can also provide calculated average values for total calories, proteins and fats that agree well with the averages from seven-day food weighings. It should be emphasized, however, that for success with the interview-questionnaire method well-trained dietitians are required and interviews must involve the wife or housekeeper as well as the subject himself. It should be noted also that the Dutch population studied in this way may be more adaptable to this method than, for example, the less sophisticated populations in some strictly rural areas.

In general, we believe that the data reported in the papers to follow are accurate estimates of the nutrient intakes of the individuals concerned at the times of the surveys. There remains the question as to how accurately these data represent the true habitual diets. In every case the men and their wives were carefully instructed at the outset not to depart in any way from their ordinary habits of food use. But it is still conceivable that there might have been some families in which the diet during the survey was "better" than their usual custom, "better" meaning more luxurious or richer. During the period of the survey, some housewives, perhaps unconsciously, might try to show how well they were feeding their husbands. Though there is no proof that such departure from the usual family custom did not occur in some cases, there is no evidence that this actually did happen. Moreover, in repeated surveys of the same individuals there was no general trend for the averages to change in respect to total meat or dairy product intakes or other indicators of relative luxury or "quality" of the diet.

If it is true that the dietary intake of lipids influences susceptibility to atherosclerosis and heart attacks, much of the effect must be a long-time influence, the cumulative effect of the diet over many years. For atherogenesis is slowly progressive and its development should be thought of in terms of decades, at least. Hence a question arises about the extent to which the diets recorded in these surveys properly represent the long-time average diets of these men. It is known, of course, that in some of the countries represented, notably Finland and the Netherlands, the average diets for some years in the 1940s were restricted in calories and were very low in fats. But such restrictions were least marked in rural areas such as studied in the present program. In the case of the Netherlands, the greatest wartime changes in the diet occurred in the metropolitan areas of the west, not in areas such as Zutphen studied in the present program.

Still, in all of the areas concerned here there undoubtedly were variations in the diets in the past twenty years and perhaps in the past decade. However, it is reasonable to believe that the differences reported here between the diets of the several areas are not merely contemporary differences; something like the same kind of differences can be postulated to have existed for the average of many past years. For a long time the diet in Finland has been high in butterfat; for still longer the diets in the Greek islands and in Dalmatia have been high in olive oil and low in meat fats. And in the rural areas of Croatia and Italy, where the people have always subsisted largely on their own local produce, it is probable that the general characters of the diet have changed little in the lifetime of the subjects studied here.

The difficulties and limitations of estimating nutrient intakes from dietary survey data are critically examined below in the article, "Dietary Survey Methods in Studies on Cardiovascular Epidemiology." In view of these limitations, and those mentioned above, it would be improper to claim that the survey data reported here are highly accurate representations of the true long-time diets of men in these areas. But, as will be seen, every effort was made to obviate controllable sources of error. Though the data on individuals have serious limitations, it is believed that the general picture is substantially correct for each of the samples, and for the differences among the samples.

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DIETARY SURVEY METHODS IN STUDIES ON CARDIOVASCULAR EPIDEMIOLOGY *)

by A. KEYS

Many methods are used in studies on the diets of populations. Though none can be universally recommended, most methods can be useful for one or another purpose; the choice of method requires careful definition of the purpose of the study as well as consideration of practicalities in the particular situation of interest. Unfortunately, practical limitations are often serious so methods that are relatively easy and cheap to apply tend to be favored over those that more nearly approach a theoretical ideal. Moreover, few studies are confined to a single, sharply defined purpose and compromise is made between methods that would be better for one or another of the purposes in mind.

The acquisition of dietary knowledge about populations generally involves several stages of refinement:

1) gross estimates of general characteristics and adequacy of food supplies emerge from food balance data on food production, import, export, spoilage and the diversion of foods from human consumption to industry and by wastage;

2) the distribution of these net food supplies between regions and between urban and rural segments of the population must be ascertained. The necessary methods, as well as those for the more general estimates in 1), above, do not concern us here;

3) nutrients rather than foods are the ultimate interest of most dietary studies so it is essential to translate foods into nutrient terms. Tables of food composition are generally used for these purposes but the available tables are not always complete or pertinent to some of the foods in local use. Moreover, such tables necessarily relate only to averages and may be grossly in error for particular areas, seasons, or food samples;

4) data on the distribution of actual nutrient intake by definable population segments are obtained by dietary surveys. The methods of choice will depend upon the population segments selected for attention — residents of particular areas, members of particular occupational or socio-economic groups, persons of particular ages, etc. — and more carefully specified purposes. The more widely used methods for dietary surveys are summarized below.

Reliability versus validity

A common criterion for judging the accuracy of any method of measurement is to repeat the measurement under the same conditions. While this test is an

*) Some data cited here were obtained with the help of research grants from the U.S. Public Health Service (HE-04697 and HE-04997), from the American Heart Association, New York, and from the California State Olive Advisory Board.

essential first step in the evaluation of a method, it allows only strictly limited conclusions about repeatability or reliability; it does not necessarily provide any evidence about validity, i.e. the true accuracy of the method as a measurement of the variable it is supposed to measure.

In pure chemistry and physics validation of measurement by independent methods, of unquestionable accuracy and specificity, is often relatively available. In nutritional surveys such validation is so much more difficult that, in fact, it is seldom attempted. But the danger of assuming validity from data on repeatability is obvious from elementary examples.

Erroneous values in tables of food composition produce estimates of nutrient intake that have low validity but such errors have no effect on reliability; erroneous estimates are repeatable. But even the best tables of food composition applied to completely accurate data on food consumption do not necessarily guarantee valid estimates of nutrient intake. In all tables of food composition the nutrient values listed are estimates of the average nutrient contents. But almost all foods vary considerably in the raw state and variation is increased by differences in preparation in the kitchen. This question is examined in more detail in the section, below, on "Tables of food composition".

When food consumption data are obtained from a survey of a proper, i.e. valid, sample of the population concerned, it is hoped that this sampling will also mean a sampling of the various foods such that the average of those foods reported in the survey will correspond with the average nutrient contents in the food tables. If the food tables have been checked by repeated analyses of local samples of the foods, the hope may be justified. Such assurance, including attention to seasonal variations, is rarely provided.

Absolute validity is the ultimate goal but for many purposes relative validity may suffice. For example, the purpose of a series of surveys may be to compare averages for populations or population segments in regard to the intake of one or more nutrients. Errors in the table of food composition used, or even in the reports of food consumption, may not invalidate comparisons if the errors are consistent and apply equally to the population samples being compared.

Unfortunately, the very nature of the population samples being compared generally tends to produce variations in the errors that apply to the different samples. Members of contrasting socio-economic classes in the same general population tend to differ in respect to the source, quality, methods of preparation and wastage of the foods they use. Though in carefully conducted surveys efforts are made to allow for such differences, few studies provide concrete evidence that such efforts have, in fact, compensated for such differential errors.

So far, we have assumed that the crude food data are accurate both as to items and as to quantity. But unless the food items have been actually recorded and measured, it is necessary to consider the accuracy of reporting. Here again repeatability is no guarantee of validity.

In response to almost all questions about personal customs, including food usage, people tend to report themselves as being more constant than they actually are.

With no effort to deceive, most people have a simplified image or their own habits and they so report themselves in questionaires and interviews. Since this image persists, they tend to give the same answers when the inquiry is repeated.

Also, in general, responses to questions about personal customs are colored by both conscious and unconscious reflections of personal attitudes. It is a common deceit, often unconscious, of fat people to under-report their food consumption. Food preferences distort recollection of food consumption; one person states he eats his favored foods more often and in larger quantity than is really the case; his opposite reports the contrary; these are the contrasts of the optimist and the pessimist. Since these attitudes tend to be maintained consistently, the result is a much higher degree of repeatability than of validity in dietary questionaires and interviews.

Another source of error which may grossly affect validity but not repeatability is the attitude of the person to the dietitian or to the survey in general. During World War II and afterward in food shortage areas gross under-reporting of food consumption in dietary surveys was the rule; though this was partly a conscious effort in the hope of getting more food, unconscious distortion because of self-pity also played a role. On the other hand, in the work of the F.A.O. Committee on Calories (6, 7) it was found that surveys to establish bench marks for establishing long time food needs in areas of chronic undernutrition often over-estimated food consumption while emphasizing the frequency of emaciation and famine edema; the goal was to arrive at acceptance of the highest possible estimate of food needs.

While errors from these particular causes are uncommon in food surveys in normal times except among severely deprived people, the attitudes of the persons surveyed always have some effect on their reports of food consumption. The interviewee commonly tries to please or impress the interviewer; the housewife reports feeding her family what she conceives to be a better diet than she actually does and this is not necessarily wholly conscious deceit. The resulting errors are not exposed by simply repeating the questions on another occasion.

These strictures are not limited to dietary surveys which depend upon interview and questionaire methods. When food purchases are being recorded and food usage in the home actually measured, the housewife is more conscious of food values, both in terms of nutrients and as related to family and personal prestige. There is no obvious way to prevent or detect such alteration from usual custom during the period of the survey but the housewife must go to expense and far more trouble to actually provide a better or more luxurious diet for seven days than is the case when only replies to a questionaire or interview are involved. The same potential motivation is present to cause error in surveys whatever the method; the question is how much effort is the housewife willing and able to devote to producing a distorted picture of the family diet. It is a good deal easier to add a kilogram of meat to the estimate of a week's usage than to buy and serve it.

Seasonal variability

Several papers in the present series of reports emphasize the somewhat unexpected fact that, on the average, seasonal variations in basic nutrients in the diets of many populations are relatively small, even where climatic variation is extreme. But over-interpretation of the dietary data must be avoided. Calorie intake is not necessarily a reliable measure of calorie expenditure; more often than not positive calorie balance rules in the winter and negative balance in the summer.

Besides the climatic aspect of the seasons and associated changes in work, especially in farming and other outdoor occupations, it is interesting to consider seasonal dietary restrictions imposed by religion. The diet of Moslems is, or should be, grossly changed for the month of Ramadan but the dietary changes prescribed for lent by the Roman Catholic Church, and more so by the Greek Orthodox Church, are also important.

The Greek Orthodox rules are much more strict than those of the Roman Catholic Church and the "fasting" period of eight weeks should, theoretically, have a major impact on the total diet for the year. However, as will be shown in the report on dietary studies in Greece, actual practice is different from Church prescription. On the Island of Corfu some farming families made concessions to Church rules and tradition during the fasting period in 1963 but the average effect on the total diet was trivial. Not only were total calories substantially unaffected; the percentages of calories from proteins and from fats were not significantly different in the fasting period than in a non-fasting period when the same families were studied.

In spite of the foregoing indications that seasonal variations in the diet are often only small, it goes without saying that assumptions about populations not previously studied are always potentially unsafe. Proper characterization of the diet of a population requires dietary surveys repeated in several seasons of the year.

Food composition

Tables of food composition give estimates of the average nutrient content of raw foods, of common prepared foods such as breads, canned vegetables and fruits, and some sausages and of a few food items after they are cooked in specified ways. These indispensable food tables are constantly being improved and enlarged but they inevitably suffer from several major limitations.

Everywhere the amount and variety of commercially prepared foods is rapidly increasing and the formulas are constantly being changed in a fiercely competitive situation. No food tables begin to cover the enormous variety of prepared foods now bought in large amounts even in rural areas. While in some countries major ingredients must be listed on the package, in no country is it necessary or customary to indicate more than the weight or volume of the total contents; the quantities of the ingredients are unspecified and even elementary data on actual nutrients are seldom available.

In many countries standards of identity, for labelling purposes, have been established for common foods including breads, ice cream, some cheeses, a few sausages, etc., but these generally simply establish upper or lower limits for certain ingredients. While there is pressure to increase the number of food preparations that must conform to certain specifications in order to carry a specified name, the majority of newly introduced prepared foods is only regulated by sanitary codes. Moreover, some segments of food industry that would like to indicate nutrients on the labels are forbidden to do so on the ground that unwarranted claims for health might be implied. For example, in the U.S.A. vegetable oil producers have been enjoined by the Food and Drug Administration from indicating the percentages of poly-unsaturated fatty acids in their products.

Accordingly, tables of food composition are grossly inadequate for commercially prepared foods as well as for meals eaten in restaurants by participants in food surveys. These limitations are most serious in big cities in the more highly developed countries where commercially prepared foods form an increasingly substantial part of the total diet and the men of the family often eat a third or more of their meals in restaurants.

Though these limitations of tables of food composition may cause little trouble in rural areas and small towns, in all areas the problem of variability of food composition is important. Regions differ in the food items available and in cookery and the same food items do not have the same nutrient composition in different areas.

For these reasons special tables of food composition have been or are being developed for use in the countries where dietary surveys are being made in connection with international cooperatieve studies on the epidemiology of cardiovascular disease. Such work is under the direction of Prof. PAAVO ROINE in Finland, Prof. FLAMINIO FIDANZA in Italy, Prof. C. DEN HARTOG in the Netherlands, Drs. RATKO BUZINA and EDWIN FERBER in Yugoslavia. In Greece special compilations are in progress under Prof. D. GALANOS.

But at best all values in tables of food composition are good estimates of averages and it is necessary to consider the variability behind the average figure that is too commonly accepted as the nutrient value. Every table of food composition mentions variability and in some cases ranges are mentioned but adequate data on variability are simply not available for most foods. By "adequate" we would mean means and standard deviations (or decile values for the distribution in cases where it is not Gaussian) for specified areas, times of the year, storage etc. Variability in the vitamin content of foods is notoriously great in the fresh raw product and is enormous in the foods as eaten but we shall not attempt to discuss this important problem. Here we shall concentrate on the fat in foods because fat consumption, and its possible relationship to heart disease, is a major interest in many recent dietary surveys, including those we are concerned with in Finland, Greece, Italy, Netherlands and Yugoslavia.

Table 1 gives percentages of fat and protein in the edible portion of meats as classed in the U.S.A. These are averages for the classes; the extreme range is greater. Except in the case of pork, the averages for fat vary by something like 3-fold; for pork the fat variability is less but a considerable amount of the fat is removed for making lard and the variability of pork eaten as meat is greater than would be inferred from table 1. It is interesting that protein variability in meat is much less: only about 20 per cent for chicken and veal, 30 per cent for lamb, 35 per cent for beef and 40 per cent for pork. In other words, fat and water percentages are inversely related. Still it must be noted that because fat is the major energy source in meats, the variation in calories per 100 grams is also very large: from 202 to 407 Cal. per 100 grams for beef, from 143 to 297 for chicken.

Table 1. Average fat content, grams per 100 grams of edible portion in the whole carcass of raw meat as classed by the common trade designation in the U.S.A. (45)

Item	Fat %	Protein %	Item	Fat %	Protein %
Beef, thin	14	19	Lamb *), thin	15	17
medium fat ..	22	18	medium ..	28	16
fat	28	16	fat	40	13
very fat	39	14			
			Pork, thin	35	14
Chicken, broilers ..	7	20	medium	45	12
roasters ..	13	20	fat	55	10
hens	25	18			
			Veal, thin	8	20
			medium	12	19
			fat	16	18

*) Excluding kidney fat.

In dietary surveys efforts are usually made to estimate the class of the meats eaten but even if this is done with high accuracy it is clear that much variability remains. For example, "medium" lamb will vary between 21 and 34 per cent fat; chicken in the "roaster" class from 10 to 19 per cent fat.

As cooked and eaten, the variability of fat in meats depends upon the method of cooking and what is done with the fat that may be cooked out or cut off. The potential range of variability in fat intake from 100 grams of raw meat is greater, therefore than indicated in table 1.

The least possible variability in cooked meats is indicated in table 2 which refers to carefully standardized cuts from animals of the same age and source selected to be in the center of the distribution of a specified quality class, cooked in specific standardized ways. The edible yield varied by about 4 per cent in baked ham and by about 19 per cent in beef brisket cooked in liquid and roast leg of lamb. The separable fat in the cooked meats was more variable; from one roasted lamb leg only 4.4 per cent of the cooked weight could be cut off as "fat" while in another 11.5 per cent could be so removed, i.e., almost three times as much.

Table 2. Yields from carcasses of animals of the same age and source, selected as in the center of distribution of given quality grade, cooked in standardized ways (31)

Lean = portion with no trace of fat marbling
Marble = portion with marbling but no separable fat
Fat = fat portion free of lean or marbled areas
Waste = inedible bone and gristle
"Meat" = cooked result as usually served
Stewed = cooked in liquid

Meat carcass		Cut	Cooking method	Percentage of raw weight Dripping	"Meat"	Lean	Marble	Fat	Waste
Beef	A	Brisket	Stewed	11.1	83.8	22.7	30.4	29.1	1.5
	B	"	"	6.7	90.5	28.2	30.6	26.6	5.1
	C	"	"	11.5	76.3	26.7	19.6	28.0	2.1
Beef	A	Sirloin steak	Broiled	7.7	77.9	38.3	18.6	14.8	6.2
	B	" "	"	6.3	76.2	34.1	17.0	16.6	8.5
	C	" "	"	3.4	85.6	30.0	26.6	15.0	14.1
Lamb	A	Leg	Roast	5.9	69.2	21.3	21.5	4.3	22.1
	B	"	"	7.9	72.0	36.2	10.2	3.2	22.4
	C	"	"	6.2	72.8	20.9	24.0	4.8	23.0
	D	"	"	2.2	82.6	31.6	14.1	9.5	27.5
	E	"	"	4.4	82.6	29.7	7.0	6.8	39.2
Pork	A	Ham	Roast	5.7	82.1	25.1	21.0	12.8	23.2
	B	"	"	5.3	85.4	28.9	21.7	10.7	24.1
	C	"	"	6.6	83.6	21.6	22.1	13.3	26.7
Veal	A	Arm steak	Braised	30.2	64.1	35.8	0	18.3	10.0
	B	" "	"	31.4	64.1	41.7	0	8.8	13.7
	C	" "	"	20.4	67.5	42.3	0	13.0	12.3
Veal	A	Breast stew	Braised	25.7	70.0	0	0	70.0	0
	B	" "	"	21.3	64.0	0	0	64.0	0
	C	" "	"	6.5	63.7	0	0	63.7	0

But table 2 does not indicate the variability in nutrient intake that would result from eating these cooked meats. Nutrient values from chemical analyses of the materials noted in table 2 are given in table 3. Here the protein content in the cooked product from 100 grams of the raw meats is found to be moderately variable, the most being some 30 per cent more than the least in roast legs of lamb, with less variability in the other materials, and it makes little difference whether all of the edible portion is eaten or only the part with the "fat" cut off (i.e. meat less waste).

But the fat intake is another matter. Suppose all possible fat is cut off and discarded (i.e. only the lean plus marble is eaten). The fat intake from the cooked product of 100 grams of raw brisket of beef would be 6.7 grams from carcass C, 15.7 grams from carcass B, i.e., 134 per cent more. In sirloin steak from carcass A, fat intake would be 53 per cent greater than from carcass B. And in veal breast stew the fat from carcass C is 71 per cent more than from cass B. If everything edible were eaten, i.e. "lean" plus "marble" plus "fat", the variation in fat intake from the cooked products from the various carcasses would still be very large. Expressing the largest intake as a percentage of the smallest, the values are 128 for beef brisket, 124 for sirloin steak, 181 for lamb leg, 131 for ham, 154 for veal steak, 173 for veal breast.

Of course, the differences between eating the separable fat and discarding it are enormous. It would not be so bad if people could be classed as those who

Table 3. Nutrients in the cooked portions, per 100 grams of raw meat, in the materials listed in table 2 (31)

Meat carcass	Cut	Lean + marble Calories	Protein, g	Fat, g	Meat less waste Calories	Protein, g	Fat, g
Beef A	Brisket	177	13.3	13.4	376	15.0	34.7
B	"	193	12.0	15.7	373	13.7	34.8
C	"	117	13.2	6.7	309	14.7	27.3
Beef A	Sirloin steak	127	14.0	7.5	243	14.6	20.1
B	" "	103	13.6	4.9	220	14.7	17.3
C	" "	113	14.4	5.7	212	15.6	16.1
Lamb A	Leg	87	13.1	3.5	111	13.7	5.8
B	"	93	13.1	4.2	110	13.6	5.9
C	"	89	12.7	3.9	115	13.4	6.5
D	"	84	12.1	3.6	149	12.8	10.5
E	"	69	10.1	2.8	114	10.6	7.6
Pork A	Ham	113	15.6	5.1	201	16.9	14.3
B	"	116	18.5	4.1	194	19.4	11.8
C	"	116	16.3	5.2	214	17.5	15.5
Veal A	Arm steak		13.1	1.5		14.0	12.2
B	" "		14.9	2.7		15.9	7.9
C	" "		14.6	2.3		15.9	10.9
Veal A	Breast stew		17.5	18.7		17.5	18.7
B	" "		19.4	11.5		19.4	11.4
C	" "		18.0	19.7		18.0	19.7

Cals. = 4.1 × prot. + 9.0 × fat.

discard all separable fat and those who consume it all. It is our experience that those who say they cut off and discard the fat actually vary greatly; some discard only a third of the fat; others remove 90 per cent.

Meat, milk fat, lard, margarine, and in some areas olive oil, account for almost all fats eaten in most of the more developed areas of the world. Lard, olive oil, butter and margarine pose no problems in regard to percentage of fat but, as will be seen below, fatty acid composition is another story.

Milk is regulated as to minimal fat content in most countries so the variability in fluid milk is small, perhaps within 10 per cent in any one area. Cheese, too, is often regulated and in most cheeses the natural variability of fat within a given cheese type is not large. Ice cream generally has a legal definition but the variability in fat content may still be substantial. In the United States the minimal legal milk fat content to allow use of the designation ice cream varies from 6 to 14 per cent in different States. And in all countries there is a substantial and increasing use of frozen sweets resembling ice cream which bear other names and are much less rigidly defined and regulated.

We conclude, then, that meat fat is by far the major source of error in the use of tables of food composition to estimate total fat intake. In areas where meats are eaten in large amounts, the possibilities of error are serious. Where only small amounts of meats are eaten, the meats tend to be lean, meat fat is seldom discarded and the errors may not be large in the estimation of total fat intake from tables of food composition (provided that the data on food consumption are accurate).

Fatty acids in foods

Fatty acid contents of foods are as yet incompletely investigated and tables of food composition that include fatty acids are neither widely available nor very reliable. But there is ample evidence to indicate variability of composition of many important food fats.

Exhaustive fully controlled dietary experiments on man show that, in general, saturated fatty acids in the diet raise the serum cholesterol concentration, poly-unsaturated fatty acids have the opposite effect with about half the potency per unit fatty acid weight, while mono-enes have little or no effect (25, 26). Recently it has become clear that not all saturated fatty acids share the cholesterol-promoting effect (27).

Saturated fatty acids with fewer than 12 carbons in the chain have little or no effect on the serum cholesterol level of man, presumably because they are much less non-polar and they are absorbed via the portal system directly to the liver rather than by way of the lacteals and the lymphatic system (12, 3, 19). Very recently, controlled experiments in several laboratories as well as in Minnesota revealed marked discrepancies from expectation in serum cholesterol response when substantial amounts of cocoa butter were incorporated in the diet. Analysis of these and all older data available by multiple regression methods indicate that stearic acid in the diet has little or no effect on the serum cholesterol level of man (27). Accordingly, it appears that it is necessary, in investigating diets in regard to serum cholesterol and coronary heart disease, to distinguish not only saturated and poly-unsaturated fatty acids, but also to distinguish saturated fatty acids with 12 through 16 carbons in the chain; in almost all natural diets it seems that these fatty acids are most important in the serum cholesterol problem.

Table 4 shows that milk fat variability is not negligible when fatty acid composition is considered. Cows of the same strain fed on different common types of winter rations produced milk markedly differing in fatty acid composition. The concentration of poly-unsaturated fatty acids, i.e., linoleic and arachidonic acid, was twice as high in the milk from cows fed silage plus either oat straw or oat straw and roots than in milk from cows fed oat straw, roots and refuse grain from distilleries. But the trivial quantities of these fatty acids at best, and their lesser effect on serum cholesterol, mean much less importance than the differences in the contents of saturated fatty acids with 12 to 16 carbon atoms. The last column in table 4 gives the estimated relative effect on serum cholesterol of ingesting the different milks. Though all of these milks would tend to raise the serum cholesterol level, the milk produced by the cows fed a ration of oat straw, roots and refuse grain from distilleries would be expected to have 45 per cent more cholesterol-raising effect than the milk from cows fed silage and oat straw.

So far there is little information about variation in the fatty acid composition of the fat in beef, lamb, chicken or other meat animals besides hogs. It has long

Table 4. Fatty acid composition of milk fat from cows of the same strain but fed on different common types of winter rations (10)

Percentages of total fatty acids represented by saturated acids with 12 to 16 carbon atoms (C12–16), by stearic acid (C18), by oleic acid (C18 : 1) and by linoleic plus arachidonic acids (C18 : 2, C20 : 4).
Chol. effect = relative value of 2s′ — p, where s′ = C12–16, p = C18 : 2 + C20 : 4.

Ration	C12–16	C18	C18 : 1	C18 : 2 C20 : 4	Chol. effect
Silage and oat straw	37.2	10.5	33.4	3.9	100
Silage, oat straw, roots ..	45.0	7.9	27.1	3.9	122
Oat straw and roots	46.5	6.7	25.6	2.4	129
Oat straw, roots, draff *)	51.9	6.1	19.6	1.8	145

*) Refuse grain from distilleries,

been known that the fatty acid composition of lard is readily altered by changes in the ration fed (13).

AHRENS et al. (2) seeking out extremes for dietary studies, obtained lards from specially fed hogs varying from 1.8 to 30.5 per cent in linoleic acid. Table 5 gives the fatty acid composition of pork fat as such (not rendered into lard), from hogs fed various rations as summarized by GODDARD and GOODALL (10). The variation in the serum cholesterol-raising saturated fatty acids with 12 to 16 carbon atoms is large (from 12.5 to 25.8 per cent) but the variations in the serum cholesterol-lowering poly-unsaturated fatty acids, linoleic and arachidonic acids, are much greater and even more important in net effect on the serum cholesterol concentration. The corn-fed hog, a favorite in the United States, has the highest concentration of C12 through C16 saturates and by far the lowest amount of poly-unsaturates; it could be confidently predicted to have a powerful cholesterol-raising effect when fed in the diet; in contrast, fat from soybean-fed hogs substituted for equal calories in the diet should tend, if anything, to lower the serum cholesterol level.

Table 5. Fatty acid composition of pork fat (10)

See table 4 for explanation of column headings.

Source	C12–16	C18	C18 : 1	C18 : 2 C20 : 4	Chol. effect
Soybean fed, high	18.4	9.4	40.9	37.3	− 7
low	14.0	7.4	37.8	32.0	−60
Corn + skim milk fed ..	25.8	12.7	54.4	7.1	664
Peanut fed	13.2	6.2	60.6	19.7	100
Corn fed	26.8	8.1	58.8	1.8	773
Garbage fed	12.5	16.6	59.9	9.2	236

In Minnesota we have made studies on fatty acid composition of some food items, selected to minimize variability, using identical methods and equipment throughout, the gas-liquid chromatograph column and recorder being checked in each case with known mixtures of pure fatty acids. Examples of results are given in table 6. Liver samples of the same price and grade were obtained from two major local retail suppliers at the same time. Olive oil samples came from wholesale suppliers, each wholesaler providing samples from his own constant source identified as to lot.

Table 6. Fatty acid composition of lipid extracts of samples of liver and of olive oil in different samples but of ostensibly the same grade

18 : 0, 18 : 1, 18 : 2 and 20 : 4 refer to stearic, oleic, linoleic and arachidonic acids, respectively. Values tabulated are % of total fatty acid. Sat. = total saturated fatty acid.

Item		Sat.	18 : 0	18 : 1	18 : 2	20 : 4
Baby beef liver, "Red Owl"		52.5	34.5	17.9	11.6	9.8
"National"		52.4	24.0	22.8	9.3	5.3
Pork liver, "Red Owl"		45.5	20.3	28.5	13.8	7.8
"National"		46.6	25.1	24.0	15.4	10.5
Olive oil, WHC,	Lot 23	16.0	3.1	69.9	11.8	—
	Lot 24AB	15.7	3.1	73.7	9.2	—
	Lot 24D	17.3	3.6	71.8	9.6	—
Paragon,	Lot 7-63	13.4	2.5	75.2	9.1	—
	Lot 8-63	14.2	3.0	76.3	8.5	—
	Lot 9-63	12.9	1.9	72.7	10.2	—
Romanza,	Lot 5-61	14.4	0.6	72.7	11.9	
AB,	Lot 5-60	14.2	2.0	62.4	16.1	
Star,	Lot 9-61	13.6	2.4	75.2	7.4	

Each of the two types of liver proved to be substantially constant in total saturated fatty acids but the individual fatty acids varied. The variations in linoleic acid were great, ranging from 7.4 per cent in "Star" brand to 16.1 per cent in "Romanza" brand, but the variation within lots from a given supplier in a given year were not great.

The fatty acid composition of olive oil seems to be more variable than that of most dietary fats but this may merely reflect the fact that it has probably been the subject of more analytical studies than any other food fat (see 13, table 57B, and 5, table 21-3). Pure olive oils in Italy are reported to range from 4 to 15 per cent in linoleic acid; in Argentina from 6 to 22 per cent. Since in the diet the serum cholesterol effect of olive oil is largely determined by the ratio of palmitic to linoleic acid in it, this ratio is of interest. Extremes of this ratio range from 2.4 (in an Italian sample) to 0.6 (in an Argentinean olive oil). Obviously, any dietary study concerned with populations whose diet contains much olive oil must obtain data on the olive oil locally used at the time.

Calorie imbalance

Almost all dietary surveys assume that the subjects surveyed are in calorie balance during the period of the survey. Probably for the average of all subjects, this assumption is justified in the majority of surveys but certainly some of the subjects will not be in exact balance at any one time and at some times of the year it is probable that most of them will be eating more (or less) food than their current energy expenditure. It is useful to consider the calorie equivalent of weight changes in healthy men.

In a careful 4-week study on 98 young soldiers in which daily food intakes were individually measured, the mean of 3669 calories per day was associated with an average body weight gain of 2.03 kg (30). KONISHI *et al.*, using data from controlled studies on weight gain of men where body composition data were available (24), estimated that, on the average, these men consumed 496 more Calories per day than their requirement for balance at the energy expenditure level maintained during the four weeks of the study.
Figure 1 calculated from table 12 in KEYS and KEYS (28) shows the estimated average effect on body weight of excess or deficit of dietary calories maintained for 60 or for 90 days, taking into account known effects on basal metabolism and specific dynamic action as well as results in controlled studies on gaining and losing weight where the composition of the tissues gained or lost could be estimated from measurements of total body water and body density. In many populations we have observed an average gain of around 2 kg during the fall and winter months, with a corresponding loss during the summer; maximum body weight is often at the end of the Christmas holidays with the minimum in July or August. Such a change of 2 kg in body weight in 2 months should be associated with an average calorie imbalance of around 240 calories per day.

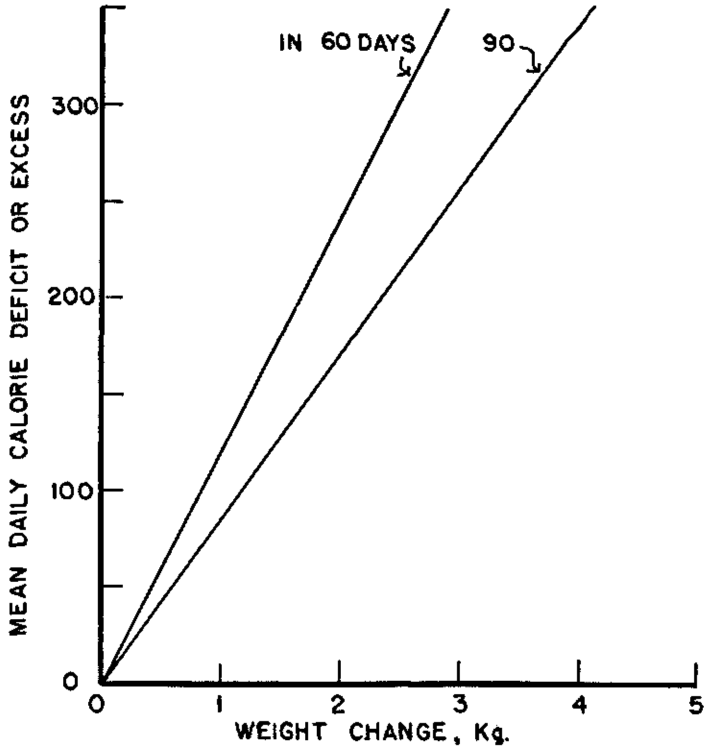

Fig. 1. Estimated average change in body weight in 60 days and in 90 days resulting from daily calorie excess or deficit in the diet (28)

It should be noted that such a weight change means an average of only 0.25 kg per week, an amount that would usually be ignored if noted at all; yet it would represent about 240 Cal. per day in the diet, or close to 10 per cent for sedentary middle-aged people.

Such considarations indicate that even if body weight appears to be relavely constant during the necessarily short period of most dietary surveys, it is unsafe to conclude that the average food consumption during the survey is a good measure of the contemporary calorie expenditure. We should question, therefore, such conclusions as made by KAWADA and KUGA (15) who carried out dietary surveys in different seasons on 15,000 persons in 34 localities in Japan and came to definite conclusions about calorie requirements as related to environmental temperature; they assumed that the reported values for food consumption were equivalent to energy expenditure.

Diet and serum cholesterol

A major new interest in dietary surveys is to obtain information relevant to the hypothesis that the diet, specifically the fat in it, via effects on serum lipids, particularly cholesterol, has a significant effect on susceptibility to coronary heart disease (16, 17, 18, 21, 22, 23, 14, 41, 42).

Under controlled experimental conditions, changes in the fats in the human diet have effects on the serum cholesterol concentration which, on the average, are predictable from details on the fatty acids in the diet before and after change (25, 26, 27). These same experiments also show that, on the same diet, individuals have striking differences in serum cholesterol concentration and when the diet is changed, though the average cholesterol level responds, the different individuals tend to retain their ranks in the array of values; there are, in fact, important intrinsic differences among individuals in this respect (op. cit.).

Many population studies show that, in general, the average serum cholesterol level in the population is correlated with the average proportion of the total dietary calories provided by fats, or at least by saturated fatty acids, in the diet (cf. references by KEYS, above). Some apparent exceptions to the rule involve problems of methodology or interpretation.

Navajo Indians were stated to eat a "typical" U.S. diet but to have remarkably low serum cholesterol values (8,36). The low cholesterol levels could be verified but the statement about the diet proved to be grossly in error (18). It is true, as reported, that most of the Navajos consume some milk; this milk proved to be only a few drops of canned milk in coffee but 59 per cent of them had even this less than twice a week. Navajos use butter, too but 79 per cent of them had no butter or margarine as often as twice a week. They have meat fairly regularly but the per capita consumption was barely a third that of the U.S. average. Yet the original misinformation about the Navajo continues to be cited (35,9).

Puzzling findings have been reported from observations on nomadic tribes in East Africa who are reported to subsist on milk, meat and blood and, therefore, on diets high in saturated fatty acids. These people are extremely thin, they undergo frequent periods of near starvation, and they generally carry heavy burdens of parasites. The Samburu warriors had a serum cholesterol average of 169 mg per 100 ml, the elders an average of 162; in contrast, the Rendille men, purportedly on much the same diet, exhibited high serum cholesterol values, averaging 247 mg per 100 ml for men estimated to be between 40 and 60 years of age (39, 38). Even lower serum cholesterol values than for the Samburu in Kenya have been reported for the Masai in Tanganyika whose diet, mode of life and other characteristics seem to be similar to those of the Samburu (32).

Observations on these primitive nomads are interesting but are not necessarily relevant to other people. Their idiosyncrasy in regard to serum cholesterol could be related to chronic undernutrition plus parasitic infestation and periodic semi-starvation, or they may be metabolically abnormal. But crude, non-quantitative dietary estimates, which are all that are available as yet, must not be labelled dietary surveys.

Acceptable dietary survey methods do not alone guarantee proper interpretation. A paper by GSELL and MAYER (11) is entitled "Low serum cholesterol associated with high calorie, high saturated fat intakes in a Swiss Alpine village population." But the serum cholesterol values were not remarkably low considering that the fat per 1000 Cal. of diet was not particularly high. True, the total calorie and fat intakes were higher in the village than in Basel but the more active life in the village accounted for the difference; the percentage of calories from fats was slightly lower in the village than in Basel and there was no significant difference in regard to saturated fatty acids (only estimated, not measured). Dietary cholesterol per 1000 Cal. of diet was also lower in the village than in Basel. The women in Basel tended to have higher cholesterol values than those in the village but there was no significant difference between the men in the two areas. Finally information is lacking as to the distribution of foods within the families.

Critically analyzing all published bad dietary surveys and far-fetched conclusions in regard to the diet and its effects would be an endless and not very fruitful task. A large compendium of uncritically cited material is provided by GLATZEL (9).

One major new point is that not all saturated fatty acids in the diet have a cholesterol-raising action. Suturated fatty acids with fewer than 12 or with 18 or more carbon atoms seem to have little or no effect on serum cholesterol level when isocalorically substituted for simple carbohydrate in the diet. In effect, this indicates that the well-known cholesterol-promoting effect in man of saturated fatty acids in the diet is really due to lauric, myristic and palmitic acid, primarily the latter in most diets. Hence in the 2S - P formulation, S should be specified as meaning only saturates with 12 to 17 carbon atoms.

A second point, now well established, is that dietary cholesterol does have a small effect on the serum level in man. In a large series of controlled experiments in five different centers the data are in full agreement that, if the diet is constant in other respects, a change in the amount of cholesterol fed produces an average response that can be predicted as $Y_2 - Y_1 = 1.5 (Z_2 - Z_1)$ where

Z is the square-root of the dietary cholesterol, in mg per 1000 Cal., Y is mg cholesterol per 100 mg of serum, and the subscripts refer to the two diets.

Third, with the same calories, proteins, fatty acid and cholesterol composition, diets containing large amounts of legumes, fruits and leafy vegetables tend to produce serum cholesterol levels somewhat lower than otherwise. Like the dietary cholesterol factor this carbohydrate factor, or factors, has only a small influence compared with that of the fatty acids in the diet but it can be significant in some natural diets as well as in experimental situations.

These considerations mean that dietary surveys that are to provide information relative to the serum cholesterol problem, must provide far more chemical detail than the usual surveys that suffice for many other purposes.

Variability of "constant" diets

In the analysis of data from dietary surveys it is generally assumed that the results apply to more than the limited period of the survey itself and that, in effect, the survey results are representative of the diet over extended periods of time. The implication is that, for the average of the sample surveyed, the diet is substantially constant. Objective evidence justifying this assumption is not often provided.

Above, in the section of "Reliability versus validity," we have indicated reasons why interview surveys tend to indicate that the individual diet is more constant than it is in reality. But even when there are seemingly excellent reasons for assuming constancy of the diet, variability may actually be significant. We have investigated diets which were supposed to be constant in nutrient composition. But critical analysis showed variations, especially in fatty acid composition, which could be important for some purposes.

Table 7 gives means, standard deviations and coefficients of variation obtained by chemical analyses of 8 five-day collections of standard servings in a mental hospital where the dietitions used a master menu supposedly providing constant nutrient composition for the average of five days. Protein variation was small; a range of less than ± 7 per cent about the mean would cover two-thirds of the samples. Fat variation was almost 50 per cent larger and variation in fatty acids was even more important. Taking the formula 2S - P (26, 27) as a measure of the influence of dietary fat on serum cholesterol, the "constant" diet proved to be substantially variable. A coefficient of variation of 25 per cent is enough to seriously disturb analysis of metabolic relationships.

Table 7. Means and standard deviations of chemical analyses of 8 samples, each made up of a replica of 5 days' standard diet as served in a mental hospital where the diet was supposed to be constant. Values are percentages of total calories (Analyses by Dr. J. T. Anderson)

Item	Mean	S.D.	S.D. as % mean
Total protein	14.41	0.96	6.7
Total fat	34.42	3.41	9.9
Saturated fatty acids	14.45	2.39	16.5
Linoleic acid	8.29	1.19	14.4
2S - P	20.61	5.24	25.4

Individual relationships

Dietary surveys are customarily made to characterize populations in regard to food and nutrient intake with emphasis on the group rather than the individuals in it. Unless repeated surveys are made covering the same individuals, the result is to over-emphasize inter-individual variability and to neglect intra-individual variability. This does not necessarily lead to errors in the comparison of population groups but can have serious effects if it is desired to examine the relationship between the individual diets and some other characteristic of the individuals. Some of the studies in the present series of dietary studies provide extreme examples of intra- versus inter-individual variation as revealed by repeated surveys on samples containing the same individual men (cf. 4, for example). Table 8 summarizes results for percentages of total fatty acids represented by particular fatty acids in 7-day replica diets collected from 18 men studied late in 1961 and again early in 1963 on the Island of Corfu, Greece.

Table 8. Variability in fatty acid composition in lipid extracts of 7-day diets, analyzed chemically, of 18 men studied on two occasions in Greece

Item	Palmitic	acid	Oleic	Linoleic
	Stearic	acid	acid	acid
1. Mean of all samples (N=36), as % of total fatty acid	19.53	4.67	62.93	9.13
2. Standard deviation of mean (average of the S.D.'s for 2 occasions)	4.36	2.11	5.93	2.89
3. Average intra-individual variance	52.25	4.57	29.81	15.85
4. Average inter-individual variance	24.57	5.04	39.36	8.76
5. % of total variance accounted for by consistent differences between individuals	3.9	37.1	41.0	0.2

The dominance of oleic acid in the fat in these diets reflects the large amount of olive oil eaten by these Greeks but the point of interest in these data is the fact that the intra-individual variance tends to be greater than the inter-individual variance. This means that the individuals in this sample are difficult to recognize in respect to the fatty acids in their diets. Line 5 in table 8 gives the percentage of the total variance that is contributed by consistent differences among individuals. Such findings as in table 8 may reflect unusual homogeneity of dietary practices among the individuals, as well as high variability within the individuals and large errors in food collection and analysis. But obviously any attempt with such material to correlate the individual fatty acid patterns with some other characteristic of these individuals will almost certainly fail, even though a highly important relationship between the dietary fatty acid pattern and the characteristic in question really exists.

Consideration of these questions helps to understand how it happens that many surveys fail to find significant relationships between dietary fat and serum cholesterol values in individuals within groups that are rather homogeneous in respect to the diets (e.g. 44, 40, 1, 33, 34, 37). Besides intra-individual variability of dietary practice and errors in recording and analyzing the diets, allowance must be made for the fact that serum cholesterol concentration is subject to high spontaneous intra-individual variability. In "healthy" adults in Europe and the U.S.A. on an ostensibly constant diet the average intra-individual standard deviation is of the order of 20 to 25 mg of cholesterol per 100 ml of serum; the **total** standard deviation is only about twice as great (25).

The relationship between the true correlation coefficient, r^*, and the value, r, found when the relationship is attenuated by error and intra-individual variability, is given by $r^2 = (r^*)^2 (1 - Vex/Vx) (1 - Vey/Vy)$, where Vex and Vey are the error variances of the variables X and Y that are being correlated and Vx and Vy are the total variances of these variables. If Y is serum cholesterol, this expression commonly would reduce to about $(r^*)^2 (1 - Vex/Vx) (0.75) = r^2$. And if Vex is half of Vx, we have $0.375 (r^*)^2 = r^2$, or $r^* = 1.64\ r$. But as table 8 shows, Vex may account for most of Vx in some series; if $Vex/Vx = 0.8$ we have $r^* = 2.57\ r$, and so on.

These same considerations apply to all variables, of course. But it is scarcely surprising that the dietary intakes of individuals as recorded in surveys often fail to explain other characteristics of those individuals, even when there is ample proof that those characteristics are affected by the diet. Besides dietary fat and serum cholesterol, examples are calorie intake and relative body weight or obesity; iron intake and blood hemoglobin concentration; iodine intake and thyroid size.

The value of dietary surveys

The foregoing considerations are not intended to depreciate the value of dietary surveys in epidemiological studies; the intent is to indicate the need to recognize limitations and to increase efforts for further improvement of dietary survey methodology and the analysis of the findings where questions of intricate metabolic relationships may be involved.

Emphasis has been placed on difficulties and limitations in such newer applications of dietary surveys as in the field of cardiovascular epidemiology. Where the major concern is to discover areas of dietary deficiencies or to compare contrasting populations in regard to general food supplies and practices, or even in regard to major nutrients, many of the strictures indicated above are of limited importance, though not irrelevant. But the problems and many of the variables are different when we come to more detailed questions of metabolism and the biochemistry of disease. Though the present discussion is focussed on cardiovascular disease, similar considerations hold for some other diseases.

Dietary surveys are certainly valuable in these specialized problems of epidemiology but three points should be stressed. First, even the most refined dietary survey cannot provide answers about biochemical relationships that need the control and isolation of variables obtainable in a metabolic laboratory unit. Second, good dietary surveys are difficult, costly and require professional expertise in planning, operation and interpretation. But because of rapidly developing interest of medical scientists in the possibility of using the epidemiological approach to gain knowledge about metabolic relationships between the habitual diet and pathogenesis of common diseases, there is temptation to promote surveys without meeting the requirements of personnel and expense to assure reliable and meaningful results. Third, in situations where nutritional factors, important as they may be, interplay with many other factors in producing the metabolic end result of interest, these other factors may not be dismissed, even if they are unknown or unmeasurable. In other words, over-simplification must be avoided.

If these points are fully appreciated, dietary surveys can be of great value. If dietary surveys on a broad scale show that the intake of iodine bears a general relationship to the incidence of thyroid dysfunction, this fact is of extreme importance in spite of the fact that the surveys fail to explain why some individuals in a population have goiters and others do not. Similarly, if good dietary surveys should support or deny the hypothesis that the blood pressure or the incidence of cerebrovascular disease tends to be directly related to the intake of sodium, such surveys would be of enormous value in spite of their failure to identify a salt-blood pressure relationship for individuals. The parallel in the case of the diet versus development of coronary heart disease is obvious.

Summary

Dietary surveys cannot substitute for controlled experiments but they are needed for testing hypotheses about relationships between the diet and certain diseases and they provide clues for further study under controlled conditions. Surveys concerned with the relationship of blood lipids and arterial disease to the diet, require detailed attention to certain nutrients that are of small importance in most ordinary dietary studies. Tables of food composition concern average nutrient contents of foods and cannot allow for variation in particular localities, seasons, food samples and individuals. Such variation is large, especially in percentages of total fat and of particular fatty acids. The use of tables of food composition results in spuriously high "reliability", i.e. repeatability. Reliability does not necessarily prove validity. Dietary surveys commonly assume calorie equilibrium but in many populations there is seasonal calorie imbalance. Relative constancy of body weight over a few weeks may conceal an average imbalance of several hundred calories daily. The analysis of relationships between dietary variables and other characteristics of the individuals concerned is greatly complicated by large intra-individual variability for one or both of the variables of interest. More favorable conditions for analysis can be found in comparisons of averages for contrasting populations studied with highly standardized methods.

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DIETARY STUDIES IN CONNECTION WITH EPIDEMIOLOGY OF HEART DISEASES: RESULTS IN FINLAND *)

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Mortality from cardiovascular disease in Finland is, according to statistics, higher than in most other countries of the world (3, 18, 7). Both mortality and disability statistics further indicate that cardiovascular disease is more common in the eastern than in the western parts of Finland (4). A high mean serum cholesterol level is known to be associated with a high incidence of coronary heart disease; the mean serum cholesterol in Finland has correspondingly been found to be rather high in comparison to other countries, and again highest in eastern Finland (6).

Since diet has been proposed to be an important etiological factor in cardiovascular disease, particular attention has been given to it in a series of international collaborative investigations on the epidemiology of cardiovascular disease, carried out in Finland since 1956, on the initiative and in cooperation with KEYS. In the summer of 1956 and in the winter of 1957 food consumption surveys of families were carried out in certain areas in eastern and western Finland (11, 12, 8, 10). It appeared important also to obtain information on the food consumption of individual men, and therefore an additional survey was made in the fall of 1959 in which both men and their families were studied at the same time. This paper presents those results of that survey which primarily concern the individual men.

The population studied

In East Finland the survey was made in the commune of Ilomantsi (see map), where according to disability statistics of the National Pension Institute (4) cardiovascular diseases are a very common cause of disability. About 80 per cent of the population in this commune earn their living from agriculture and forest work (13). The farms are small, and a great number of the farmers are occupied in forest work part of the year. The 30 men included in the food consumption survey in fall 1959 represent very well the occupational distribution within the area. The type and amount of physical activity performed by them were assessed with the aid of an interview, using a scale with 4 work grades; grade 1 corresponds to sedentary work, grade 2 to light work, grade 3 to heavy work, and grade 4 to very heavy work. Of the men surveyed, 10 were forest workers (grade 4), 11 farmers (8 of them classified to grade 3 and 3 to

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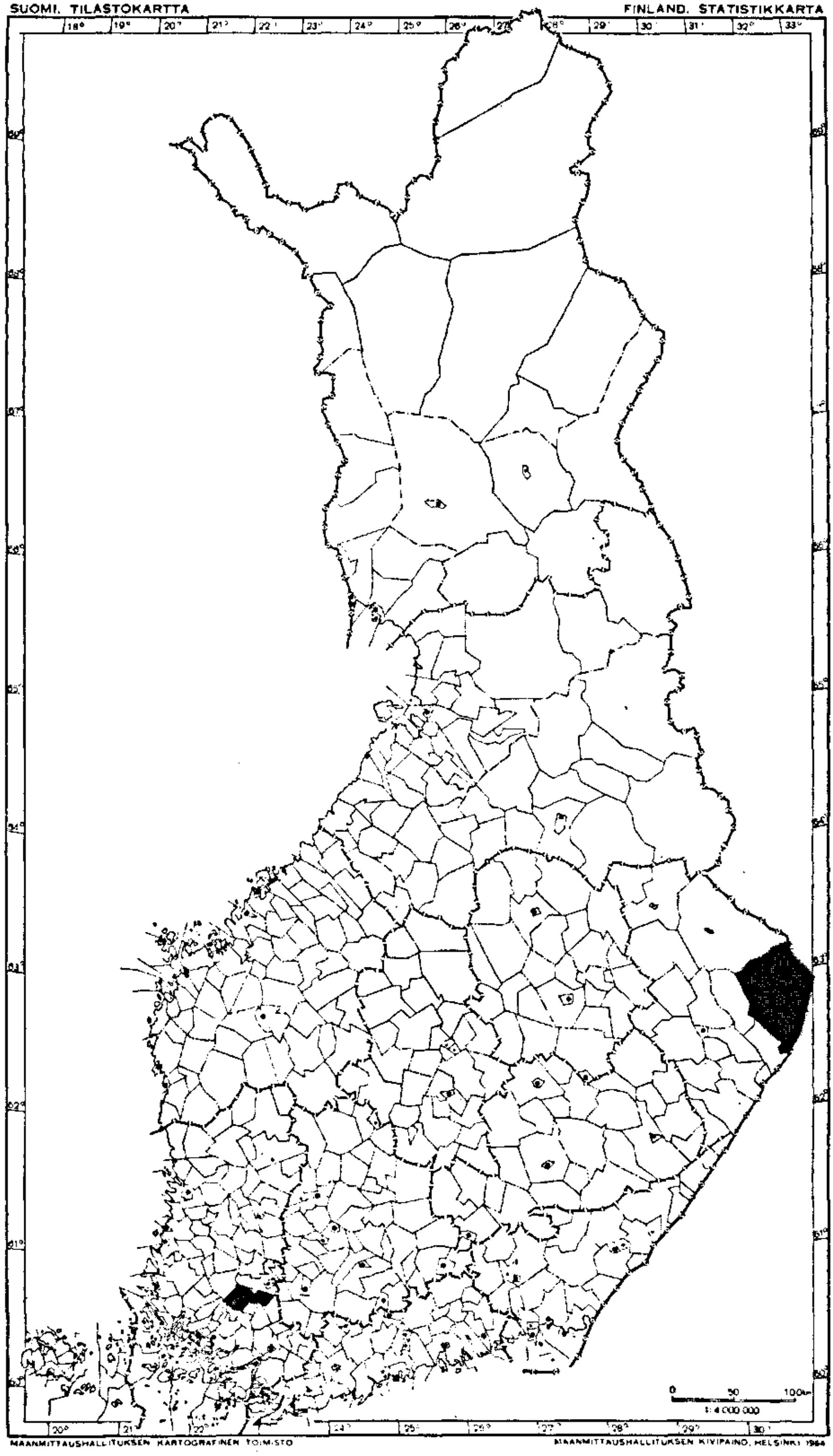
grade 2), and 9 had other occupations: 3 were road and other workers (grade 3), 3 truck drivers (grade 2), 1 shopkeeper (grade 2), 1 carpenter (grade 2), and 1 car mechanic (grade 2). The average age of the men was 48.9 years. In West Finland the survey was carried out in two communes, Pöytyä and Mellilä (see map), where cardiovascular diseases are a less common cause of disability. Of the population in this area, 65 per cent earn their living in agriculture and forest work. The farms are larger and the soil more fertile than in East Finland. The farming also is more mechanized with the result that farm work is not as heavy as in East Finland. Of the men surveyed in West Finland, 25 were farmers (1 classified to work grade 4, 22 to grade 3, and 2 to grade 2), a somewhat higher percentage than that of farmers in the entire population of this area. Two of the men included in the survey were forest technicians, one was a mill technician, one a railway worker (all work grade 2), and one a blacksmith (grade 3). The average age of the men was slightly higher than in East Finland, 49.9 years.

Method

The medical investigation undertaken simultaneously with the food consumption survey was a total population study which covered substantially all men aged 40 through 59 in the area. The number of men studied was 819 (98.4 per cent of the total number of men in this age range) in the eastern area and 852 (96.8 per cent of the total) in the western area. From these samples a total of 60 men were selected for the food consumption survey, 30 in each area. Half of them as well as their alternates were selected at random; the other half of the survey participants were chosen on the basis of being their closest neighbours participating in the medical investigation. This was done so that one dietitian would be able to record the food consumption of two men during the same survey week, even in sparsely populated districts.

The survey was conducted in September, 1959. The food consumption of each of the 60 men was recorded on 7 consecutive days by field workers who were university students majoring in nutrition. The precise weighing method was used. All the ingredients used in the preparation of the food, the waste, the total amount of food cooked, and the amount consumed by the men were weighed daily for each meal. Also uncooked food was weighed. If the men took meals outside the home during the survey period, the amounts were recorded and taken into account when calculating the total intake of food. The intake of different foodstuffs was calculated as fresh raw material excluding waste.

With the aid of Finnish Food Composition Tables (14) the values of energy and the following nutrients were calculated: proteins, fats, carbohydrates, calcium, iron, vitamin A, thiamine, riboflavin, nicotinamide, and ascorbic acid. The amino acids, the fatty acids in most of the foodstuffs, vitamin E, pyridoxine, pantothenic acid, and choline were calculated according to estimates fixed on the basis of an extensive survey of the literature. For iodine, analytical values of foodstuffs in West and East Finland (16) were used. Special attention was paid to the



Map of Finland; the areas surveyed in East and West Finland are darkened

quantity and quality of fats; since milk fat is the main source of fat in the Finnish diet, the fat content of the milk used by each man was determined. The fatty acid composition of milk produced in these areas was calculated on the basis of analyses made by ANTILA (1). The fatty acid values for margarine were obtained from analyses of the most common brands of margarine used in Finland.

The calculated values of nutrients were partly checked by chemical analyses. Twenty men were included in the checking study, ten from each area. Samples corresponding to the mean daily amounts of food consumed by each man during the survey period were taken; the foodstuffs were then mixed, homogenized, and freeze-dried. The 20 food samples thus obtained were analyzed for water, ash, protein, total fat, and fatty acids. The fatty acid determinations for this purpose were carried out in the Laboratory of Physiological Hygiene, University of Minnesota, USA.

In connection with the medical investigation, the height and weight as well as the upper arm and subscapular skinfold of each man were measured using a caliper with a pressure of about 10 g/mm^2.

Results

Diet

Tables 1 and 2 give a general picture of the composition of the men's diet. The main sources of energy were milk products including butter, cereals, sugar, meat products, and potatoes. The consumption of other vegetables, fruit, berries, fish, and eggs was relatively small. Some regional differences were observed in the diet; for instance, the men in East Finland used more butter, milk and fish but less eggs than the West Finnish men.

Table 1. Consumption of different foodstuffs (excluding waste), grams per person per day

Foodstuff	West	East
Flours and grains	81	61
Bread, dark: soft	212	266
dry	23	3
Bread, white	133	113
Cakes and biscuits	12	16
Potatoes	296	273
Other vegetables	112	110
Starch (potato)	8	5
Fruit and berries	47	41
Margarine	7	8
Sugar	87	85
Milk, whole	872	860
Milk, skim, butter milk	178	312
Cream	46	53
Butter	65	88
Cheese	19	18
Meat and meat products	107	105
Fish	7	59
Eggs	35	11
Beverages (incl. alcohol)	56	15

Table 2. Percentage contribution of different foodstuffs to total energy

Foodstuff	West	East
Cereal products	32.7	30.2
Potatoes	6.1	5.6
Other vegetables	1.5	1.0
Fruit and berries	2.1	0.9
Sugar	10.0	9.6
Margarine	2.8	2.7
Butter	12.4	16.7
Other milk products	21.3	23.0
Meat and meat products	7.6	7.0
Fish	0.4	1.7
Eggs	1.8	0.7
Beverages (incl. alcohol)	0.6	0.4
Other products	0.7	0.5
Total	100.0	100.0

The mean energy intake was 3760 kcal. per day for the West Finnish men, and 3805 kcal. for the East Finnish men. Men employed in heavy forest work (work grade 4) in East Finland consumed on an average 4200 kcal. per day, men working mainly on farms (grade 3), 3745 kcal., and men doing light work (grade 2), 3445 kcal. per day. In West Finland the mean daily energy consumption of men employed in farm work (grade 3) was 3805 kcal. and of those in other occupations (grade 2), 3670 kcal.

Table 3. Intake of calories and energy yielding nutrients (grams per person per day, mean ± standard error) and their percentage contribution to total energy

	West	East
Energy, kcal.	3760 ± 100	3805 ± 160
Fats, g	148 ± 6.2	166 ± 9.0
Fatty acids, g:		
Saturated	78.5 ± 3.4	93.3 ± 5.2
Monoethenoid	48.2 ± 2.1	51.0 ± 2.8
Linoleic	9.4 ± 0.3	9.0 ± 0.4
Other polyethenoid	3.7 ± 0.2	3.5 ± 0.2
Proteins, g	114 ± 3.6	120 ± 4.5
Carbohydrates, g ..	509 ± 14.9	479 ± 19.9
% from total energy		
Fats	35.4	39.2
Fatty acids		
Saturated	18.8	22.1
Monoethenoid	11.5	12.1
Linoleic	2.3	2.1
Other polyethenoid	0.9	0.8
Proteins	12.2	12.6
Animal	6.7	7.8
Vegetable	5.5	4.8
Carbohydrates	52.4	48.2

The men used 148-166 grams of fat per day (table 3). In West Finland fats contributed 35.4 % of the total energy, in East Finland 39.2 %; the difference is statistically significant ($P < 0.01$). The fats were highly saturated; the ratio of polyethenoid to saturated fatty acids was 0.17 in West and 0.13 in East Finland. This difference is also significant (P = 0.02). The main source of fat was milk; 74 % of the total fat was derived from milk fat in East Finland and 67 % in West Finland. Other sources were meat (13-15 %) and margarine (7-8 %).

The intake of proteins was abundant in both areas, 114-120 grams per day. Since more than half of the proteins were of animal origin, mainly from milk and meat, the intakes of essential amino acids also were generous (table 4).

Table 4. Intake of amino acids (grams per person per day)

Amino acid	West	East
Valine	6.6	7.0
Leucine	9.3	9.8
Isoleucine	6.1	7.7
Threonine	4.5	5.9
Methionine	2.3	2.5
Cystine	1.6	1.6
Phenylalanine	5.5	5.6
Tyrosine	4.5	4.5
Tryptophan	1.4	1.7
Lysine	6.6	7.6
Arginine	5.5	5.7
Histidine	2.8	2.7

The diet included an adequate, even abundant, amount of vitamins and minerals with the exception of iodine (table 5); in both areas its intake was lower than

Table 5. Intake of some vitamins and mineral elements (per person per day, mean ± standard error)

Nutrient	West	East
Vitamin A, I.U.	4630 ± 235	5750 ± 415
Vitamin E, mg	13.8 ± 0.5	13.3 ± 0.7
Thiamine, mg	2.25 ± 0.07	2.11 ± 0.08
Riboflavin, mg	3.38 ± 0.14	3.78 ± 0.17
Nicotinamide, mg	18.4 ± 0.9	17.2 ± 0.9
Pyridoxine, mg	4.27 ± 0.14	4.32 ± 0.18
Pantothenic acid, mg	9.32 ± 0.34	9.36 ± 0.38
Choline, mg	1075 ± 43.5	1010 ± 89.4
Ascorbic acid, mg	105 ± 7.1	102 ± 7.9
Calcium, mg	1760 ± 83	1865 ± 81
Iron, mg	18.7 ± 0.6	18.5 ± 0.8
Iodine, µg	97.7 ± 3.1	73.2 ± 3.9

the World Health Organization (19) recommended minimum allowance, particularly in the East. A highly significant difference ($P < 0.001$) was observed in the intake of iodine between the two areas. The intake of vitamin A was somewhat higher in East Finland ($P < 0.05$). There were no marked differences in the intakes of other nutrients between the two areas.

Anthropometric characteristics and the diet

As seen in table 6, the West Finnish men included in the survey were somewhat

Table 6. Anthropometric characteristics of men investigated (mean ± standard error)

	West	East
Number of men	30	30
Mean height, cm	171.3 ± 0.96	167.4 ± 0.77
Mean weight, kg	72.2 ± 1.76	64.0 ± 2.34
Mean skinfold, mm:		
Triceps	9.4 ± 0.86	7.3 ± 1.04
Subscapular	12.3 ± 0.91	9.1 ± 1.34

taller and heavier than the East Finnish men. The mean relative body weight of the total population investigated was in all age groups in West Finland slightly higher than in East Finland. This is in accordance with the finding that the mean energy consumption was about the same for both groups, although the men in East Finland are reported doing heavier work.

The skinfold measurements are in agreement with these observations. Both the upper arm and subscapular skinfolds of the West Finnish men were thicker. There was a low positive correlation between the percentage of calories derived from fats and the thickness of the skinfolds in both areas, +0.35 in the East and +0.28 in the West.

Chemical analyses

A comparison of the results obtained by chemical analyses with those obtained by calculations for total fat, fatty acids, and total protein (table 7), shows that

Table 7. Comparison between values obtained by calculations according to Food Composition Tables, and by chemical analyses (average daily consumption of 20 men)

	Calculated	Analyzed
Proteins, g	121.3	121.2
Fats, g	162.8	166.3
Fatty acids, g		
Saturated	89.5	90.9
Monoethenoid	51.3	46.6
Linoleic	9.7	6.6
Other polyethenoid	3.6	5.4

the mean figures for the two groups are in a very good accordance with each other. Only for linoleic acid is there a statistically significant difference between the analyses and the calculations, but if all the polyunsaturated fatty acids are considered as one group, no significant difference is observed. However, although the mean figures tally with each other, there are differences in the intakes of individual men, about ± 10 % for proteins, ± 30 % for fats, and still higher for fatty acids in some individual subjects.

Discussion

In an attempt to throw light on the relations between the diet and a disease, it is essential to obtain as reliable data as possible about the food of the population or population group being surveyed. Various methods may be employed for collecting food consumption data, such as interviewing, the dietary record, and the weighing method. In our food consumption surveys we have used the precise weighing method despite the fact that it demands much work and is costly, so that the population surveyed cannot be very large. On the other hand, a great advantage of the method is its dependability, it evidently gives more accurate data about the food consumption than any other method.

In our earlier studies on diet and cardiovascular diseases (11, 12) the food consumption of families only was investigated. The question of whether the results of a family survey can be used for judging the diet of the men can partly be answered by comparing the nutrient compositions of the diets of men, on one hand, and of families, on the other. Table 8 gives the amounts of nutrients per 1000 kcal. both for men and for other members of family in the present survey. It can be seen that the diet of the men in both East and West Finland contains relatively more fat and less carbohydrates than the diet of the rest of the family members. The differences are greater in East than in West Finland. Because of the abundant use of milk fat, the diet of the eastern men is also richer in vitamin A than their mean family diet. In other respects the men's diets and the family diets do not differ much in either area. On the basis of these findings it is evident that in order to obtain results on the diet reliable enough for comparisons with the results of medical investigations, it is essential to study the food consumption of individual men and not only that of the families. However, the results of family studies can also give important information. E.g., we can see that the results of the three different family studies made in 1956, 1957 and 1959 (8, 10) are strikingly similar (table 9) which not

Table 8. Intake of different nutrients per 1000 kcal by men and other family members

Nutrient	West		East	
	Men	Other family members	Men	Other family members
Proteins, g	30.3	29.0	31.5	29.6
Fats, g	39.4	37.0	43.6	38.3
Carbohydrates, g	135.4	142.7	125.7	139.3
Calcium, mg	468	443	449	481
Iron, mg	5.1	4.7	4.9	4.9
Vitamin A, I.U.	1231	1252	1511	1279
Thiamine, mg	0.60	0.56	0.55	0.56
Riboflavin, mg	0.90	0.86	0.99	0.95
Nicotinamide, mg	4.9	4.5	4.5	3.8
Ascorbic acid, mg	27.9	28.1	26.8	26.1
(Total energy, kcal.)	(3760)	(2620)	(3805)	(2455)

Table 9. Daily intake of energy and different nutrients in the diets of families

	West Finland			East Finland		
	Summer 1956	Winter 1957	Fall 1959	Summer 1956	Winter 1957	Fall 1959
Total energy, kcal.	2600	2550	2915	2635	2730	2705
Proteins, g	78.6	79.1	86.0	86.9	82.9	81.4
Fats, g	99.3	98.9	110.2	102.7	105.0	107.3
Carbohydrates, g	357	346	409	348	376	367
Calcium, mg	1200	1200	1320	1410	1220	1310
Iron, mg	11.7	12.4	13.9	11.9	12.5	13.2
Iodine, µg	71.2	62.2	72.5	54.8	50.6	51.0
Vitamin A, I.U.	2830	3200	3630	2760	2820	3620
Thiamine, mg	1.51	1.57	1.67	1.53	1.69	1.51
Riboflavin, mg	2.33	2.26	2.54	2.74	2.48	2.60
Nicotinamide, mg	12.1	12.8	13.5	11.5	13.6	10.9
Ascorbic acid, mg	44.4	68.2	80.7	36.7	54.3	71.8

only reveals the stability of the general diet but also gives strong support to the reliability of the method used. In addition, it is seen that the seasonal variations in the diet of families, and evidently of men as well, are relatively small. There are considerable differences only in the intakes of vitamin A and ascorbic acid, mainly due to the more abundant use of fruit and vegetables in the fall season. When judging the diet of Finnish rural men from the results of the present survey, it can be seen that it abundantly meets the requirements of energy and of most nutrients (14). With increased physical activity the men seem to increase the proportion of fat in their food intake. Thus the percentage of fats from the total energy amounted to 42 % for the lumberjacks included in the survey, which is in good accordance with the 44.7 % obtained in an earlier survey of lumberjacks in the same area (5, 9).

Although the mean fat content of the diet is somewhat lower for the entire group of men surveyed than for the lumberjacks, it is still rather high. Since the largest part of the fat is obtained from milk, the fats are highly saturated. There is a significant difference in both the quantity and quality of the fats consumed in the two areas; in East Finland the total fat consumption is higher and the fats are more saturated than in West Finland. To what extent this is connected with the higher incidence of heart disease in East Finland still remains to be solved. It is evident, however, that the abundant use of fats in general and of saturated fats in particular is one of the causes for the high mean serum cholesterol values found in Finland.

The intake of all nutrients except iodine is sufficient to meet the requirements even when taking into account losses in food preparation and incomplete absorption. Although, according to ANTILA (2), nutritional anemia is rather common in these areas, even among the men, the diets were found to contain enough iron to meet the accepted requirements. On the other hand, the intakes of iodine are lower than the WHO recommended allowances in both areas, but especially in East Finland, where endemic goitre is known to be common (17).

As we have earlier pointed out (11, 12) this may partly account for the higher serum cholesterol values in East Finland.

Summary

In order to study the reasons for the difference in the frequency of cardiovascular diseases in East and West Finland, an individual food consumption survey was carried out simultaneously with a medical investigation in fall, 1959. A total of 60 men, 30 from each area, were selected at random from the men participating in the medical investigations. The men were 40-59 years old, and most of them were farmers and forest workers. The individual food consumption of the men was recorded by the precise weighing method during 7 consecutive days. The mean energy and nutrient intakes were calculated with the aid of Food Composition Tables and chemical analyses. The results were checked by analysing equivalent composites of uncooked food corresponding to the food consumption of 20 men.

The diet of the men included in the survey was a typical Finnish diet, the main energy sources of which are cereals, milk and milk products, sugar, meat and potatoes. The consumption of other vegetables, fruit and berries, fish and eggs was relatively small. The daily energy intake was 3760 kcal. for West Finnish men, and 3805 kcal. for East Finnish men. The contribution of proteins to total energy was 12.2 % in West Finland and 12.6 % in East Finland; more than half were of animal origin. In East Finland 39.2 % of the calories were derived from fats and in West Finland, 35.4 %; the difference is statistically significant. The fats were significantly more saturated in East Finland, the ratio of polyunsaturated to saturated fatty acids being 0.13 in East Finland and 0.17 in West Finland.

The intake of vitamin A was significantly higher in East Finland; that of other nutrients did not present any regional differences. The intake of iodine was low in both areas, significantly lower in East Finland.

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A COMPARISON OF THE FOOD INTAKE OF RURAL FAMILIES ESTIMATED BY ONE-DAY RECALL AND PRECISE WEIGHING METHODS

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Introduction

When investigating the diet of population or individual groups or individuals one has to consider which method would be most suitable for collecting food consumption data, so that the results concerning the nutritive value of the diet would be reliable. Recently, dietary recall as a quick and cheap method has more and more superseded traditional methods based on more or less accurate weighing and recording. In order to test the reliability of the results several comparative studies, especially on the food consumption of individual groups, have been carried out by comparing the results of dietary recall with those of other methods (1, 2, 5, 7). The results have shown that the recall method gives a fairly reliable picture on the average nutrition of the group investigated, but on the other hand not always on the nutrition of individuals.

Because in Finland we did not have any experience in recall as a research method in dietary surveys, we took the opportunity which offered itself in connection with dietary surveys associated with coronary heart disease studies to use both recall and weighing in collecting food consumption data. The purpose of this comparison was to find out whether the results of the food intake of families obtained by recall would be reliable enough, so that this method could be used in the future instead of weighing, which is a time consuming and very expensive method.

Method

In connection with international coronary heart disease studies, a dietary survey using the weighing method was carried out in Finland in the autumn of 1959 (3). Sixty rural families from two different comparison areas participated in this survey. The survey lasted one week in each family. The daily weighing and recording was done by outside investigators. The interviews were done on two separate days. The first one was concerned with the food intake of the day preceding the weighing survey and the second one with the food intake of one particular day of the recording week. The purpose of this was to find out whether the results on recall differ from each other and/or from the average results of the whole week. The interviews did not concern Saturdays or Sundays, since the diet of these days may differ from the diet of the usual weekdays. When interviewing, the investigator asked the housewives to recall the amounts of all food and foodstuffs the whole family had eaten the previous day. The nutrient content of the diets was calculated with the aid of Finnish Food Composition Tables (6). The results thus obtained were compared with those of the weighing method using the mean weighing values of the whole week as well as the values of the day corresponding to the second day recall as a comparison.

In the winter of 1965 a repeated dietary survey (4) was done by the method used earlier, the recording period, however, was cut down to five days. This time 48

Table 1. Mean nutritive values of food intakes on one-day recalls and weighings in East and West Finland in 1959 and 1965

	Energy-Cal.	Proteins g	Fats g	Carboh. g	Calcium mg	Iron mg	Vit. A I.U.	Thiamine mg	Ribo-flavin mg	Niacin mg	Ascorbic acid mg
1959 East											
7-day weighing	2705	81	107	367	1310	13.2	3620	1.5	2.6	10.9	72
1st day recall	2503	79	102	333	1247	12.4	3244	1.4	2.4	10.8	49
2nd day: weighing ..	2692	83	112	367	1282	13.8	3891	1.5	2.5	10.8	61
recall	2411	75	95	319	1169	12.1	3256	1.4	2.3	9.5	62
West											
7-day weighing	2915	86	110	409	1320	13.9	3630	1.7	2.5	13.5	81
1st day recall	2954	90	120	393	1490	16.1	3645	1.8	2.7	13.3	74
2nd day: weighing ..	3026	88	111	449	1350	13.8	3878	1.6	2.6	13.2	71
recall	3273	94	122	462	1492	15.4	4201	1.9	2.9	14.4	84
1965 East											
5-day weighing	3026	92	122	406	1400	14.3	3757	1.6	2.8	13.2	73
1st day: weighing	2944	86	115	405	1316	13.3	4156	1.6	2.8	12.6	65
recall	3047	96	121	409	1393	15.2	4163	1.8	3.0	14.0	77
2nd day: weighing ..	2829	85	112	385	1268	13.2	3390	1.5	2.5	12.7	60
recall	2830	87	109	388	1321	13.6	3272	1.6	2.6	12.8	61
West											
5-day weighing	2812	84	111	380	1282	13.0	3490	1.6	2.4	13.3	71
1st day: weighing	2744	83	110	368	1283	12.8	3041	1.6	2.4	12.8	57
recall	3407	100	132	471	1335	17.1	3977	2.0	2.6	16.5	62
2nd day: weighing ..	2584	77	103	349	1255	11.3	3162	1.4	2.2	11.5	62
recall	2733	85	107	372	1253	13.6	3665	1.6	2.4	12.3	71

of the families of the previous study participated. An interview concerning the food intake of two different days was performed also in connection with this study. Both of these days were included in the recording period, wherefore the weighing data were available for comparison with the results of the interviews.

Results

The methods were compared in the first place by using as a basis the results of the nutritive value of the daily diet. Because the precise weighing of food is the most accurate method in the estimation of food consumption, the results on recall were compared with those obtained by weighing. The intakes of energy and different nutrients according to recall and weighing are shown in table 1.
When comparing the results of the first day recall with the mean results of the whole recording week, in the study of 1959, it can be seen that the differences are very small. The intake values of nutrients according to the recall method were less than 10 % lower than those obtained by weighing in East Finland, with the exception of ascorbic acid, the intake of which was 32 % lower. In West Finland only the intake values of calcium and iron differed more than 10 % from the weighing data. The first day recall would thus have given a highly reliable picture on the average food intake of families for most nutrients. On the other hand, the results of the second day recall differed more from the mean results of the whole week, the differences being, however, less than 16 % for all nutrients. When comparing the results of the second day recall with the corresponding results of the weighing method it can be seen that in the East the greatest difference (—15 %) was in the amount of fats and the smallest (+2 %) in ascorbic acid. In the West the greatest difference (+19 %) was in thiamine and the smallest (+3 %) in carbohydrates respectively (table 2). Statistically significant divergences were found in the East in the amounts of energy, proteins, fats, carbo-

Table 2. The results of one-day recall as a percentage from those of corresponding one-day weighing in 1959 and 1965

Nutrient	East Finland			West Finland		
	1959	1965		1959	1965	
	2nd day	1st day	2nd day	2nd day	1st day	2nd day
Energy	90	104	100	108	124	106
Proteins	90	111	103	107	121	110
Fats	85	105	98	110	120	104
Carbohydrates	87	101	101	103	128	106
Calcium	91	106	104	111	104	100
Iron	88	114	103	112	134	120
Vitamin A	84	100	97	108	131	116
Thiamine	93	112	104	119	125	118
Riboflavin	92	107	103	112	110	108
Niacin	88	112	101	109	129	107
Ascorbic acid	102	119	102	118	109	115

hydrates, iron and thiamine. In the West, however, there were no differences of statistical significance.

An important fact to be mentioned is that the results on recall in the East were altogether lower than the results of the weighing method, whereas in the West the recall method, as a rule, gave higher values. This indicates that the housewives in East Finland tended to underestimate and those in West Finland to overestimate the consumption of most foodstuffs. In the event that the whole material, East and West, had been treated as one group, the divergences in the results would have levelled down. Thus for instance the results of the second day recall would have differed from the results of the corresponding weighing day as follows: energy —0.6 %, proteins —1.4 %, fats —3.7 %, carbohydrates —4.4 %, calcium +1.5 %, iron ±0 %, vitamin A —5.4 %, thiamine +2.5 %, riboflavin +2.1 %, niacin —0.2 % and ascorbic acid +11.9 %.

According to the study performed in the winter of 1965 the results on recall concerning the nutritive value of the diet were in both areas higher than the results obtained by the weighing method for nearly all nutrients on both recall days. Overestimation was stronger on the first day recall, and distinctly stronger in the West than in the East. The mean results of the second day recall in the East can ever be regarded as excellent, since the divergence from the weighing data was no more than ± 4 % in respect of all nutrients. There was no statistical difference between the weighing and recall results on either area.

When comparing the results on recall with those obtained by the weighing survey, it can be established that in the West the results of both recall days deviate less from the average results of the whole five-day recording period than from the weighing data of the correponding days. The agreement of the second day recall results with the average results of the whole survey period is nearly complete in respect of several nutrients. On the other hand, in the East the results of the first day recall, with the exception of thiamine, agree very well with the average results of the whole survey period.

When comparing the recall data of the two different years it can be seen (table 2) that the housewives in East Finland were able to estimate the food consumption, on the average, better in the second study than in the first one. On the contrary, the housewives in West Finland estimated the consumption of the first day recall markedly poorer in the second study, whereas the second day recall gave equally good results in both studies.

Although the mean nutrient intakes estimated from recalls agreed rather well with those estimated from the results of the weighing method, there were, however, very wide variations between the results of these two methods in individual families. As can be seen from table 3, the agreement was greatest in energy and in proteins, since 53-76 % of the recalled diets agreed within ± 20 % of the weighed diets in energy and in the case of proteins 46-70 % of the recalls were within ± 20 % of the weighed diets. Although the mean results of the second recall in 1965 were closer to the results of the weighing survey than those of the first recall, the distribution was almost the same in both cases.

Table 3. Distribution ranges of intakes of nutrients within certain agreements between recall and weighing in 1959 and 1965

Nutrient	Agreements within		
	± 10 %	± 20 %	More than ± 20 %
	Per cent of recalls		
Energy	17 — 53	53 — 76	24 — 47
Proteins	21 — 50	46 — 70	30 — 54
Fats	8 — 42	25 — 66	34 — 75
Carbohydrates	13 — 42	42 — 62	38 — 58
Calcium	17 — 38	40 — 63	37 — 60
Iron	17 — 53	38 — 70	30 — 62
Vitamin A value	12 — 38	33 — 62	38 — 67
Thiamine	12 — 40	46 — 58	42 — 54
Riboflavin	17 — 50	38 — 75	25 — 62
Niacin	17 — 37	33 — 62	38 — 67
Ascorbic acid	8 — 27	25 — 46	54 — 75

The differences in the accuracy of estimation were not very great between the two areas. In some cases there were a few more recalls in the East than in the West, where the agreement was within ± 10 % and ± 20 %.

Discussion

The reliability of the results on recall in estimating the nutritive value of food intake greatly depends on the fact how accurately the interviewed persons are able to recall and estimate their own or their families' food consumption. In our study those interviewed were mainly the housewives of farm and lumberjack families. Most of them were between 40 to 55 years old. Some housewives were able to estimate the food consumption especially well, whereas most of them had more or less under- or overestimated the amounts of food consumed. Nearly all housewives both in the East and West had most of all overestimated the amounts of grain products. The overestimation of butter, milk, potatoes and meat was also very common, more so in the West than in the East. In the study of 1959 the housewives in East Finland distinctly tended to underestimate the consumption of most foodstuffs. This was also partly true in the later study, although not as obviously as in the first one. The differences in the estimates between the housewives in East and West Finland may partly be due to differences in national character, partly to different food habits. In West Finland the diet is more varied than in East Finland. In addition, food is there prepared for several meals or days at a time, which easily causes errors in estimation, since it is easier for a housewife to recall the amounts of foodstuffs used in a whole dish than to estimate from these the consumption of the day in question. Evidently this fact has caused the greatest overestimations. Since in the study of 1965 the second day recall values agreed with the results of the weighing survey better than the first day recall values, it is evident that the errors in estimation have become smaller. In the estimations of some housewives there was a certain kind of consistency, either to over- or underestimate, whereas some others overestimated at one time and underestimated another, the consumption of the same foodstuff.

The errors made in estimating the amounts of different foodstuffs are naturally reflected in the results concerning the nutrient content of the diets. Over- and underestimations are often levelled down in the average results of the whole group, for which reason the average values of the recall results did not to any noteworthy degree deviate from those of the weighing results, although the differences in these results in many individual cases were rather great. The agreement between the averages of recall and weighing was so good, especially in East Finland, that recall would have been used instead of weighing in studying the nutrient content of the food intake of families on a group basis. A similar conclusion was also made by YOUNG and co-workers (7) and later by TRULSON (5) and ADELSON (1). Based on the results it can be said that any day of the survey period could have been chosen for recall, although there were some deviations in the results obtained on the two different days.

In a few cases the recall method could have been used when studying the diet of individual families, though in most cases, it did not turn out to be a satisfactory method in this respect. Although for some nutrients about half of the recalls agreed within ± 10 % of the weighings, for some other nutrients less than 10 % of the recalls had the same agreement. In some families the amounts of nutrients estimated from recalls were only one half or even one third of the ones estimated from weighing, in other families three times as great or even more. Also other investigators (1,5) have found more or less differences between the dietary information provided by recall and other more precise methods. Thus the value of recall in estimating the diet of individuals or families may be in many cases questionable, although it often gives fairly reliable results of the average diet of indivdual or family groups.

Summary

In 1959 and 1965 dietary surveys were carried out in 60 and 48 rural families in two areas in Finland by using the weighing method. In connection with these studies the housewives were asked to recall all the food consumed by their families during two separate days. The nutrient content of the diets was calculated and the results obtained by these two methods were compared. The results show that the average values of most nutrients on recall differed from those of the weighing method in the East by less than 10 %. The agreement was not quite as good in the West. In many cases the recalls were closer to the averages of the whole weighing period than to those of the corresponding weighing days. Although the results in most cases agreed very well with each other on a group basis, there were, however, large differences in the amounts of nutrients on recall and weighing in individual families.

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CHEMICAL ANALYSIS IN CONNECTION WITH DIETARY SURVEYS IN FINLAND *)

by M. PEKKARINEN

Introduction

In dietary studies, the data indicating the nutritive value of the diet are usually based on the use of food composition tables. Besides the method of collecting the information of food consumption, the reliability of the results depends on the applicability of food composition tables to the calculation of nutrient contents. As the diet nowadays may contain more and more processed foods, the exact composition of which is unknown, or if we want to find out the intake of such nutrients as the food composition tables give no figures of, it is necessary to make a chemical analysis. However, it is seldom used as the only method in dietary studies. Mostly it is used to supplement some other method, whereby it can be used for controlling the results obtained by other methods.

The reliability of the results obtained from analysis much depends on how well the food samples analyzed correspond to the diets under study. The samples can be collected by different means.

1. At each meal a duplicate of food eaten or part of it is collected, and the composite equalling one day's consumption is analyzed. This type of sample has been called a ready-to-eat food sample in this paper.
2. The composites as above are collected daily and combined into one composite equalling the diet of the whole study period. This method has been used e.g. in Yugoslavia and the Netherlands (1, 4). Samples of this kind have been called food composites by BUZINA et al. (1).
3. Samples of raw foods are collected equalling the mean daily diet of the whole study period (samples called equivalent composites). This method has been used in studies carried out in Yugoslavia, Italy and Finland (1, 2, 8). The food samples can be collected either at local shops or at the homes of the persons examined.

Depending on circumstances, different means of collecting samples must be used. With the aid of studies carried out in Finland, attempts have been made to find out whether the results obtained when analyzing samples that have been collected and treated in different ways disagree so greatly that they cannot replace each other. The nutrients compared have been proteins, fats, carbohydrates and energy, and, in some cases, fatty acids. The results of analyses have furthermore been compared with the corresponding calculations. The results of these comparisons are presented in this paper.

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Material

Chemical analysis has been used in Finland when examining the diet of individuals, the data of food consumption having been based on the daily weighing of all food (8). The first study (Study I) was carried out in 1959 in two rural areas of the country. It comprised a total of sixty men (aged 40 to 59), whose food consumption was studied during a period of seven days. In 1965 the study was repeated (Study II), 48 men of the previous study taking part, their food consumption during a period of five days being studied (7). In 1964 the individual food consumption of four intellectual workers was examined during four to seven days, the total number of recording days being 24 (Study III). In addition to this, the average food consumption of patients has been ascertained in a long term study in two mental hospitals (12) by using the annual food account (Study IV). In all studies the nutritive value of the diets has been calculated by the use of Finnish Food Composition Tables (11). As far as the most important fat sources (milk fat and margarine) are concerned, the calculation of fatty acids has been based on our own analytical data. For hospital studies there has been further analysis of the fatty acids in beef, pork and salad oils. The fatty acids of other foodstuffs have been calculated by using the figures published by others (3, 5).

Collection and preparation of food samples

After calculating the amounts of different foods consumed, composites of raw, wasteless foods equalling the average daily consumption of the whole study period of a total of 20 men in Study I and that of all the 48 men in Study II, were collected separately at the home of each man. In Study III, ready-to-eat food samples equalling one day's consumption were weighed. Furthermore, on the evening of each day of study, equivalent composites of raw foods were collected. The purpose of this step was to compare with each other the results of analyzing the ready-to-eat food sample and those of analyzing the equivalent composite. The samples were collected only according as there was time to analyze them. In Study IV, the food samples were collected at each hospital separately as equivalent composities on the basis of the annual consumption. Two identical samples were weighed out of these foodstuffs, one being analyzed raw and the other boiled. All weighed food samples were packed in paraffinized pasteboard jars or in plastic boxes in which they were conveyed to the laboratory. In Studies I and II, the samples were transported a distance of several hundred kilometres.

In Studies I and II, the food samples were first ground, whereafter they were homogenized in a Warring blendor. In other studies the samples were homogenized immediately in a one gallon size Warring blendor. In Study I portions of the homogenate were preserved by lyophilization and thus the analysis could be carried out at any time. In Study II on the other hand, the homogenized samples were kept tightly sealed in 2 to 3-decilitre boxes of styrene plastic at a temperature of —20 °C, and were analyzed within two months after being collected.

According to previous studies (6), a storage as short as this was not considered to cause changes in the fatty acids of the samples.

Chemical analysis

The proximate composition of all mixed food samples and the fatty acid content of the samples in Studies II and IV were analyzed in the Department of Nutritional Chemistry at the University of Helsinki. The fatty acids of lyophilized samples in Study I were analyzed in the Laboratory of Physiological Hygiene at the University of Minnesota. *) In analyses made in Finland, water, ash, total fats and proteins were determined by using generally accepted standard methods. The total fat was determined by the S-2 R (Röhrig) method (9) using ethyl ether and petroleum ether as solvents, the method being a modification of that described by Söderhjelm and Söderhjelm (10). The fatty acids were isolated from the fats and esterified with a mixture of methanol and sulphuric acid. The dried methyl ester mixtures were analyzed by gas-liquid chromatography using a "Perkin Elmer M 116 E" chromatograph and a 400 × 0.6 cm metal column containing 15 % EGS as stationary phase at a temperature of 198 °C. The contents of individual fatty acids were estimated from the areas of the peaks measured by the values of the triangle method.

Results

Ready-to-eat diets vs. equivalent composites

As we wanted to know whether ready-to-eat food samples (A) could be replaced by collecting the equivalent composites of raw foods (B), a comparison was carried out between the results of samples of different types. As can be seen from table 1, the averages concerning the studied nutrients agree very well with each other. The differences of results between the methods were small also as far as individual samples were concerned, as is shown by the values indicating a range

Table 1. Daily intakes (mean ± standard deviation) of energy and energy-yielding nutrients according to chemical analyses in ready-to-eat food samples and in equivalent composites as raw foods (n = 24)

Type of food sample	Proteins g	Fats g	Carbohydrates g	Energy Cal.
A. Ready-to-eat food samples	88.4 ± 19.9	83.6 ± 32.0	289 ± 66	2261 ± 537
B. The equivalent composites as raw foods	88.6 ± 20.4	83.2 ± 31.7	299 ± 73	2298 ± 567
Range between methods A and B ..	0 — 7.2	0 — 9.0	0 — 65	5 — 303

of differences. In few cases only did the results of analysis of sample A differ from those of sample B with more than 10 %. In most cases the difference was less than 5 %. No statistically significant differences were observed in the results in regard to any nutrient.

*) In Finland the fatty acid analyses were done by Dr. R. Rashid and in Minnesota by Dr. J. T. Anderson, to whom I am very grateful for their co-operation in the study.

When samples are collected as raw foods, it is advised to boil them first in order to make the homogenization easier. As we did not know whether boiling would improve the results or not, we carried out an experiment. In Study IV, of identical samples some were homogenized raw and others were boiled in a closed vessel for five hours; the latter, having cooled down, were homogenized immediately before being analyzed. The results in table 2 show that the amounts of proteins and fats are identical in samples of both types. Also the fatty acids of the same

Table 2. Proteins, fats and fatty acids in raw and boiled food samples according to chemical analyses

	Sample 1		Sample 2	
	Raw	Boiled	Raw	Boiled
Proteins, g	97.4	97.4	100.3	100.3
Fats, g	99.3	99.2	117.7	117.6
Fatty acids, g				
saturated	54.3	54.1	35.2	34.1
monoethenoid	29.1	28.4	35.6	34.6
linoleic	9.4	9.8	36.7	37.8
other polyethenoid	2.1	2.2	5.4	6.3
Ratio: polyethenoid/saturated	0.21	0.22	1.20	1.29

samples were analyzed, and it was observed that their amounts in different samples differed very slightly from each other. The amounts of saturated fatty acids and monoethenoids in the boiled samples were slightly smaller and those of polyethenoids were accordingly slightly larger than in the raw samples. The differences mentioned were somewhat bigger in samples containing an abundance of polyunsaturated fatty acids.

Calculated vs. analyzed

Energy-yielding nutrients. In all four studies the results obtained from analyses and those given by calculations based on food composition tables were compared with each other. As the results of the equivalent composites of raw foods did not differ from those of the cooked ones to a degree worth mentioning, the comparision in table 3 contains with respect to all studies only the results of the analyses of raw samples. As can be seen, the mean intake of proteins and fats given by calculations and obtained from analyses are nearly identical in all studies. Neither did the mean intakes of carbohydrates and energy obtained through the two methods differ greatly from each other. The differences were neither great with respect to individual samples, for in the case of most nutrients, over 50 % of the diets calculated differed less than 5 % from the results obtained by analysis. The greatest differences between methods were observed in the amounts of fats in Studies I and III. In the statistical testing no significant differences were observed between the results of these methods.

Table 3. Daily intakes (mean ± standard deviation) of energy and energy-yielding nutrients according to calculations and chemical analyses and ranges between methods in comparative studies made in 1959-1966

Study	Proteins g	Fats g	Carbo-hydrates g	Energy Cal.
I. 1959 (n = 20)				
Calculated	121.3 ± 23.8	162.8 ± 48.2	523 ± 92	3964 ± 756
Analyzed	121.2 ± 26.5	166.3 ± 52.8	510 ± 91	4024 ± 803
Range	0.2 — 13.6	0.3 — 61.6	8 — 88	6 — 521
II. 1965 (n = 48)				
Calculated	112.7 ± 26.4	151.4 ± 37.5	452 ± 89	3562 ± 679
Analyzed	115.5 ± 26.6	154.7 ± 39.2	470 ± 87	3734 ± 676
Range	0.6 — 16.1	0.5 — 54.4	0 — 61	6 — 421
III. 1964 (n = 24)				
Calculated	86.7 ± 20.9	84.3 ± 32.7	279 ± 60	2171 ± 542
Analyzed	88.6 ± 20.4	83.2 ± 31.7	299 ± 73	2298 ± 567
Range	0 — 12.9	0 — 13.6	0 — 55	2 — 325
IV. 1966				
Sample 1: Calculated	97.3	99.2	418	2905
Analyzed	97.4	99.3	475	3183
Sample 2: Calculated	100.3	117.8	372	2920
Analyzed	100.3	117.7	446	3244

Fatty acids. Only during the last decade more attention has been paid to the fatty acid composition of food. Particularly when analyzing fatty acids in different diets, chemical analysis has proved to be necessary, for fatty acid values based on analyses of local foods are seldom available for calculations. In Finland, the fatty acid composition of the diet has been calculated together with Studies I and II (7, 8) and in connection with a long-term hospital study (12), of which Study IV is a part. The fatty acids of the same diets have been determined chemically. The saturated fatty acids, monoethenoids and polyethenoids, except for linoleic acid, are given in the calculations in groups, not specifying individual fatty acids. For comparison also a similar grouping has been made in the results of the analysis.

When comparing the results based on analyses and those based on calculations (table 4), it can be seen that in all studies analysis gives a smaller total of polyunsaturated fatty acids than the calculations. Statistically, there is a highly significant difference (P = 0.001) given by these two methods as to the amounts of linoleic acid in Study I and as to that of the other polyethenoids in Study II. If on the other hand all polyethenoids are examined as one group, the significance of differences decreases. In Study IV, the difference between the amounts of polyunsaturated fatty acids given by calculations and that obtained by analysis is smaller than in Studies I and II. This is due to the fact that the calculations have been based on the fatty acid figures of analyzing more of the major fat sources in Study IV. No statistically significant differences between the methods were observed in the amounts of saturated fatty acids, but in monoethenoids on the other hand a hardly significant difference was observed in Study II (P = 0.05).

Table 4. Daily intakes (mean ± standard deviation) of fatty acids and the ratio of polyunsaturated (P) to saturated (S) fatty acids according to calculations and chemical analyses

Study	Saturated g	Mono-ethenoids g	Linoleic g	Other poly-ethenoids g	Ratio P/S
I. 1959					
Calculated	89.5 ± 30.1	51.3 ± 14.3	9.7 ± 1.8	3.6 ± 1.2	0.15
Analyzed	90.9 ± 29.4	46.6 ± 15.2	6.6 ± 1.9	5.4 ± 5.7	0.13
II. 1965					
Calculated	87.3 ± 22.8	45.2 ± 11.3	7.9 ± 1.8	3.7 ± 1.8	0.13
Analyzed	87.5 ± 22.8	51.1 ± 13.9	7.4 ± 2.3	1.0 ± 0.6	0.09
IV. 1966					
Sample 1: *Calculated*	47.6	32.8	11.5	2.7	0.30
Analyzed	54.3	29.1	9.4	2.1	0.21
Sample 2: *Calculated*	30.3	39.3	36.6	6.8	1.43
Analyzed	35.2	35.6	36.7	5.4	1.20

In all studies, chemical analysis gave a lower ratio of polyethenoids to saturated fatty acids than given by calculations. Even though the average results of the methods agreed fairly well with each other in several cases, there were considerable differences between individual results, the differences being greatest with respect to polyunsaturated fatty acids.

Discussion

The aim of the present study has been, with the aid of comparative studies, to find out the use of chemical analysis in dietary surveys for determining the proximate composition and fatty acid contents of the diet. In the studies, the results obtained by analyzing mixed food samples collected in different ways were compared with each other. Likewise, the results of the nutritive value of the same diet based on analysis and calculation were compared.

The results showed that in Finland food samples for chemical analysis can be collected in composites of raw foods equalling the daily food consumption, for the results of analysis of the samples mentioned did not differ from those of ready-to-eat food samples. Thus, instead of several food samples, only one mixed food sample is required, this facilitating the collection of samples, particularly in studies that must be carried out in sparsely populated regions far from laboratory and without proper means of preserving the food samples. As the quality of foodstuffs may vary even greatly in different families, as e.g. the fat content of whole milk in our own studies from 3.6 % to 5.0 %, it is important that the food samples are collected separately at the home of each person examined, so that they correspond to the diet as well as possible. In studies carried out in e.g. Yugoslavia and Italy (1, 2), corresponding samples have been collected at local markets. This may be one reason for the differences that BUZINA et al. (1) observed in their studies to exist between the results of food composite and equivalent composite samples.

According to our experiments, raw food samples need not necessarily be boiled before homogenization, supposing that it can be done effectively enough and that the homogenization of foodstuffs difficult to grind is done separately when necessary.

The comparison between chemical analysis and calculations showed in all of our studies so great an agreement, particularly in the mean values of proteins and fats but also in those of carbohydrates and total energy, that chemical analysis is not necessary for the control of calculations. The reliability of calculations is obviously due to the Finnish food composition tables being composed very largely on the basis of the analytical data of our local foods. Our studies agree with those carried out in Italy by FIDANZA and FIDANZA ALBERTI (2), who also found a very great similarity in the mean values of proteins and fats by calculations and chemical analysis of the equivalent composites of the same diet. On the contrary, in a comparison of corresponding methods in Yugoslavia, BUZINA et al. (1) observed that the results obtained from chemical analysis of equivalent composites gave significantly higher values than the calculations. The differences between the methods are probably due to the food composition tables not being as applicable for calculations as ours and perhaps means of collecting samples being a little different from those used in Finland. In corresponding studies carried out in the Netherlands by DEN HARTOG et al. (4) the comparison was made between calculations and chemical analysis of one week ready-to-eat food samples. The results showed very close agreement in the intakes of calories and energy-yielding nutrients by these two methods.

No comparisons of the amounts of fatty acids based on calculation and chemical analysis have appeared in the literature. This is obviously due to the fact that there are no existing tables of the fatty acids in different foodstuffs reliable enough to be used as such as a base to calculations. Particularly the quality of animal fat is influenced quite decisively by the feeding of cattle and thus e.g. the fatty acid composition of milk fat may vary considerably from time to time. For this reason, the results of the chemical analysis of the fatty acid composition of a certain diet are more reliable than calculations based on tables.

In spite of the limitations connected with the use of fatty acid tables we have carried out a comparison between the results obtained by calculation and chemical analysis. In order to get the results of calculations as reliable as possible we have used when calculating the fatty acid composition of the diet our own analytical data with respect to the most important fat sources such as milk fat, margarine and meat fat. The results obtained by chemical analysis and calculations showed that the total amounts of saturated fatty acids, the quantity of which in the diet is very great, were surprisingly alike by the two methods. On the other hand, the differences are more distinct for polyunsaturated fatty acids, the proportion of which in the total of fatty acids is very small in the Finnish diet. According to our experience it is evident that calculations can give a fairly reliable picture of the fatty acid composition of the diet, provided that the calculations are based on the analyzed fatty acid values of such local foods which constitute the major sources of fats.

Summary

In connection with four dietary surveys, the food consumption data being based on weighings or an annual food account, samples equalling the daily food consumption were collected and analyzed for proteins, fats and fatty acids. The results obtained from analyses of ready-to-eat food samples and those of equivalent composites were compared with each other. For comparison, in connection with one study, one of the two identical raw food samples was analyzed as boiled and the other without boiling. The results obtained by analyzing the equivalent composites were compared with those of calculations based on tables. Analyzed values for energy and energy-yielding nutrients were almost the same in ready-to-eat food samples and in the corresponding equivalent composites showing that samples for analysis can be collected using the latter, less complicated method. The amounts of proteins and fats were exactly the same and those of fatty acids nearly the same in samples treated as raw and in those which were boiled. Also the calculated values agreed well with those obtained from analysis of equivalent composites, and no statistically significant differences were found. For fatty acids chemical analysis gave lower values from the totals of polyunsaturated fatty acids, and also the ratio of these acids to saturated ones was lower by analysis than by calculations. Statistically highly significant differences were observed in the amount of polyunsaturated fatty acids, a hardly significant difference in monoethenoids and none in saturated fatty acids.

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THE DIETS OF MIDDLE-AGED MEN IN TWO RURAL AREAS OF GREECE *

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Surveys of the habitual diet are an integral part of a cooperative international study on the epidemiology of heart disease in men aged 40-59 when first examined (6). In Greece, one sample embraces the men in villages on the island of Crete centered about 30 kilometers east of the port city of Iraklion. Another sample in Greece in made up of men living in villages northeast of the town of Corfu on the island of the same name. These areas have been described and some characteristics of the men have been reported elsewhere (6). The present paper reports the results from 3 dietary surveys in Crete and 2 in Corfu. In Crete the villages involved were Agies Paraskies, Thrapsano, and Kastelli. In Corfu the villages were Ano Korakiana, Skriperon, and San Marco.

Subjects and field operations

The medical studies cover practically all (over 95 per cent) of the men in the defined areas aged 40-59 at the start of the program in 1960 in Crete and in 1961 in Corfu. From these cohorts random sub-samples of men were selected for detailed studies of the diet. A few men selected at random for this work were unwilling or unable to cooperate because of special circumstances in their homes and these men were replaced by further random selections from the cohort. The selections of the subjects and the general organization of the dietary studies were similar to the parallel programs in other countries (7, 2, 3, 4, 5). Each survey involved recording the weights of all foods eaten by the individual subjects during 7 successive days and a quantitative replicate of each meal was collected for subsequent chemical analysis of the composite 7-day diets. The amounts of alcoholic beverages were also recorded but these materials were not included in the composites for chemical analysis.

A team of dietitians from Athens, with local help, carried out or supervised all measurements and food collections in the households. In general, each dietitian worked in only two neighboring households in the same period so close contact was maintained at all times. In several of the surveys this work was checked in the field by dietitians engaged in similar work in this program in other countries.

Covered enamel jars were provided to each household for each day's collection of the replicate of food eaten by the man in the study. These composites were collected in the evenings, stored overnight in a refrigerator, and in the morning, after the composite was finely ground and well mixed, a 10 per cent sample was transferred to a glass jar and stored in a low-temperature refrigerator. At the

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end of the 7 days of food collection the combined samples were well mixed with a heavy-duty electric mixer and 240 grams, as a 7-day sample, was flown to Athens where it was again mixed and sub-samples were lyophilized at the Department of Chemistry of the University of Athens. Aliquots of these lyophilized samples were sealed in small glass containers and shipped to the University of Minnesota for chemical analysis.

Chemical analyses—proximate analysis

Weighed aliquots of the lyophilized homogenates of the 7-day diets were analysed for water by drying to constant weight in a vacuum oven, for nitrogen, using the Kjeldahl method, for lipids („fat") by Soxhlet ether extraction, and for ash by incineration in a muffle furnace. Protein was estimated as 6.25 times nitrogen weight. Carbohydrate was estimated by subtracting from the weight of lyophilized homogenate the weights of water, protein, fats, and ash.

Calories were computed from the weights, in grams, as 4 times protein, 9 times fat, and 4 times carbohydrate. Total calories were estimated as the sum of protein, fat and carbohydrate calories plus 7 times grams of alcohol the weight of alcohol being computed from the volumes of alcoholic beverages consumed and tables of alcohol concentration in the various beverages.

The diet samples were analysed in the United States but in some cases duplicates were also analysed in Athens (Prof. D. S. GALANOS) and in Naples, Italy (Prof. F. FIDANZA). Table 1 summarizes the results from 31 sets of duplicates

Table 1. Comparison of the results of chemical analyses of 31 sets of aliquots of diet composites in Athens and in the U.S.A.

Item	% of total calories from: Proteins	Fats
1. Mean, Athens	10.06	34.63
2. Mean difference, Athens minus U.S.A.	— 0.75	— 0.43
3. Standard error of 2)	± 0.256	± 0.739
4. 100 x 2) / 1)	— 7.46	— 1.24
5. t = 2) / 3)	2.93	0.58
6. p	< 0.01	n.s.

analysed in Europe as well as in the United States. The average calories provided by proteins is indicated to be 7.46 per cent lower from the European than from the U.S. analyses and the difference is statistically significant (p = less than 0.01). For fats, the European results are also lower than those obtained in the U.S. but the difference, 1.24 per cent, is not statistically significant. In the subsequent presentation of the analytical data in this paper only the U.S. values are used.

Chemical analyses—fatty acids

The lipid (ether) extracts of the lyophilized homogenates were methylated by treatment with methanol and sulfuric acid as described by ANDERSON (1). The resulting methyl esters were analysed by gas-liquid chromatography (GLC) in three different systems, the carrier gas being helium in all cases.

Method 1 used a Beckman GC-2 GLC machine with a column 150 cm. long packed with 30 per cent butanediol succinate (Craig) polyester on acid-washed Celite, 60-80 mesh, operated at 220-230 °C. Method 2 used an F and M model 810 GLC machine with flame ionization detector, the column being a 6-foot length of 1/4 inch (internal) diameter stainless steel tube packed with 10 per cent ethylene glycol succinate on Deatoport S, 80-100 mesh. The column temperature was 200 °C., detector 300 °C., and injection port 280 °C. Method 3, at Athens, used an F and M model 720 GLC machine, the column being 6 feet in length, ½ inch outside diameter, packed with 15 per cent ethylene glycol succinate on Chromisorb S, 80-100 mesh. The column temperature was 188 °C., detector 315 °C., injection port 300 °C.

Table 2 summarizes results from duplicate GLC analyses. In general, the standard error of measurement, S.E.M., was less than one per cent of the total fatty acid except for oleic acid (18:1), and linoleic acid (18:2). When Method 1 was compared with Method 2 the mean values for the several fatty acids were not significantly different except for oleic and linoleic acids. Compared with Method 1, Method 2 gave lower values for oleic and higher values for linoleic acid. On the other hand, Method 3 results (Athens), are higher in oleic and lower in linoleic

Table 2. Duplicate analyses for fatty acids as percentage of total fatty acids in the lipid extracts of the lyophilized diet homogenates

Average differences Method 1 minus Method 2, and Method 1 minus Athens, and the standard error of measurement for pairs of duplicates, $(S.E.M.)^2 = (\sum\Delta^2) / 2N$, where Δ = difference between duplicates and N = number of pairs of duplicates.

	Fatty Acid							
Item	12 : 0	14 : 0	16 : 0	18 : 0	16 : 1	18 : 1	18 . 2	18 : 3
Method 1 - method 2	0	0	0.8	0.4	0.4	3.5	—0.6	—0.1
S.E.M.	±0.1	±0.4	±0.9	±0.5	±0.6	±2.6	±0.8	±0.7
Method 1 - Athens	0.1	—0.4	—0.1	—0.4	—0.4	—3.9	1.8	0.7
S.E.M.	±0.2	±0.4	±0.7	±0.9	±0.3	±3.6	±1.4	±0.9

acid than those from Method 1. Pure fatty acid standards were used in all 3 methods. It was not possible to make sure which system provided the most accurate results but there was indirect evidence that Method 3 underestimated the linoleic acid content of the diet. Since Method 1 and Method 3 results were available only on some of the samples, in general the subsequent presentation of fatty acid data relies on Method 2 data.

General character of the diets

As indicated in table 3, both in Crete and Corfu the diets of the rural men are dominated by olive oil and bread, these two items alone accounting for 50 to over 60 per cent of the total calories. Olives as such, chiefly the black kind, are eaten only in small amounts, the daily average being of the order of 5 grams (edible portion). Besides the use in bread, cereal grains contribute substantially to the diet in the form of macaroni, rice, and barley soup or porridge.

Alcohol makes an appreciable part of the total calories for most of the men but around one-fifth of the men are abstainers. Almost all of the alcohol is taken in the form of light wine, averaging about 12 per cent alcohol by volume. Very small amounts of a kind of brandy („raki") and liquors („ouzo" etc.) are con-

Table 3. Sources of calories in the diets of the men in Crete and Corfu

Calories estimated from tables of food composition applied to the weighed quantities of all foods and alcoholic beverages consumed by each individual in 7 consecutive days. Means and, in parentheses, standard deviations.

	Crete			Corfu	
Item	1960	1962	1965	1961	1963
Period	Sept.	May-June	Feb.	Sept.	Mar.-April
Number of men	32	34	30	40	34
% Cal., bread	28.9	28.1	22.6	38.1	40.8
	(7.1)	(6.6)	(7.9)	(7.0)	(6.8)
% Cal., olive oil	32.6	33.0	28.5	25.7	22.1
	(6.8)	(6.5)	(7.2)	(6.0)	(5.5)
% Cal., animal protein	2.6	2.8	4.0	3.3	3.2
	(1.6)	(2.8)	(1.6)	(2.1)	(1.7)
% Cal., animal fat	5.5	5.8	6.7	3.9	3.9
	(3.8)	(2.8)	(3.3)	(3.2)	(2.3)
% Cal., alcohol	2.1	5.2	5.7	8.4	7.2
	(2.7)	(6.8)	(5.5)	(5.7)	(5.2)
All other sources	28.3	25.1	32.5	20.6	22.8

sumed. The use of beer is increasing in these areas but the average intake is still trivial.

Animal products generally contribute less than 10 per cent of the total calories. On the average, only 3 to 4 per cent of the total dietary calories are provided by animal proteins. In Crete the meat is mostly goat, beef and mutton, with an occasional chicken or rabbit. In Corfu, the meat is mostly beef and veal. In both areas fish is eaten, chiefly sardines and salt codfish, the latter especially in the winter. In the several surveys in Crete the average was from 15 to 23 grams of fish a day. In Corfu the average consumption of fish was more than twice as great, 53 to 65 grams a day.

Milk as such is little used by the men in these areas and is generally considered as a food only for children and sick people. Cheese, mostly of the soft, fresh variety, is eaten regularly, the averages for the several surveys running from 8 to 20 grams per day. In addition, yogurt is popular but the total consumption is small. Butter is rarely used and then only in trifling amounts. Margarine is also seldom used. Instead of using a spread, these Greeks prefer to dip bread in olive oil, though most of the bread in eaten plain.

Eggs are eaten is small quantities in both Crete and Corfu. In the Crete survey of 1962 the average consumption was 3 eggs a week. In Corfu in 1961 a total of 26 eggs were eaten by 40 men in 7 days.

Potatoes are eaten in large amounts both in Crete and Corfu, especially in the fall and winter. In Corfu, sweet potatoes as well as white potatoes are popular. In the several surveys the average per capita daily consumption of potatoes ranged from 100 to 197 grams.

Pulses of various kinds, also form an important part of the diet, especially in the winter, the average being about 30 grams daily. Broad beans (**Vicia fava**) are most common but dried peas, lentils, ordinary beans (**Phaseolus vulgaris**), and chick peas (**Cicer arietinum**) are also widely used.

Nuts, mainly almonds and filberts, are popular throughout the year but the total consumption is small, 6 or 8 nuts being eaten at a time, often as a snack, especially when there are visitors in the household. Raisins and dried figs are eaten in the same way, commonly with nuts.

Sugar, as such, is used in very small amounts, the average daily per capita consumption in the several surveys being only 8 to 17 grams. Honey is popular and is frequently used on special occasions. In the summer an occasional small dish of ice cream and a sweetened soft drink add a few grams of sugar.

Both in Crete and Corfu garlic and onions are generously used throughout the year. Leaks and scallions also contribute flavor in the season. The average intake of salt (NaCl) is of the order of 5 to 10 grams daily.

The similarities between the diets in Crete and those in Corfu are striking but there are a few differences. The Cretans use more olive oil and goat milk; the men of Corfu more fish and macaroni. The bread in both areas is mostly rather dark; that in Crete often is made of whole wheat and barley; in Corfu 85 to 95 per cent extraction wheat flour is more common.

Calories, proteins and fats—calculations versus analyses

Table 4 gives means, and standard errors of those means, for total calories per day and percentages of calories from proteins and from fats. The averages for calories from calculations and from chemical analysis are not significantly

Table 4. Men in Greece, 7-day survey data, means and standard errors

Area	Date	No. Men	Cal./Day Tables	Cal./Day Chem.	% Protein Cal. Tables	% Protein Cal. Chem.	% Fat Cal. Tables	% Fat Cal. Chem.
Crete	Sept. 1960	30	2769	2654	10.2	10.6	41.8	35.7
			±78	±91	±0.2	±0.4	±1.2	±1.2
Crete	May-June 1962	33	2848	2781	9.8	9.2	41.8	37.9
			±97	±97	±0.3	±0.3	±1.0	±1.1
Crete	Feb. 1965	30	2626	2566	11.6	9.6	37.4	34.6
			±98	±82	±0.4	±0.3	±0.9	±0.9
Corfu	Sept. 1961	40	2796	2632	11.2	10.3	34.2	26.2
			±89	±90	±0.4	±0.4	±0.9	±0.9
Corfu	Mar.-April 1963	34	2877	2712	11.5	10.0	31.2	28.2
			±62	±116	±0.2	±0.2	±0.9	±0.9

different in any one of the 5 surveys but in every survey the average calculated value is somewhat higher than the chemical value, the average difference for 5 surveys being 114 Cal. per day or 4.1 per cent of the average calculated value and for all surveys combined the difference is statistically significant.

The agreement in regard to protein is less satisfactory and in three out of five surveys the calculated values for percentages of calories from proteins are significantly higher than the values from chemical analysis. On the average, the calculations indicate 5.1 per cent more protein calories than recorded by chemical analysis.

For fats, the calculated values are consistently higher than the analytical values and this difference accounts for the discrepancy in calories noted above. For all

5 surveys combined, the calculations indicate an average of 37.3 per cent of calories from fats while the corresponding analytical average is only 32.5 per cent. Two possible explanations for this discrepancy can be entertained. Conceivably the food composition tables used may not allow for the fact that the meats used in Crete and Corfu are very lean. However, as will be seen, at most this would account for only a small part of the discrepancy because the amount of meat is so small in these diets and olive oil accounts for much the largest amount of fat in these diets. It seems more likely that the fat values from chemical analysis are erroneously low. Besides the possibility that the ether extraction of the lyophilized material was incomplete, note should be made of the problem of obtaining true aliquots from the composites before lyophilization. Loss of fat on the walls of the containers is a distinct possibility.

Area and season comparisons

The data in table 4 do not indicate any significant differences between Crete and Corfu in regard to total daily calories or percentage of calories from proteins. But the proportion of fat in the diet is distinctly higher in Crete than in Corfu, the general averages, in terms of percentage of total calories, being 40.3 for Crete and 32.7 for Corfu. These values are from calculation; the chemical analyses indicate a similar difference.

The surveys covered three seasons in Crete, early summer, late summer, and winter, while in Corfu the surveys covered spring and late summer. In regard to total calories and percentages of calories from proteins and from fats the data indicate no significant seasonal differences. It should be observed, however, that dietary surveys were not carried out during the periods of heaviest farm work because the men, and their wives, were too busy in the fields at those times.

Fatty acids in the diet

As would be expected from the large use of olive oil, oleic acid dominated the fatty acids in the diets of both Crete and Corfu. In Crete, oleic acid represented an average of from 70.7 to 72.7 per cent, of the total fatty acids. In Corfu the average was 67.7 per cent.

Table 5 summarizes the principal fatty acid data, expressed as per cent of total fatty acids, in 3 surveys in Crete (N = 30, 33, and 30, for number of men) and in one in Corfu (N = 34). Data from Corfu in 1961 are not included because it was evident that some of the poly-unsaturated fatty acids had been destroyed in processing. In general, however, the Corfu 1961 data seem to have been similar to those obtained in 1963.

Oleic acid accounted for close to 70 per cent of the total fatty acids in all cases and palmitic was the next most abundant fatty acid in the diets. The third most important fatty acid, linoleic, may have been under-estimated in some of the 1962 Crete samples. On the average, 3 to 5 per cent of the total fatty acids was made up of stearic acid. Myristic acid, not tabulated in table 5, and palmitoleic acid, were the only other fatty acids that amounted to as much as 1.0 per cent

Table 5. Fatty acid composition of the ether extracts of the diets, expressed as percentage of the total fatty acids

Means and, in parentheses, standard deviations.

Fatty acid	Crete 1960	Crete 1962	Crete 1965	Corfu 1963
Short-chain saturates	-	0.4	0.1	0.1
(10 or fewer carbons)	-	(0.4)	(0.1)	(0.1)
Palmitic (16 : 0)	14.6	17.0	13.9	14.7
	(3.3)	(3.8)	(2.0)	(2.6)
Stearic (18 : 0)	5.2	5.1	4.1	3.2
	(2.2)	(1.2)	(1.4)	(1.2)
Other saturates	1.4	1.5	1.4	1.5
	-	-	-	-
Palmitoleic (16 : 1)	1.0	1.2	1.3	1.6
	(0.4)	(0.4)	(0.4)	(0.5)
Oleic (18 : 1)	69.6	69.7	71.3	65.9
	(5.0)	(5.2)	(4.2)	(3.6)
Linoleic (18 : 2)	7.5	4.2	7.1	12.0
	(1.5)	(2.0)	(1.3)	(2.4)
Linolenic (18 : 3)	0.4	0.5	0.3	0.7
	(0.3)	(0.9)	(0.3)	(0.3)

of the total fatty acids. Most of the diets showed traces of caprylic, capric and lauric acids, as well as of negligible amounts of fatty acids with odd numbers of carbon atoms in the chain.

Table 6 summarizes the major fatty acid data, as averages for all surveys, expressed as percentage of total calories in the diets. Both in Crete and Corfu the diets, though moderately high to high in total fats, are definitely low in both

Table 6. Average percentage of calories provided by specified groups of fatty acids in the diets of the men of Corfu and Crete in the period 1960-65

Calculated from chemical analyses.

Fatty acids	Crete	Corfu
Saturates with fewer than 18 carbon atoms	6.0	4.5
Stearic acid	1.7	0.9
Mono-enes (palmitoleic plus oleic)	25.8	18.3
Linoleic	2.3	3.3
Total poly-unsaturates	2.5	3.5

saturated and poly-unsaturated fatty acids. Compared with typical U.S. diets, as well as those in Finland, saturated fatty acids are of the order of only one-third as high in these Greek diets. Though the averages for linoleic and total poly-unsaturates in table 6 may be somewhat under-estimated for Crete, it is clear that these Greek diets are lower in these constituents than most U.S. diets.

Diet of the man versus that of the family

In most dietary surveys in which the foods are actually weighed in the household, the family, sometimes excluding infants, is the unit. Though it is realized that the individuals differ in total calories, it is generally implicitly assumed that the

composition of the diet is the same for all members of the family, or at least for all adults.
The question of the distribution of the nutrients between the man of the family and the rest of the family was investigated in a separate survey in Crete in 1957.

Table 7. Percentage of total calories provided from specified sources in the diets of middle-aged men and of their families in villages of central Crete in 1957
Data from records of food weights consumed in 7 successive days in October-November

Item	Man	Rest of family
% Calories from proteins	10.2	10.4
" " " total fats	32.9	38.6
" " " olive oil	26.6	31.6

The method was essentially the same as that used in the later surveys summarized above except that records of food weights were kept separately for the family as a whole and for the man of the family. Food values were estimated by the use of tables of food composition. Salient results are summarized in table 7.
Proteins provided substantially the same proportion of the total calories for the man and the other members of his family but there was a significant difference in regard to fats, the men having a diet relatively lower in fats than eaten by the rest of the family. The data show that olive oil was responsible for this difference. Compared with the rest of the family, the men consumed relatively less olive oil and more bread and potatoes.
This result is in contrast with the findings in similar surveys in Finland and in Yugoslavia. Both in East and in West Finland the diets of the men, compared with those of the other family members, provided a larger proportion of total calories from proteins and from fats (7). For both areas of Finland combined, the averages were 12.4 and 37.4 per cent of calories from proteins and from fats, respectively, for the men, the corresponding figures for the other members of the families were 11.7 and 33.9. In Yugoslavia, however, five similar surveys in Dalmatia and Slavonia failed to show any significant difference between the heads of families and the other members of the families in regard to proportion of calories from either proteins or from fats (2).

Intra- versus inter-individual variations

The principal purpose of these dietary surveys was to characterize, as groups, the middle-aged men in the populations concerned. At the same time it was interesting to examine the stability of the positions of the individuals within the group so some of the same men were included in repeated surveys. The question at issue is as to how well the individual men are distinguished from their fellows in terms of their dietary characteristics.

18 MEN OF CORFU % CALORIES
FROM FATS IN REPEATED SURVEYS

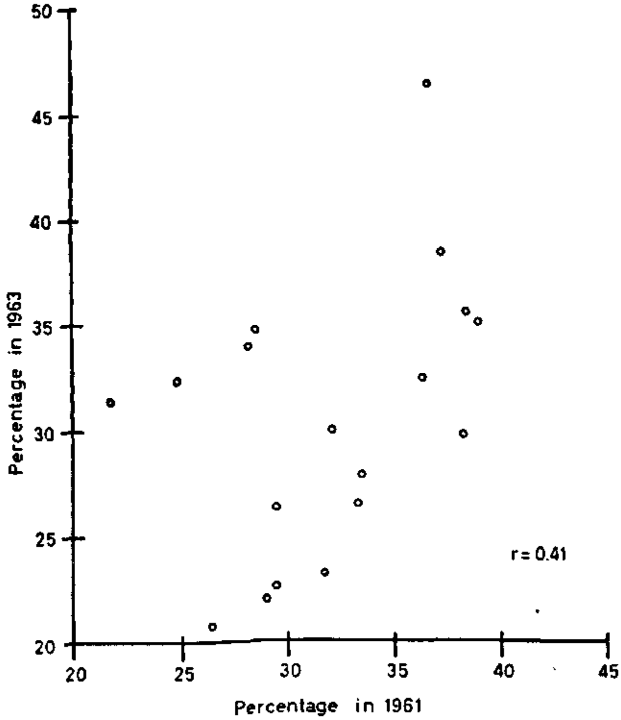

Figure 1

Figure 1 gives an example. Among the men in the surveys in Corfu, 18 men were included in the studies both in 1961 and 1963. The average percentages of calories from fats in the diets of these 18 men were 31.9 in 1961, 30.6 in 1963, so the averages agree very well. But figure 1 shows that the individual variation from survey to survey was large. Among the 9 men who were below the median in 1961, 4 were **above** the median in 1963. This means that the ranking of the men from lowest to highest fat diets in the first survey is practically useless for predicting their rank in this respect in the next. This is the result using nutrient values calculated from tables of food composition; the data from chemical analysis of the diets produces almost exactly the same result.

Figure 2 concerns the individual data on the proportion of calories from proteins in repeated surveys on the same 15 men in Crete. In this case there is a substantial difference between the averages for the same men in the two surveys — 9.2 in 1962, 11.5 in 1965 — but it is again clear that the relative positions of the individuals in regard to protein calories in 1965 bear little relationship to those observed in 1962.

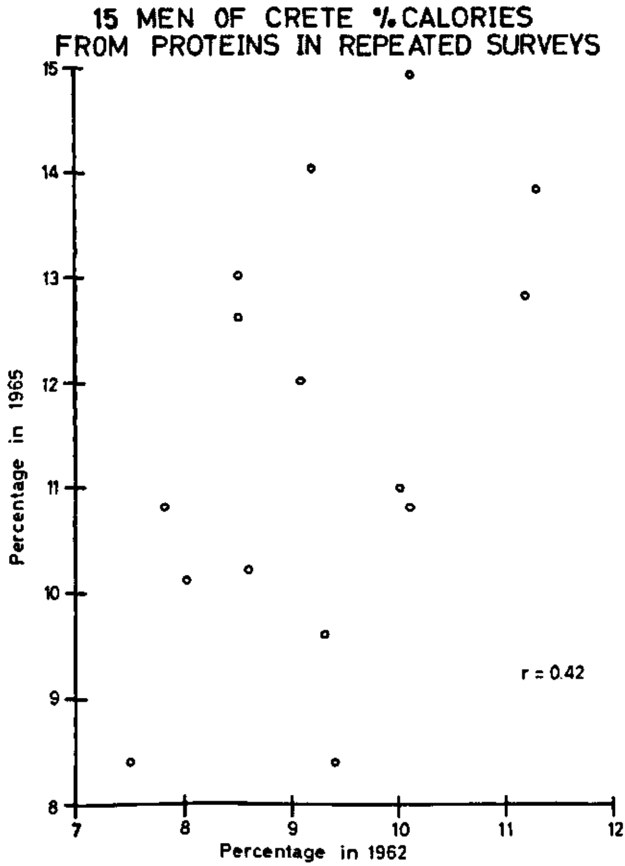

Figure 2

Table 8 summarizes the data from repeated surveys in regard to total calories and to percentages of calories from proteins, fats and alcohol. Essentially, the degree to which an individual can be distinguished among his fellows in regard to the diet, or any other characteristic that may be the item of measurement, depends on his intra-individual variability as compared with the variability between the individuals of the group of which he is a part. With the present material, the standard deviation about the mean value in a survey is a measure of the inter-individual variability of the group. When the survey is repeated, the geometric mean of the 2 values for the S.D. may be taken to be a better estimate of the average inter-individual variability; in table 8 this value is designated as $S.D._b$. The average intra-individual variability can be similarly computed from all the pairs of first and second survey values for the individuals. For most items in table 8 this statistic, $S.D._w$, is of much the same order of magnitude as $S.D._b$.

Table 8. Comparison of intra- and inter-individual variations in the dietary data on the same men surveyed on each of 2 occasions

N = number of men. $S.D._w$ and $S.D._b$ are the average standard deviations within and between individuals, respectively. The coefficient of correlation between values from the same men on the 2 surveys is given in the lines designated by r.

Area Years	N	Food table data Calories	Food table data % Calories Protein	Food table data % Calories Fat	Food table data Alcohol Calories	Chemical analysis Calories	Chemical analysis % Calories Protein	Chemical analysis % Calories Fat
Crete, 1960-'65	9							
$S.D._w$		200	1.9	4.7	63	236	1.3	5.3
$S.D._b$		643	1.2	4.7	84	463	1.2	5.4
r		0.83**	0.01	0.02	0.58	0.71*	−0.21	−0.04
Crete, 1962-'65	15							
$S.D._w$		598	2.0	7.3	40	494	1.6	4.9
$S.D._b$		525	1.6	6.1	186	451	1.6	7.7
r		−0.08	0.42	0.17	0.91**	0.13	0.05	0.47
Corfu, 1961-'63	18							
$S.D._w$		572	1.3	4.5	139	414	2.1	5.0
$S.D._b$		688	1.5	5.9	203	731	2.6	6.5
r		0.34	0.31	0.41	0.51*	0.66**	0.33	0.41

* Statistically significant at $p < 0.05$
** Statistically significant at $p < 0.01$

These findings mean, of course, that only a relatively small part of the differences observed between individuals in any one survey is truly dependent upon consistent differences between individuals. Similar results were obtained in repeated surveys on the same men elsewhere (2). The values for the correlation coefficient, r, in table 8 further show the importance of intra-individual variability. Using food table data, the average value of r is 0.30 for calories, 0.29 for protein per cent calories, 0.24 for fat per cent calories, and 0.67 for alcohol. The higher reliability of the alcohol values for characterizing the individual would be expected because some of these men never drink and a few are always heavy drinkers. The men are more variable in other aspects of the diet.

From time to time there are reports of dietary surveys made in the interview method that suggest less intra-individual variability than found in Greece or in the comparable surveys in Yugoslavia. This would be expected because when people simply are queried about their diets their answers from time to time necessarily reflect their own ideas of their stereotypes; they tend to repeat the same answers whether or not they truly correspond to current reality.

Summary

Seven-day dietary surveys were made on random samples of middle-aged men in villages on the island of Crete (3 surveys) and on the island of Corfu (2 surveys) in Greece. All foods consumed were weighed and replicates of the meals were collected for chemical analysis.

In both areas the diets were dominated by olive oil and bread, with low intakes of foods of animal origin and of sugar. Calculations using tables of food composition gave average results in fair agreement with those from direct chemical analysis but the chemical analyses indicated lower values than the tables for calories, proteins and especially for fats. In both areas the intake of saturated and of poly-unsaturated fatty acids was low.

There were no significant seasonal differences in the diets in regard to calories or percentage of calories from proteins or from fats. The diets of the men in these two areas of Greece did not differ significantly in calories or protein but the diet in Crete provided a higher proportion of fat calories (40.3 by calculation, 36.1 by analysis) than in Corfu (32.7 and 27.2, respectively).

Repeated surveys on the same men showed intra-individual variability to be a large part of the total variability in regard to total calories, alcohol calories, and percentages of calories from proteins and from fats. The individual values from a single survey are of very limited use in predicting individual values in a second survey.

Acknowledgments

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DIETARY SURVEYS IN CONNECTION WITH THE EPIDEMIOLOGY OF HEART DISEASE: RESULTS IN ITALY *)

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The basic idea of the international cooperative study on the epidemiology of heart disease is to study, in parallel and with identical methods and criteria, practically all men, initially aged 40-59, in a number of geographical areas, each selected to represent a culturally relatively homogeneous set, embracing in all a wide range of dietary and/or heart disease patterns.

In Italy three rural areas were selected: Crevalcore in the province of Bologna, Montegiorgio in the region of Marche and Nicotera in the region of Calabria. Crevalcore, an example of a rich rural area, is located in the south part of the fertile Po valley just at the north border of the province of Bologna, the city commonly called "la grassa" ("the fat") because of richness of the land and the diet. The farms produce wheat and other grains, tomatoes, apples and other fruits, cattle, milk and milk products (butter and parmesan cheese). The climate is hot in summer and humid and cold in winter. Besides the main center of Crevalcore with a population of 8109 inhabitants, one third of them living in the village and the remaining spread out in the farms, there are two attached hamlets: Caselle and Palata. These have a population of 6237 inhabitants, but they were excluded from the study in order to limit the size of the total sample for the epidemiological study to about 1000 men of 40-59 years of age. The population is fairly stable but there is a little emigration to big cities such as Milan and Turin. The distribution of occupation is reported in table 1.

Table 1. Percentage distribution of occupation of the men in the dietary survey and, in parentheses, of all men in the clinical survey

	Crevalcore N = 36	(N = 997)	Montegiorgio N = 38	(N = 720)	Nicotera N = 37	(N = 597)
Professional, business, government	0	(6.9)	0	(8.5)	18.9	(11.3)
Foremen	2.7	(0.5)	0	(0.1)	0	(0 2)
Clerical	2.7	(4.5)	2.6	(1.1)	2.7	(5.1)
Protection	2.7	(0.6)	2.6	(1.3)	2.7	(2.5)
Food handlers	2.0	(1.6)	2.6	(1.5)	2.7	(1.7)
Skilled light crafts	2.0	(2.3)	5.2	(2.2)	2.7	(2.1)
Transportation	8.3	(7.1)	0	(4.6)	5.4	(2.6)
Building trades	19.4	(15.9)	5.2	(6.0)	13.5	(6.6)
Metal work	2.7	(5.0)	0	(1.0)	0	(0.2)
Farming, logging	38.8	(44.1)	65.7	(68.2)	35.1	(50.0)
Fisherman	0	(0)	0	(0)	0	(3.4)
Factory work	5.5	(8.7)	0	(0.1)	0	(0)
Services and general labor	5.5	(2.1)	5.2	(1.1)	5.4	(6.4)
Miscellaneous	5.5	(2.5)	10.5	(2.4)	2.7	(0.9)
Not working	0	(2.9)	0	(1.8)	8.1	(7.0)

*) Research aided by grants from the Italian Ministry of Health and U.S. Public Health Service (No. HE 04672 from the National Heart Institute).

Montegiorgio, an example of a moderately prosperous rural area, where the consumption of olive oil and pork fat is fairly high, is a farming village in the rolling hills of the region of Marche, 22 km inland from the small town of Fermo on the Adriatic coast, and about 60 km south of Ancona. It is centered on a hill at about 400 meters in altitude, above the valley of the Tenna river. Agriculture is the only industry; the small farms, mostly operated by share-cropping, produce wheat and corn, some fruits, vegetables and little olive oil at a high cost of labor. There is a rather large production of high quality beef and pork but this is mostly exported to Rome and the north of Italy. The climate is mild-temperate with rather high humidity in the fall and winter. The people, living on the farms, are self-sufficient for everyday needs from their own farms but the cash income is low. Besides the main center, the village of Montegiorgio has three attached hamlets (Alteta, Cerreto and Monteverde) with a total population of 7033 inhabitants, three-fourths of them living in scattered farms. There is some emigration both to neighbouring villages and to urban centers of the same region, but mostly to Rome and the north of Italy. The distribution of occupation is reported in table 1.

Nicotera, selected as an example of a poor rural area, with rather high consumption of olive oil and legumes, is perched on a spur of the mountains overlooking the Tyrrhenian Sea about 60 km north of Reggio Calabria near the toe of Italy. The main farm products are olives, grapes, figs, oranges, tomatoes, pulses, wheat, some flowers for the perfume trade, and for local use, a little meat and poultry. In the hamlet of Nicotera Marina few families are engaged in fishing. There is no manufacturing industry. The population is poor and there is a steady migration of young people away from the area to other parts of Italy and overseas, but beyond the age of 40 the population is more stable. The climate is warm-temperate, with a long hot dry season. Besides the main center of Nicotera and the hamlet of Nicotera Marina, three other nearby hamlets were included: Comerconi, Badia, and Preitoni; the total population of the entire survey area was 9043 inhabitants at the time of the survey. About 80 percent of the people live in the centers, and go out daily to work in their small fields as far as several kilometers away. The distribution of occupation in Nicotera is reported in table 1.

The dietary surveys started in January 1960 in Nicotera and were continued in different seasons, as shown in table 2.

Table 2. Schedule of the dietary surveys in Italy

Nicotera	January	1960	May-June	1960	October	1960
Montegiorgio	April	1960	Aug.-Sept.	1960	Jan.-Feb.	1961
Crevalcore	June-July	1960	Nov.-Dec.	1960	March	1961

Methods

The dietary survey method used was that of the recipe weighing for seven days for the individual men and also for each other component of the family. The dietitians went to the selected households, with a scale having a capacity of 10 kg and sensitive to 5 grams, one day before, to collect information on the composition of the family reported on form 1 (1). At the same time the dietitian talked with the housewife to learn the eating habits of the family, asking general questions on cooking and the timetable of preparation and serving of meals. The next morning she started the survey, collecting on form 2 the data on the food eaten for breakfast by each component of the family. During the late morning she observed the preparation of the cooked food in the kitchen, recording on form 4 the weight of each ingredient, the garbage and also the final weight of the cooked food just before it was served. During the noon meal the dietitian recorded, again on form 2, all food eaten as such and cooked deducting for plate waste such as bones, fruit peelings, etc. The same was done in the afternoon for the evening meal. If somebody in the family ate away from home, he was given form 3 where he recorded the food eaten with some description about the quantity and preparation if possible. This collection of data was continued for seven successive days recording all the useful information for processing the data. For the food eaten as such there was no problem. In the cooked food, the weight in grams of each ingredient was divided by the weight in grams of the cooked food just before serving. This factor for each ingredient was multiplied by the actual quantity of the cooked food consumed by the subject so as to obtain the real quantity of raw food consumed. All the amounts of these food items were added for every day and the total amounts were converted into nutrients and calories using a food composition table specially developed for this study. Chemical analyses on local foodstuffs were carried out to assure that the table of food composition was properly applicable (2).

This type of dietary survey is time-consuming and very expensive, so the number of subjects has to be limited. For each of the three areas 32 families were chosen as follows. From the roster of all men examined in an area, sixteen names were chosen at random. Then for each family so selected, the family living closest to it was also selected. In this way one dietitian could carry out the study on two families at the same time and the total of 32 families would constitute a random sample of all families in the area whose family heads were in the general study. In the few cases where the randomly selected family was unable or unwilling to cooperate or had special and unusual dietary problems (illness in the family, etc.), the subject preceding in the roster was chosen.

In this paper we are reporting only the results on the men 40-64 years of age that have been clinically examined. Table 1 shows the percentage distribution of the occupation of the selected subjects. The difference between the dietary and clinical study is very small and in all areas farmers are the dominant group.

The techniques for anthropometric data are reported elsewhere (3).

Results

The percentage distribution of calories according to food groups, as three-season averages , is given in table 3.

Table 3. Percentage distribution of calories according to food groups (average for three seasons)

Food Groups	Crevalcore N = 28	Montegiorgio N = 34	Nicotera N = 21-31
Milk and cheese	7.9	0.9	2.8
Meats, eggs, fish	11.6	9.9	6.3
Fats and oils	13.3	16.8	14.9
Cereals	46.4	52.6	54.3
Legumes	1.0	3.0	4.7
Vegetables incl. potatoes	1.4	3.8	5.4
Fruits	3.0	1.0	1.9
Alcoholic beverages	10.1	10.0	5.3
Sugar and sweets	5.2	1.8	4.3

Milk and cheese consumption was relatively high in Crevalcore; meats, eggs and fish less common in Nicotera; total fats and oils were almost the same in all three areas. The high value for cereals decreases from Nicotera to Crevalcore, and the same trend holds for legumes and vegetables. The consumption of fruit was relatively low in Montegiorgio and higher in Crevalcore and the same holds for sugar and sweets. Alcoholic beverages were consumed to about the same extent in Crevalcore and Montegiorgio while Nicotera was low in this respect. The consumption of nutrients and calories per man and per day in Crevalcore, Montegiorgio and Nicotera for each of the three seasons is summarized in table 4, 5 and 6, respectively. Because the analysis of variance showed no statistically significant differences for proteins, fats, carbohydrates

Table 4. Consumption of nutrients and calories per man per day in Crevalcore (means and standard deviations for 28 men in each of the 3 seasons)

		Nov.-Dec. 1960	June-July 1960	March 1961	Mean for the 3 seasons
Proteins	g	105.8 ± 23.3	109.3 ± 20.9	103.7 ± 16.3	106.3
Fats	g	95.4 ± 25.5	109.1 ± 22.2	94.0 ± 18.3	99.5
Carbohydrates	g	428.5 ± 104.7	430.8 ± 96.6	396.8 ± 81.6	418.7
Total Calories		3272.1 ± 703.9	3486.0 ± 675.9	3209.9 ± 542.0	3322.7
Ca	mg	894 ± 292	909 ± 331	887 ± 232	897
Fe	mg	17 ± 4	19 ± 5	16 ± 5	17
Thiamine	mg	2.3 ± 0.6	2.3 ± 0.6	2.1 ± 0.5	2.2
Riboflavin	mg	1.8 ± 0.5	1.9 ± 0.5	1.8 ± 0.4	1.8
Niacine	mg	17.6 ± 3.8	18.2 ± 3.5	16.6 ± 3.1	17.5
Vitamin A	I.U.	4511 ± 2979	5280 ± 3016	5416 ± 2520	5249
Ascorbic Acid	mg	40 ± 33	40 ± 24	52 ± 37	44

and total calories, these tables also give the mean values for the three seasons. In Crevalcore there was a higher consumption of almost all nutrients and

Table 5. Consumption of nutrients and calories per man and per day in Montegiorgio (means and standard deviations for 34 men in each of the 3 seasons)

		April 1960	Aug.-Sept. 1960	Jan. 1961	Mean for the 3 seasons
Proteins	g	85.0 ± 11.8	84.4 ± 12.3	82.5 ± 10.8	84.0
Fats	g	83.9 ± 15.4	81.8 ± 17.2	79.5 ± 11.2	81.7
Carbohydrates	g	392.3 ± 112.0	472.2 ± 110.1	376.9 ± 65.0	413.6
Total Calories		3003.9 ± 516.0	3130.8 ± 642.2	2865.7 ± 395.2	3000.1
Ca	mg	411 ± 85	406 ± 124	329 ± 68	382
Fe	mg	17 ± 4	19 ± 5	16 ± 5	17
Thiamine	mg	1.5 ± 0.2	1.6 ± 0.4	1.6 ± 0.4	1.6
Riboflavin	mg	1.1 ± 0.2	1.2 ± 0.4	1.0 ± 0.2	1.1
Niacine	mg	14.4 ± 2.4	16.9 ± 2.5	15.2 ± 3.4	15.3
Vitamin A	I.U.	3718 ± 1634	3312 ± 1018	3658 ± 3252	3563
Ascorbic Acid	mg	33 ± 31	84 ± 38	55 ± 28	57

Table 6. Consumption of nutrients and calories per man and per day in Nicotera (means and standard deviations for 24 men in Jan.-Feb., 31 in May-June and 29 in October)

		Jan.-Feb. 1960	May-June 1960	Oct. 1960	Mean for the 3 seasons
Proteins	g	82.8 ± 18.8	75.4 ± 16.7	77.1 ± 20.6	78.4
Fats	g	64.5 ± 18.3	64.3 ± 22.3	66.2 ± 19.2	65.0
Carbohydrates	g	399.4 ± 86.1	376.9 ± 93.4	400.6 ± 99.7	392.3
Total Calories		2643.8 ± 563.6	2529.4 ± 571.7	2568.1 ± 571.6	2580.4
Ca	mg	467 ± 163	441 ± 189	368 ± 132	425
Fe	mg	16 ± 5	17 ± 7	16 ± 5	16
Thiamine	mg	1.3 ± 0.5	1.0 ± 0.3	1.0 ± 0.3	1.1
Riboflavin	mg	1.1 ± 0.4	1.0 ± 0.3	0.9 ± 0.3	1.0
Niacine	mg	13.7 ± 4.3	13.7 ± 3.8	14.4 ± 4.4	13.9
Vitamin A	I.U.	4786 ± 2337	3641 ± 3452	2652 ± 1273	3693
Ascorbic Acid	mg	73 ± 34	62 ± 41	74 ± 47	70

calories, with a tendency to the highest intake in summer; this corresponds to the period of heavy harvest work of the farmers. In Montegiorgio the values are lower than in Crevalcore with the same situation for the summer season. In general, the lowest values were found in Nicotera except for calcium, vitamin A and ascorbic acid; local production of citrus fruits and tomatoes accounts for the high intakes of vitamin A and ascorbic acid.

In Nicotera there were some difficulties with the subjects in the dietary study. For the first survey one dietitian became ill at the start and it was impossible to find a replacement so the total number of subjects had to be reduced from 32 to 24. In the second follow-up, six of the families refused to continue to cooperate; six substitute families were statistically selected from the roster. The final result is that only 18 families were surveyed for all three seasons. But, as shown in table 7, the analysis of variance for the head of the family for proteins, fats and calories showed no statistically significant difference. Accordingly, all the data for the three seasons for the total number of subjects surveyed in each season are reported here.

Table 7. Analysis of variance of results for proteins, fats and calories in Nicotera from the same and from different groups of subjects in different seasons (the table shows F values)

Period	Groups	Proteins	Fats	Calories
January 1960	a, b	—2.82	—0.017	—1.54
May—June 1960	c, d	—0.87	—0.23	—0.036
October 1960	e, f	—0.30	—0.63	—0.01
All three a, b, c, d, e, f		—1.20	—0.20	—0.41

Groups a, c, e = the same 18 men for all three surveys
Group b = 6 men
Group d = 13 men
Group f = 11 men

From a nutritional point of view there were no great deficiencies in any of the three areas. There was a rather low intake of calcium and riboflavin in Montegiorgio and Nicotera; this is related to the low milk consumption in these two areas. However, the intake of calcium in the drinking and cooking water is significant and this is not included in the tables.

The percentage of total calories from proteins was almost the same in all three areas (table 8), but the percentage of animal proteins was relatively high in Crevalcore. The percentage of total calories from fats (table 8) decreases from an intermediate value in Crevalcore to a rather low value in Nicotera. On the other hand, the percentage of animal fat is relatively high in Montegiorgio and Crevalcore as compared with Nicotera.

Table 8. Percentage of total calories from proteins and fats and percentage of animal proteins and fats (average for 3 seasons)

Area	Proteins		Fats	
	% Tot. Cal.	% Animal	% Tot. Cal.	% Animal
Crevalcore .... N = 28	12.8	56.4	26.9	55.5
Montegiorgio .... N = 34	11.2	34.6	24.5	63.9
Nicotera .... N = 24–31	12.1	30.4	22.7	25.3

Table 9. Total calories and percentage of total calories from fats in the diet, relative body weight and sum of skinfolds for all men in the dietary study and correlation coefficient among these variables

Item	Crevalcore N = 35	Montegiorgio N = 38	Nicotera N = 37
Total Calories	3401	2993	2601
% Total Calories from fats	28	26	22
Relative body weight	103.4	100.6	94.3
Sum of skinfolds, mm.	22.6	18.8	17.3
r (Cal. - R.B.W.)	0.38	0.22	0.08
r (% Cal. Fats - R.B.W.)	0.03	0.09	0.30
r (% Cal. Fats - Skinfolds)	—0.06	0.38	0.30
r (Cal. - Skinfolds)	0.19	—0.10	—0.03

It is interesting to compare (table 9) total calories and total calories from fats with the anthropometric data on relative body weight and the sum of two skinfolds (on the right arm over the triceps and below the tip of the right scapula). There is a significative correlation coefficient (at the 5 % level) between total calories and relative body weight in Crevalcore and between percentage of total calories from fats and the sum of skinfolds in Montegiorgio.

Discussion

The three areas of Crevalcore, Montegiorgio and Nicotera are not necessarily representative of the rural parts of the general regions in which they are situated but there is no reason to suggest that they are atypical. Taken together, the areas studied may indicate something of the general dietary pattern and its variations in a substantial part of rural Italy in recent years.

From this study there are no indications of serious deficiencies or excesses of the several nutrients or of total calories in the diet. In general, the middle-aged men in Montegiorgio and Nicotera tend to be thin while the proportion of men in Crevalcore who are obese, is rather high. In fact, the mean, median and 90th centile values of the sum of the two skinfolds for the men in Crevalcore is considerably higher than in any of the other rural areas covered in parallel surveys in this cooperative study: two rural areas each in Yugoslavia, Finland, Greece and Japan (3). In this respect of body fatness, the men in Nicotera and Montegiorgio are very similar to the men of the same age in the rural areas of the other countries studied (3). It is interesting to note that, with the exception of Crevalcore, the men in all these rural areas tend to be much thinner than men in urban areas (3). This fact does not seem to depend on any shortage of energy yielding foods but probably reflects the difference in physical activity between farmers and inhabitants of cities.

When this study was planned it was thought that important seasonal differences in the diet might be found. It will have been observed, however, that the seasonal differences are small, the major difference being an increased total food consumption during the period of heaviest work on the farms.

The general study, in which the present dietary survey work is a part, is focussed on the incidence of heart disease. For this reason there was special interest in the dietary fat. It is well known that dietary fat has a strong influence on the level of cholesterol in the blood and that, in turn, the susceptibility to coronary heart disease is related to the serum cholesterol concentration (4, 5). With this in mind, it will be noted that the percentage of calories supplied by dietary fats, and especially fats of animal origin, is considerably lower in all three of the areas studied in Italy than in such countries as the U.S.A., the United Kingdom and Finland in which the incidence of coronary heart disease is high. Also, as will be reported elsewhere, the level of cholesterol in the blood in the three Italian areas tends to be relatively low compared with the countries with high animal fat intakes.

When the three areas of Italy are compared, it is observed that in Crevalcore and

Montegiorgio the total fat intake is moderate, with fats of animal origin providing a fair proportion of the total. On the other hand, in Nicotera the total fat consumption tends to be somewhat lower and large part of this fat is in the form of olive oil. As will be reported in detail separately, these dietary differences are associated with corresponding differences in the serum cholesterol level and in the apparent frequency of coronary heart disease in these areas.

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DIETARY SURVEYS IN CONNECTION WITH THE EPIDEMIOLOGY OF HEART DISEASE: RELIABILITY, SOURCES OF VARIATION AND OTHER DATA FROM NINE SURVEYS IN ITALY *)

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As reported earlier (5), 7-day dietary surveys were made in three seasons of the year in each of three geographically defined rural areas of Italy. In each of the three areas substantially all men aged 40-59 at the start of the work were medically examined and statistical samples of those men were studied in the dietary surveys. The present report concerns variability between seasons, between methods, and within individuals in regard to calories and the proportions of total calories provided by proteins and fats in the diets. In addition, data on fatty acids in the diets are presented.
As emphasized elsewhere (9), it is practically impossible to assure, or even to test, the absolute validity of dietary survey data as being truly representative of the habitual long-time diets of free-living individuals. However, by repeated surveys and by the use of different methods applied in parallel, it is possible to examine reliability, that is to say repeatability of the data, and to assess relative validity. Such material also allows examination of intra-individual variability.

Methods

The surveys

Each survey covered seven consecutive days during which all foods consumed by each man were weighed daily in the household. For sub-samples of these men equivalent composites of their 7-day diets were made up from local foodstuffs and the composites were analysed chemically, by methods described elsewhere (3) for water, mineral ash, protein, total lipids and, by difference, carbohydrate. The nutrient intakes of all men were estimated by the application of tables of food composition to the weights of the foods consumed. The food tables used were especially devised for such surveys in Italy and included data from original chemical analyses of some food items (4) as well as the data available in Italy or other European countries. The food composition table of the Istituto Nazionale della Nutrizione (8) was not used because it was made with criteria considered not suitable for us and also because it includes values, chiefly for minerals and vitamins, largely based on the tables of the U.S. Department of Agriculture Handbook no. 8 (14) and of Food and Agriculture Organization of the United Nations (6). In devising the table used here the aim was to have food composition

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values that would be most truly representative of the foodstuffs in the three localities.

Fatty acids in the diet

The lipid extracts of the lyophilized homogenates of the equivalent composites for each man were methylated with methanol and sulfuric acid (1). The methyl esters so obtained were analysed with a Beckman Model GC-2 gas-liquid chromatograph machine, using a ¼ inch diameter column, 150 cm. long, packed with 30 per cent butanediol succinate polyester on acid-washed Celite, 60-80 mesh, operated at 220-230 °C. Standardization was made with pure fatty acids obtained from the Hormel Institute, Austin, Minnesota, U.S.A. The proportions of the several fatty acids recorded on the chromatogram were calculated by planimetric estimation of the areas under the peaks.

Results

Calculation versus chemical analysis

Table 1 compares the data on daily intakes, grams per man, of total protein and of total fat obtained by the use of the food composition table and by chemical analysis of the equivalent composites for the same 7-day period. In each of the three areas the two methods yielded similar mean values. It will be noted, however, that in all three areas and for both proteins and fats the averages are slightly higher from chemical analysis than indicated by calculation. For proteins, chemical analysis gave a grand average 5.8 per cent higher than that calculated by the food tables. For fats, the grand average by chemical analysis is 3.1 per cent higher than indicated by the food tables. The agreement between the values on the individual subjects obtained by the two methods is indicated by the correlation coefficients which average r = 0.86 for protein and r = 0.85 for fats.

Table 1. Comparison of results from chemical analysis and from calculations using tables of food composition. Mean values, in grams, per man per day and the coefficient of correlation, r, between estimates by the two methods

Area and	Proteins			Fats		
Number of men	Analysis	Calculation	r	Analysis	Calculation	r
Crevalcore (N = 41)	116	109	0.86	105	101	0.78
Montegiorgio (N = 39) ..	90	86	0.84	89	88	0.86
Nicotera (N = 38)	85	80	0.89	72	69	0.92
All, (N = 118)	97.4	92.1	0.86	89.1	86.4	0.85

Comparison of seasons

Table 2 summarizes the nutrient intake values, obtained by computation using the food composition tables, for the same 80 men surveyed in each of three seasons. In none of the three areas is there any significant seasonal difference in calories or percentages of calories provided by proteins or by fats in the diet. For all areas considered together, the nearest approach to a seasonal trend is a slight indication that the percentage of calories provided by proteins may be a trifle lower in the warmest periods. The grand average value for the months of May-September is 11.77 per cent calories from protein compared with 12.28 for the months October-November and January-February, the difference being significant at p = 0.05.

Table 2. Seven-day dietary repeated on the same men in each of three seasons
Data calculated from tables of food composition.

Item	Crevalcore, N = 28 Season	Mean	S.D.	Montegiorgio, N = 34 Season	Mean	S.D.	Nicotera, N = 18 Season	Mean	S.D.
Calories	June-July	3486	676	April	3004	516	Jan.-Feb.	2725	591
"	Nov.-Dec.	3272	704	Aug.-Sept.	3131	642	May-June	2546	652
"	March	3202	553	Jan.	2836	395	October	2576	628
Protein % Cal.	June-July	12.7	1.6	April	11.4	1.4	Jan.-Feb.	12.7	1.1
" " "	Nov.-Dec.	13.0	1.7	Aug.-Sept.	11.0	1.6	May-June	11.6	1.3
" " "	March	13.1	2.1	Jan.	11.7	1.7	October	11.8	1.6
Fat % Cal.	June-July	28.7	5.7	April	25.5	4.6	Jan.-Feb.	21.0	3.4
" " "	Nov.-Dec.	26.3	4.5	Aug.-Sept.	23.9	4.6	May-June	23.0	6.3
" " "	March	26.5	4.5	Jan.	25.4	3.6	October	24.0	4.0

Table 3 gives the corresponding data from chemical analysis of the equivalent composites. Again there is no significant seasonal trend in any of the areas. Moreover, the slight indication from the food table data in the combined material of lower protein percentage of calories in the warmer months is not borne out in the chemically analyzed composites.

Table 3. Seven-day dietary surveys repeated on the same men in each of three seasons
Data from chemical analysis.

Item	Crevalcore, N = 10 Season	Mean	S.D.	Montegiorgio, N = 7 Season	Mean	S.D.	Nicotera, N = 7 Season	Mean	S.D.
Calories	June-July	2894	364	April	3063	549	Jan.-Feb.	2407	752
"	Nov.-Dec.	3124	641	Aug.-Sept.	3192	690	May-June	2247	410
"	March	3019	548	Jan.	2806	305	October	2722	1003
Protein % Cal.	June-July	15.1	2.1	April	11.1	1.8	Jan.-Feb.	14.3	1.7
" " "	Nov.-Dec.	14.3	2.1	Aug.-Sept.	11.8	1.9	May-June	13.5	2.8
" " "	March	14.4	1.5	Jan.	12.1	3.1	October	13.5	1.2
Fat % Cal.	June-July	31.8	5.0	April	24.9	4.7	Jan.-Feb.	25.3	4.7
" " "	Nov.-Dec.	26.3	5.5	Aug.-Sept.	23.0	4.8	May-June	25.7	11.2
" " "	March	30.5	3.3	Jan.	26.9	7.6	October	24.0	4.5

Three-day versus seven-day surveys

The method of collecting data for each single day in these surveys allows comparison of the results from the full seven days with those from the first three days of the survey. Table 4 summarizes the comparison with a total of 267 sets of data obtained by the use of tables of food composition. In all three areas and for all four of the nutrient items concerned the averages from the 3-day data are in close agreement with the 7-day averages. Further, the correlation coefficients for the individual 3-day versus 7-day values are high; for all three areas combined, the average coefficient is r = 0.86 for protein, r = 0.79 for fats, r = 0.91 for carbohydrate, and r = 0.86 for calories.

Characterization of the individuals

The data obtained in these studies allow calculation of the variances between

Table 4. Comparison of data from seven days and from the first three of the seven days
Means, standard errors, and coefficient of correlation, r

Area and Number of men		Proteins g	r	Fats g	r	CHO g	r	Calories	r
Crevalcore	7 days	106 ± 2	0.89	99 ± 2	0.83	419 ± 10	0.89	3317 ± 70	0.88
N = 84	3 days	105 ± 2		98 ± 3		418 ± 11		3269 ± 73	
Mntegiorgio	7 days	84 ± 1	0.82	81 ± 2	0.72	406 ± 9	0.92	3015 ± 57	0.82
N = 99	3 days	83 ± 2		81 ± 2		414 ± 11		3049 ± 69	
Nicotera	7 days	78 ± 2	0.88	65 ± 2	0.83	392 ± 10	0.91	2580 ± 62	0.88
N = 84	3 days	79 ± 2		63 ± 3		383 ± 11		2535 ± 69	

individuals and within individuals for the same men in three repeated surveys. The corresponding standard deviations are given in table 5 for calories and percentages of calories from proteins and from fats for each area for the values from food tables and for the values from chemical analysis of the equivalent composites. In general, the variation within individuals (intra-individual standard deviation) tends to be almost as large as the variation between individuals; the difference is statistically insignificant. With the data from chemical analysis on percentage of calories from porteins, the intra-individual standard deviation is slightly larger than the between-individual values for Montegiorgio and Nicotera. With the data from chemical analysis on percentage of fat calories at Crevalcore the intra-individual variation also exceeds the between-individual value.

These findings indicate that the dietary customs of the individual men vary from time to time to much the same extent that there are differences between

Table 5. Analysis of 7-day dietary data from 3 surveys repeated on the same men for calories per day and percentages of calories from total proteins and total fats
SD = standard deviation, wi = within and bi = between individuals. rc = coefficient of consistency, the average coefficient of correlation between individual values, occasion 1 versus 2, 1 versus 3, and 2 versus 3

Area, No. Men	Item	Method	SD_{wi}	SD_{bi}	rc
Crevalcore, N = 28	Calories	Tables	452	648	0.55
	Prot. % Cal.	"	1.12	1.79	0.61
	Fat % Cal.	"	3.47	4.91	0.55
Crevalcore, N = 10	Calories	Chem.	455	530	0.23
	Prot. % Cal.	"	1.31	1.92	0.54
	Fat % Cal.	"	5.22	4.70	0.41
Montegiorgio, N = 34	Calories	Tables	367	528	0.58
	Prot. % Cal.	"	1.22	1.56	0.43
	Fat % Cal.	"	3.35	4.30	0.42
Montegiorgio, N = 7	Calories	Chem.	358	538	0.64
	Prot. % Cal.	"	2.45	2.34	—0.23
	Fat % Cal.	"	5.09	5.85	0.24
Nicotera, N = 18	Calories	Tables	416	624	0.55
	Prot. % Cal.	"	1.18	1.35	0.40
	Fat % Cal.	"	3.78	4.74	0.44
Nicotera, N = 7	Calories	Chem.	743	761	0.06
	Prot. % Cal.	"	2.05	2.01	—0.14
	Fat % Cal.	"	6.96	7.49	0.07

the individual men. Accordingly, it follows that it is not possible to characterize with any security individual men as being habitually big eaters, or high-protein or high-fat men as compared with the average of the group of men. Further analysis of these data yields the coefficients of consistency, rc in table 5. Combining all areas and both methods, the values for average intra-individual consistency are rc = 0.51 for calories, rc = 0.40 for percentage protein calories, and rc = 0.43 for percentage fat calories. Though these values indicate statistically significant correlations for N = 104, they are so small as to mean that very low accuracy can be expected in predicting, for individuals, the values for one survey from the values from another. On the average, the amount of individual variance accounted for by the observed correlations is only 26 per cent for calories, 16 per cent for proteins, and 18 per cent for fats.

Fatty acid composition

Table 6 summarizes the fatty acid data obtained by gas-liquid chromatography of the methyl esters of the ether extracts of the equivalent composites of the 7-day diets of 23 men. The fatty acids in all of the diets are dominated by oleic acid and this is particularly striking for Nicotera where 65 per cent of the total identified fatty acids are accounted for by oleic acid. This reflects the dominance of olive oil among the fats in the diet at Nicotera. All of the diets are relatively low in short-chain saturated fatty acids, reflecting the relatively low consumption of milk and milk products in these areas.

Table 6. Fatty acid composition of the ether extracts of the diets, expressed in grams per person per day

	Fatty acids								
Area, no., men	Short-chain saturates	Palmitic	Stearic	Other saturates	Palmitoleic	Oleic	Linoleic	Linolenic	Arachidonic
Crevalcore (N = 9)									
June-July	0.8	21.8	6.8	5.0	2.8	46.6	7.5	0.6	—
Nov.-Dec.	0.8	23.0	6.8	5.5	2.6	37.0	7.6	1.0	—
March	1.4	22.3	7.3	6.2	2.8	36.9	17.6	2.1	—
Montegiorgio (N = 8)									
April	0.2	16.6	8.2	2.3	1.8	36.4	9.1	0.9	1.6
Aug.-Sept.	0.2	17.6	8.7	1.9	1.7	37.3	8.6	0.7	1.8
Jan.	0.5	19.7	9.6	3.0	2.2	41.6	10.1	0.9	4.8
Nicotera (N = 6)									
Jan.-Feb.	0.2	9.7	2.5	1.2	1.2	40.0	5.0	0.5	—
May-June	0.4	9.0	2.3	1.3	0.8	43.2	5.5	0.4	—
Oct.	0.6	11.5	3.5	1.8	1.7	43.9	8.4	1.1	—

Table 7 shows the percentage of total dietary calories provided by types of fatty acids. All of the diets are relatively low in saturated and in polyunsaturated fatty acids. The values in table 7 for saturated and for polyunsaturated fatty acids are only about half those commonly reported for diets

Table 7. Average percentage of calories provided by specific groups of fatty acids in the diet of the men of Crevalcore, Montegiorgio and Nicotera

	Fatty Acids				
	Saturates excluding stearic	Stearic	Mono-enes	Linoleic	Total poly-unsaturates
Crevalcore (N = 9)					
June-July	8.1	2.0	14.5	2.1	2.3
Nov.-Dec.	6.9	1.8	9.9	2.1	2.4
March	8.0	2.0	10.3	4.5	5.0
Mean, 3 seasons	7.7	1.9	11.6	2.9	3.2
Montegiorgio (N = 8)					
April	5.8	2.2	11.3	2.6	3.5
Aug.-Sept.	5.0	2.2	10.1	2.1	2.8
Jan.	8.7	2.7	12.8	2.9	3.7
Mean, 3 seasons	6.5	2.4	11.4	2.5	3.3
Nicotera (N = 6)					
Jan.-Feb.	4.3	1.0	15.6	1.8	2.0
May-June	4.2	0.9	17.2	2.2	2.3
Oct.	4.3	1.1	14.6	2.7	3.1
Mean, 3 seasons	4.3	1.0	15.8	2.2	2.5

in the United States. No seasonal trends are indicated in table 7. When the areas are compared the only significant peculiarity is the fact that saturated fatty acids are decidedly lower in the diets at Nicotera than in the diets of the other two areas.

Cholesterol

Table 8 gives the mean dietary intakes of cholesterol as milligrams per 1000 calories per day. On the average, the men of Crevalcore had the highest concentration of cholesterol in the diet while the men of Nicotera had the least. But even at Crevalcore the dietary intake of cholesterol must be considered low in comparison with averages in the United States.

Table 8. Dietary cholesterol (mg. per 1000 Cal.) — three seasons. Number of men shown in parentheses

Area				Average
Crevalcore	Nov.-Dec. 139 (28)	March 117 (28)	June-July 149 (28)	135
Montegiorgio	Jan. 105 (34)	April 72 (34)	Aug.-Sept. 86 (34)	88
Nicotera	Jan.-Feb. 64 (24)	May-June 102 (31)	Oct. 59 (24)	75

In controlled experiments on man the average effects of differences in fats and cholesterol in the diet on serum cholesterol concentration are predictable (10, 11, 13). It is interesting, therefore to apply to the present data the formulae derived from controlled experiments. Results of these calculations are summarized in table 9. For Montegiorgio there is a significant correlation between the serum cholesterol values of the individual men and the fatty acids estimated in the diets of those men but this is not true for Crevalcore. In neither of the areas is there any significant correlation between individual serum chloresterol level and such dietary factors as egg cholesterol, calories from leguminous seeds, sucrose or lactose.

Discussion

The results of the present dietary surveys are gratifying in some respects but they illustrate the limitations of single surveys on free-living men as indicators of the habitual diets of those men. It appears that a dietary survey on free-living men, carried out with meticulous care, can usefully indicate the average calories and percentages of calories from proteins and fats in the diets at the time the survey is made. Further, if tables of food composition adopted for the local foods are applied to the weighed diets, the results are closely comparable to those obtained by the method of chemical analysis of equivalent composites of the foods. There is no certainty, however, that similar success can be achieved in regard to fatty acids or cholesterol in the diet.

The second finding of importance from these studies is the fact that, in these populations, a three-day survey is substantially as good as a seven-day survey. It should be emphasized, however, that this may not hold for other populations that have different customs for the distribution of their weekly foods among the days of the week. Obviously serious errors could arise in a population in which one day of the week is truly a feast day, with far more calories and richer foods than other days of the week.

Table 9. Correlation coefficient, r, between serum cholesterol and some dietary factors

Serum cholesterol and:	N	Crevalcore	N	Montegiorgio
2 S — P *)	39	0.026	34	0.54
2 S' — P **)	39	0.004	34	0.55
mg. of egg cholesterol	87	0.18	91	0.005
% total Cal. from legumes	43	—0.20	68	—0.19
% total Cal. from sucrose	83	0.096	51	0.14
% total Cal. from lactose	77	0.097	—	—

*) S = % total Cal. from saturates fatty acids; P = % total Cal. from poly-unsaturates fatty acids.
**) S' = % total Cal. from saturates fatty acids less stearic acid.

The absence of seasonal differences in the diets of the men in these studies is interesting and conforms to findings on other groups of rural men reported in this series of studies (2, 12). The indication is that for the nutrient items considered here it may not be necessary to make surveys in different seasons in order to estimate the average nutrient picture for the year. But three notes of caution must be observed. **First,** the absence of seasonal differences is established only for calories, proteins and fats; vitamins and minerals, as well as specific proteins and fatty acids, could yield a different picture. **Second,** these surveys did not cover the periods of the greatest calorie expenditure during the periods of the heaviest farm work; at those times the farmers are too busy to be attentive to the demands of surveys. Third, these findings about lack of seasonal differences apply only to these samples of men, that is to say middle-aged rural men, mostly farmers, in Italy.

Analysis of the data on the same men surveyed on three occasions showed very substantial spontaneous intra-individual variability not accountable by differences in methods or in general seasonal trends. Similar intra-individual variability has been found in parallel studies in Greece (12) and in Yugoslavia (2). Since this intra-individual variability is of the same general magnitude as the inter-individual differences observed in these studies, it is clear that little success can be achieved from any one survey in trying to characterize the average place of the individuals within the group. The men differ almost as much within themselves from time to time as they do from each other in regard to their diets. These limitations are obvious in regard to calories, proteins and total fats; direct evidence is lacking but it may be expected that intra-individual variability in fatty acids, cholesterol, etc., in the diet should be at least as great, perhaps considerably greater.

These facts alone would be enough to lead to the prediction that individual serum cholesterol values on one occasion would be poorly correlated at best with the dietary composition recorded in a 7-day survey. Even if the serum cholesterol and dietary nutrient values were absolutely accurate for the occasions of their measurement, three facts must be noted. First, even on an identical diet individuals show highly significant average differences in serum cholesterol, reflecting intrinsic individual differences in metabolism. Second, on a constant diet, individuals show substantial variation from time to time, even from day to day, the average intra-individual standard deviation under these conditions being of the order of 12 to 20 mg. of cholesterol per 100 ml. of serum (10). Third, insofar as the diet influences the serum cholesterol value, variations in the diet are not immediately reflected in the serum level; the full response to the diet requires 3 to 4 weeks (10); ideally the need would be for a survey covering 3 or 4 weeks and several blood samples in the last days of the survey.

With groups of men, these variations — intrinsic differences between individuals, intra-individual variation independent of diet, dietary variations over time — should tend to be randomly distributed so that averages of groups of men and their diets should be much more meaningful; the confidence to be attached to those averages, method errors aside, can be estimated by the standard deviations and the numbers of men.

Accordingly, significant relationships between dietary variables and other characteristics such as serum cholesterol concentration may be shown by the analysis of averages of contrasting groups of men though not by the analysis of data on individuals in a culturally relatively homogeneous population. The requisite data include serum cholesterol values at the start and end of the dietary surveys and reliable figures for the relevant dietary variables. Though the data in the present studies do not allow detailed examination of this question, it may be noted that, in general, the serum cholesterol averages recorded for the men of Crevalcore and Montegiorgio are closely similar while the average for the men of Nicotera is substantially lower. These facts are in accordance with expectations from the data in tables 7, 8 and 9.

Summary

Seven-day dietary surveys covering weighings of all foods were made in each of three seasons on the same middle-aged men in three rural areas of Italy. Intakes of calories and percentages of calories from proteins and fats were calculated with a special table of food composition and also from chemical analysis of equivalent composites of the local foods. Fatty acids in the composites were estimated by gas-liquid chromatography.

The coefficients of correlation between calculated and analyzed values for proteins and fats averaged $r = 0.86$ and $r = 0.85$, respectively. Chemical analysis yielded average values 5.8 per cent higher for proteins and 3.1 per cent higher for fats than the calculated values. No seasonal differences were found for calories, proteins or fats in any of the three areas.

Average nutrient intakes calculated from the first three days of each survey agreed closely with the averages for the full seven days. For these populations three-day surveys are suitable for estimating population averages.

Intra-individual variation in nutrient intakes approached inter-individual variation, so distinction of individuals within the group was difficult. For the nutrient items considered, correlation between values from repeated surveys on the same men was only $r = 0.4$ to 0.5.

All diets were relatively low in both saturated and poly-unsaturated fatty acids. Particularly low intakes of saturated fatty acids were found for the men of Nicotera, Calabria. Dietary cholesterol was also relatively low in all diets and lowest in Nicotera.

A significant correlation between the relevant dietary variables and serum cholesterol was found only in one area. This conforms to the facts of intra-individual variabiliy in both diet and serum cholesterol.

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THE DIET OF VOLUNTEERS PARTICIPATING IN A LONG TERM EPIDEMIOLOGICAL FIELD SURVEY ON CORONARY HEART DISEASE AT ZUTPHEN, THE NETHERLANDS *)

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Introduction

In 1960, 1961 and 1963 a dietary study was carried out at Zutphen under the auspices of the Netherlands Nutrition Council and in cooperation with the investigators of an international long term field survey on coronary heart disease conducted by Prof. Ancel Keys of the University of Minnesota, U.S.A. [1]).

Zutphen has been of old a small commercial town among agricultural surroundings in the eastern part of the Netherlands. It is a centre of trade and traffic, in which since the second world war a considerable industry has developed (engineering works, iron works, leather factories, bookbinding establishments, printing offices and ready-made clothing factories).

The town of Zutphen was selected because the older population is rather sessile and, as from experience in earlier surveys, good collaboration could be expected, both from the population and the local authorities. The collaboration in particular has been very important for the accomplishment of the study.

The total population of Zutphen, amounting to 25 000 in the beginning of 1960, included about 2300 men aged from 40 to 59 inclusive, living at Zutphen for at least five years. A roster of these men was extracted from the official population register [2]). From this a random sample of 1088 men was selected, of whom 906 (83.3 %) volunteered in the field survey. In addition 144 (some 12.5 %) participated in the dietary study only and 2 in the medical examination only.

The complete field survey included:

a medical history and examination inclusive fluoroscopy of the thorax, electro-

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Members of the Nutrition Council's Committee on Nutrition and Atherosclerosis: Prof. P. Muntendam, M.D. (chairman); E. F. Drion, Ph.D.; M. van Eekelen, Ph.D.; E. H. Groot, Ph.D.; Prof. C. den Hartog, M.D.; Prof. G. A. Lindeboom, M.D.; Prof. H. Mulder, Ph.D.; T. Mulder (secretary).

[2]) This register is very closely in accordance with the results of the 1960 census, the difference being only 8 persons.

cardiograms at rest and post exercise, and examination of the eyes by an oculist; determination of the serum-cholesterol level and
a dietary study.

The medical examination and the determination of the cholesterol level in the blood serum of the volunteers (S Summer 1960, table 1) have been repeated every year since 1960.

The methodology and first results of the medical examinations are reported elsewhere (1, 2, 3, 6). The present paper deals with the diet of the volunteers and with the relations between food intakes and serum-cholesterol levels of the men involved.

In the dietary study several methods were used to measure the consumption of the various foodstuffs, viz.:

the cross-check dietary history

the recording of weighed and estimated food intake during seven consecutive days

the sampling of the food during seven consecutive days and chemical analysis.

The intake in terms of calories and nutrients was determined either by simple calculation using the Netherlands food composition table, or by applying chemical analysis on the food consumed.

In 1960 the dietary survey was carried out among 1050 volunteers (S Summer 1960) with the cross-check dietary history method. For the one week record of weighed and estimated food intake a sub-sample (SS Summer 1960) of 54 men was selected; in addition the food as consumed by these men was sampled and analysed. In 1961 and 1963 the survey was repeated with a sub-sample (SS Winter 1961; SS Winter 1963) of 49 and 53 men respectively. In 1961 the one week record method was applied, whereas in 1963 the food-intake was studied by the cross-check dietary history method and by one week recording, as well as by the method of one week sampling and chemical analysis.

The men in the sub-sample (SS Summer 1960) were to be a true representation of the full sample (S Summer 1960). However, the high requirements for carrying out food measurements at home (e.g. a willingness to take the trouble of weighing food several times a day during a week and a sufficient interest in the survey) led to the refusal by a number of selected families to take part and made it impossible to achieve a random sample.

With respect to measurements of personal anthropometric and biological characteristics, the sub-sample seems to be a reasonable representation of the full sample (S Summer 1960). It was found for instance that, as far as the skinfolds, relative body weights and serum cholesterol levels of the men in the sub-sample are concerned, 53 %, 48 % and 53 % respectively, were above the median for the total sample. These deviations from the 50 percent (median) are statistically insignificant. The sub-sample, however, proved to be unrepresentative in regard to physical activity, mainly due to differences in the social circumstances of the men involved. In the total sample (S Summer 1960) 24 percent of the men were sedentary or did light work only; in the sub-sample, however, 63 percent belong to this physical activity class. There was no difference between the total sample and the sub-sample as to the representation of men with heavy physical activity; the percentage being 11 and 9 respectively.

Of the originally selected sub-sample of 54 men (SS Summer 1960), 37 participated in all three dietary studies (SS Summer 1960; SS Winter 1961; SS Winter 1963) and 12 solely in the dietary studies of 1960 and 1961. In 1963 the remaining 37 men of the sub-sample were supplemented by 16 persons from the total sample; the sub-sample SS Winter 1963 therefore was not entirely composed of the same persons as the samples SS Summer 1960 and SS Winter 1961. In total 65 men were enlisted once or more times in the various sub-samples. In annexe 1 the persons, constituting the sub-samples in the various studies as well as their anthropometric data and personal characteristics are mentioned.

The framework of the dietary study, which was repeated with the sub-sample in 1961, and a sub-sample in 1963, is specified in table 1.

Table 1. Frame-work of the survey on coronary heart disease
S = sample, SS = subsample

		Dietary study			Medical examination	Serum cholesterol determination
Date	group	methods applied		number of men	number of men [1])	number of men [1])
1960 May/July	S Summer 1960	A.	Dietary history (cross-check dietary history)	1050 (906 [2]) + 144)	908	865
	SS Summer 1960	A[1].	Dietary history	49		
		B.	One week record	54 [2])		
		C.	One week sampling and analysis	54		
1961 Jan/Febr.	SS Winter 1961	D.	One week record	49		
May/June	S Summer 1961				819	828
1962 May/June	S Summer 1962				805	806
1963 Jan/Febr.	SS Winter 1963	E.	Dietary history	53 [3])		52
		F.	One week record	53 [3])		
		G.	One week sampling and analysis	53 [3])		
		H.	Analysis of average data obtained by method E	50		
1963 May/June	S Summer 1963				789	789

[1]) In regarding the figures in this column it should be kept in mind that there is a natural decline through death, illness, removal and the like. The response of the subjects since 1960 is excellent (well over 90 %).
[2]) Data of 5 men were excluded from elaboration for statistical reasons.
[3]) Data of 3 men were excluded from elaboration for statistical reasons.

Methodology

Applied dietary study methods (9, 10)

Cross-check dietary history method (A and E) *)

This method, developed by BURKE (4, 5, 7), is a form of dietary anamnesis, directed to making an accurate estimate of the average amount of food consumed in a period of 6 to 12 months preceding the study. In each dietary study the method is adapted to the prevailing conditions.

The Zutphen study was carried out as follows. The wife or housekeeper of the volunteer was visited by a dietitian; she enquired as to the man's living habits during the whole day, from morning to night, whereby particular attention was paid to the food intake during, but also beyond the meals. Differences between summer and winter diets were also taken into consideration. The data were recorded by the dietitian on form A (annexe 2) and then verified with the aid of form B (annexe 2), expressing the normal daily or weekly consumption of the various foodstuffs. The dietitian, specially trained in this work, estimated the consumed quantities by help of particular indications of the housewife, e.g. showing the thickness of slices of bread and the size of the milk jugs used. Quantities hard to estimate were weighed on a letter balance which the dietitian carried with her. As a second check, the housewife was asked to state the quantities of the various foodstuffs purchased per day and per week for the whole family. Mostly this test revealed not yet detected errors such as incorrect estimates. Finally, an interview was held with the volunteer himself asking for a true statement of what he was accustomed to consume daily; the statement was checked with the data already obtained. The interview with the housewife lasts about one hour, that with the volunteer about 20 minutes.

In 1960 six dietitians were employed in the cross-check dietary history study, whereas in 1963 the study was carried out by only one dietitian.

The nutritive values of the diets were determined by calculation (vide page 92). In 1963 also a chemical analysis was applied on a sample representing the average of the data obtained (see below and see table 5, H).

Recording of weighed and estimated food intake during one week (B, D and F)

In 1960 and 1963 when during one week the food was sampled and collected for chemical analysis (C) the weights of all the drinks, dishes and snacks that were consumed daily by the volunteers were recorded by themselves or by their wives (B and F). The ingredients of the dishes were estimated. As far as possible this week was scheduled before the time of the medical examination and blood-sampling for the serum cholesterol determination. In 1960 and 1963, 1 and 5 dietitians respectively took part in the study

In 1961 when no analysis was applied the volunteers or their wives recorded during one week on a special form all that had been consumed daily at and

*) See for these and similar indications table 1.

outside the regular meals (D). The quantities were mainly recorded in portions. The enquiry was conducted by 2 dietitians, who estimated or weighed the portions and ingredients.
The nutritive value of the average consumption per person per day in 1960, 1961 and 1963 was calculated (vide page 92).

Sampling of the food during one week and chemical analysis (C)

During one week a tenth part of all the food and drink consumed was collected daily in containers and kept in a deep freezer. A fifth part of this was lyophilized

Comparison of methods applied in the dietary study

The cross-check dietary history (A, A[1] and E)	Recording of weighed and estimated intake (B, D, F) Sampling of the food and chemical analysis (C) during one week
The data obtained relate to the food habits for ½ to 1 year and are particularly suited for epidemiological research.	The data relate to the consumption for one week in a given season.
The data relate partially to a quantity by weight of raw, undressed or unprepared products.	The weights relate to the products in prepared condition. This means, among other things, that most waste factors have been accounted for – meat and fish have lost about 20 % in weight, – vegetables may have changed in weight by —50 % to +10 %, – pulse, rice, etc. have gained in weight. In calculating the nutritive value, the differences of the condition of the various products measured by cross-check, by weighing and recording, recording and estimating, and sampling and chemical analysis should be taken into account.
This method makes heavy demands on the dietitian. She has to gain the confidence of the participant, to make good estimates, should not suggest and be able to judge whether the answers are reliable or not. She needs special training.	Makes less heavy demands on the dietitian. No special training is needed.
This method does not require a high educational level of the participant.	For weighing the participant should possess a certain amount of diligence. Accuracy is required so that a selection must be made, which means that rarely the sample is a true representation of the population.
The information supplied may be unreliable, but the diet itself is not influenced when this survey is the first one.	The food habits may be influenced but the quantities consumed can be stated accurately.
The study is not a great burden on the participant, who generally cooperates well.	The study is a burden on the participant and/or his family. Families with small or many children often do not like to participate.
One dietitian can question about 25 persons a week.	One dietitian can help about 5 persons a week in weighing or estimating.

and analysed in the laboratories of the van den Bergh's en Jurgens' Fabrieken N.V. (a Unilever factory) in Rotterdam (The Netherlands) and in the Laboratory of Physiological Hygiene of the University of Minnesota. In the latter laboratory the summer 1960 food samples were analysed for nitrogen (Kjeldahl method), total lipids, ash and fatty acids. At the Unilever Laboratories the samples were analysed for total lipids and for fatty acids. In both laboratories the analysis for fatty acids was done by gaschromatography. The caloric values of the food samples were calculated.
The chemical analysis which was applied on an average sample of the diet consumed by the volunteers examined by the cross check method (sub-sample winter 1963) was carried out in the Unilever laboratory; nitrogen, total lipids and fatty acids were determined with the methods mentioned above.
The cross-check dietary history method is generally preferred in the Netherlands. A sufficient number of well-trained nutritionists (the dietitians) are available for this work. They have received a training of high standard and are specially instructed for carrying out surveys. As Dutch people like privacy and an undisturbed family life it has to be considered in the Netherlands a serious difficulty if a dietitian stays for a week in a family weighing foods as is done in other countries. A number of housewives told the dietitian that their husbands had changed their food pattern since the weighing of the food had started, whereas to diminish the work simpler meals, less variations or less servings were eaten.

Calculation of nutritive value

For the determination of the nutritive value of the diet the Netherlands food composition table (12) has been used. In this table the composition in terms of calories and nutrients of selected foods is expressed per 100 grams of the uncooked edible portion. Therefore, in calculating the nutritive value of cooked and fried products conversion factors have been used. An exception, however, exists in the food composition table regarding vitamin C; for this nutrient the figures are mostly given for both the uncooked edible portion and the cooked or otherwise prepared edible portion as well.
To save labour a simplified method has been applied to the calculation of the caloric value and the quantity of nutrients of the foods consumed. In stead of calculating the nutritive value of the diet by adding all the separate constituents of the various foods consumed, the simplified method unites, whenever possible, the various more or less similar foods in groups, and figures with average compositions of food groups. The average compositions of the food groups, however, are weighed averages, calculated with aid of the food composition table and taking into account the average consumption of each of the separate foods. The method included all varieties of the following main food groups: brown bread, white bread, milk, cheese, lean meat, fat meat, vegetables, pulse, citrus fruits, fats, jam, marmalade and candy, cookies and gingerbread and other pastry, cereals and cereal products and flour etc. A total of 30 foodgroups and separate foods were used in the ultimate calculation.

Serum cholesterol

Blood sampling for serum cholesterol analyses was carried out in summertime in connection with the physical examination and without direct connection with the dates of taking the dietary histories. In the winter 1963, the blood samples were taken 0-3 days after the 7-day sampling of food was completed.

Results

Comparison of results obtained by various dietary study methods

Food consumption

Table 2 shows the average consumption of foodstuffs per person per day in 1960 and 1961 of the sample S Summer 1960 and the sub-samples SS Summer 1960 (B) and SS Winter 1961 (D), obtained by various methods.

Table 2. Average consumption of foodstuffs per person per day of sample and sub-samples in 1960 and 1961

Date and group	Method	Number	Foodstuffs in grammes: bread	coffee milk	cream	milk	butter-milk	cheese	ice-cream
S Summer 1960	A dietary history	852	260	25	0.4	464	30	30	1.0
SS Summer 1960	A[1] dietary history	49	260	31	0.3	503	37	38	1.0
	B one week record	49	255	13	0.6	469	28	28	—
SS Winter 1961	D one week record	49	247	12	0.6	465	19	28	—
	Average B + D	49	251	13	0.6	467	24	28	—

Date and group	Method	Number	lean meat	fat meat	bacon	lean fish	fat fish	egg	potatoes
S Summer 1960	A dietary history	850	45	52	14	13	6	34	342
SS Summer 1960	A[1] dietary history	49	52	58	11	13	4	30	310
	B one week record	49	38*	44	9	6*	6	25	251
SS Winter 1961	D one week record	49	27*	72	12	8*	4*	22	255
	Average B + D	49	33*	56	11	7*	5	24	253

Date and group	Method	Number	vege-tables	pulse	citrus fruits	other fruits	butter	marga-rine	lard dripping
S Summer 1960	A dietary history	850	196	28*	49	71	14	64	8
SS Summer 1960	A[1] dietary history	49	188	33*	50	70	13	67	4
	B one week record	49	196*	5*	33	57	19	56	6
SS Winter 1961	D one week record	49	171*	19*	41	102	18	52	10
	Average B + D	49	184*	12*	37	80	19	54	8

Date and group	Method	Number	oil	cooking fat	cereals, cereal products, flour	sugar	jam, marma-lade, candy	peanut butter	cake and cookies
S Summer 1960	A dietary history	856	1	0.5	15	65	15	1	19
SS Summer 1960	A[1] dietary history	49	2	0.8	19	62	14	3	26
	B one week record	49	1	—	19	67	13	2	31
SS Winter 1961	D one week record	49	1	—	22	63	13	2	38
	Average B + D	49	1	—	21	65	13	2	35

* cooked

As the cross-check dietary history method gives an idea of the average food pattern during a year, whereas the other methods show the consumption for one week in a particular season, a comparison of the different methods needs careful consideration. One might, in our opinion, compare the consumption figures of the complete sample S Summer 1960 and the sub-sample SS Summer 1960, both obtained with the cross-check dietary history method (A and A[1]), and also the average figures of samples SS Summer 1960 and SS Winter 1961 obtained by one week weighing and recording (B and D respectively). Some discrepancies are then becoming manifest; they are partly imputed to the method applied, partly to possible real differences in consumption. The variations in the consumption of lean meat and lean fish for instance are due to the fact that with the cross-check dietary history method the consumed quantity of the raw product is estimated, whereas with the weighing and estimating method the prepared product is measured.

It is however impossible to resist the impression that the average consumption figures for the various foods estimated with the cross-check dietary history method and thus based on information supplied by the housewife are often higher than those obtained with the other methods. Remarkable is, for instance, the higher consumption of milk and milk products estimated by the

Table 3. Average consumption of foodstuffs per person per day of sub-samples in 1961 and 1963

			Foodstuffs in grammes						
Date and group	Method	Number of men	bread	coffee milk	cream	milk	butter-milk	cheese	ice-cream
SS Winter 1961	D one week record	49	247	12	0.6	465	19	28	—
SS Winter 1963	F one week record	50	224	13	1.0	445	13	28	—
	E dietary history	50	231	14	0.5	444	15	34	—
			lean meat	fat meat	bacon	lean fish	fat fish	egg	potatoes
SS Winter 1961	D one week record	49	27*	72	12	8*	4*	22	255
SS Winter 1963	F one week record	50	30*	52	7	5*	5	17	231
	E dietary history	50	46	60	11	8	3	27	259
			vege-tables	pulse	citrus fruits	other fruits	butter	marga-rine	lard dripping
SS Winter 1961	D one week record	49	171*	19*	41	102	18	52	10
SS Winter 1963	F one week record	50	150*	26*	44	64	6	52	—
	E dietary history	50	180	35*	55	81	16	55	5
			oil	cooking fat	cereals, cereal products, flour	sugar	jam, marma-lade, candy	peanut butter	cake and cookies
SS Winter 1961	D recording/estimating	49	1	—	22	63	13	2	38
SS Winter 1963	F cross-check	50	—	—	21	55	12	1	37
	E weighing/recording	50	0.9	—	18	62	18	1	34

* cooked

cross-check dietary history method. It may be possible that by applying the weighing and estimating method not all the ingredients used for preparing a dish have been taken into account.

Of greater importance is, however, a comparison of the individual data of the sub-sample SS Summer 1960 obtained by the cross-check dietary history method (A¹) and those obtained by the other methods separately (B, D) and of their average ($\frac{B + D}{2}$). The individual data on the consumption of bread, potatoes, fat and margarine were correlated, but no mutual relation was found.

Table 4. Average consumption of calories and nutrients per person per day in 1960, 1961 and 1963

Date and group	Method	Number of men	Calories	An. prot. g	Veg. prot. g	Total prot. g	Fat g	Carbo-hydr. g	Vitamins A mg	Car. mg	Thiam. mg	Ribo-fl. mg	C mg
S Summer 1960	A dietary history	852	2941	51	38	89	141	329	1.03	2.47	1.33		
SS Summer 1960	A¹ dietary history	49	3009	56	39	95	145	331	1.10	2.40	1.36		
	B one week record	49	2765	47	37	84	129	317	0.96	2.44	1.06		
SS Winter 1961	D one week record	49	2895	48	38	86	139	325	0.87	2.20	1.17		
	Average B + D	49	2830	48	38	85	134	321	0.92	2.32	1.12		
SS Winter 1963	F one week record	50	2426	43	33	76	106	292	0.62	2.92	1.03	1.52	70
	E dietary history	50	2763	48	35	83	131	313	0.78	3.55	1.15	1.66	84

Table 5. Calorie, protein, fat and carbohydrate intake as determined by dietary calculation or chemical analysis, and the serum-cholesterol level in 1960, 1961 and 1963

Date and group	Method	Number of men	Calories	Protein, g	Fat, g	Carbo-hydrate, g	Cal. % protein	Cal. % fat	Cal. % carbo-hydrate	Serum cholesterol, mg %
S Summer 1960	A dietary history	852	2941	89	141	329	12.1	43.1	44.7	237
SS Summer 1960	A¹ dietary history	49	3009	95	145	331	12.2	43.4	44.0	230¹)
	B one week record	49	2765	84	129	317	12.1	42.0	45.9	230¹)
	C one week sampling and analysis	54	2832	87	128	333	12.3	40.7	47.0	230¹)
SS Winter 1961	D one week record	49	2895	86	139	325	11.8	43.2	44.9	235²)
SS Winter 1963³)	E dietary history	50	2763	83	131	313	12.0	42.7	45.3	242
	H analysis dietary history sample E	50	2787	82	124	335	11.7	40.2	48.1	242
	F one week record	50	2426	76	106	292	12.5	39.3	48.2	242
	G one week sampling and analysis	53					12.2	39.2	48.5	242

¹) The average is fairly low because one subject with a level of 93 mg% happened to be in the group (see also annexe 1).

²) Summer 1961 level.

³) From the 1960 sampling group 11 felt off in 1963. Their average cholesterol level in 1960 was 221 mg%. Ten new participants were incorporated in the group. They had an average serum cholesterol level of 233 mg% in 1960.

It cannot be ascertained to what extent this is due to the already mentioned fact that a number of men have changed their food pattern since the one week record method by weighing and estimating was applied, or to false information obtained by the method.

Table 3 states the average consumption of foodstuffs per person per day of the sub-sample SS Winter 1961 and the sub-sample SS Winter 1963 obtained with various methods (D, F, E). Also in the 1963 study it is shown that the consumption figures, obtained by the cross-check dietary history method (E) appear to be higher than those obtained by recording of weighed and estimated intake (F).

Nutritive value of the diet

Table 4 shows the average daily intake of calories and of nutrients of the sample S Summer 1960, the sub-sample SS Summer 1960, the sub-sample SS Winter 1961, and the sub-sample SS Winter 1963, obtained with the cross-check method (A, A', E) and the one week recording (B and F respectively). A comparison of the results of the cross-check dietary history method (A', E) and those of the one week recording method (B, F) shows for both years a greater intake of calories and nutrients in favour of the cross-check dietary history method: it reflects, as may be expected, the higher consumption of foods shown in tables 2 and 3.

In 1960 the quantity of thiamin consumed in mg per 1000 calories according to the cross-check dietary history method shows to be the highest, and in 1963 the lowest (table 6).

Table 6. Intake of thiamin in mg per 1000 calories in 1960, 1961 and 1963

Date and group		Method		Thiamin
S	Summer 1960	A	dietary history	0.45
SS	Summer 1960	A'	dietary history	0.45
		B	one week record	0.39
SS	Winter 1961	D	one week record	0.40
SS	Winter 1963	E	dietary history	0.41
		F	one week record	0.43

The percentage of calories supplied by proteins and fats (table 5) is greater in the sub-sample SS Summer 1960 when examined by the cross-check dietary history method (A') than examined by the one week recording and estimating (B). Table 5 includes also the intakes of calories and nutrients calculated from the chemical analyses. A comparison shows that the results obtained by chemical analysis of the weighed foods (C) give higher figures for the intake of proteins and carbohydrates by the SS Summer sample 1960 than by calculations from records of weighing and estimating (B). There is no difference in the fat intake as found by analysis and by calculation.

Food intake and nutritive value of the diet in 1960, 1961 and 1963

Food consumption

It appears that the average consumption of the sample S Summer 1960 and sub-sample SS Summer 1960 obtained by the cross-check dietary history method (A and A[1]) is in good harmony with regard to 8 foodstuffs, viz. bread, icecream, lean fish, fruits, butter, margarine, sugar and sweet bread-spread. In the consumption of the other 20 foodstuffs the two groups show a slight variation, the differences expressed in percentages amount mostly 10 to 20 %, with the exeption of the consumption of fats and oils. As the latter foodstuffs are consumed in small quantities, differences of 4 grams may already mean a difference of 100 %. The average consumption of shortening per person per day was less than 1 gram for both groups. The conclusion seems to be justified that the food pattern of the sub-sample SS Summer 1960 fairly corresponds with that of the sample S Summer 1960.

When comparing the foodconsumption for one week of the sub-sample SS Summer 1960 obtained by one week recording of weighed and estimated intake (B) with the food consumed in one week by the sub-sample SS Winter 1961 (D) the differences generally found were small, with the exception of the consumption figures for buttermilk, lean and fat meat, bacon, pulse and fat. The consumption for these foodstuffs shows marked differences due to typical seasonal influences.

As the composition of the sub-samples SS Winter 1963 and SS Winter 1961 was not similar, it has been ascertained whether it would be justifiable to compare the data about the diet of both sub-samples SS Winter 1961 and SS Winter 1963. For this purpose the average consumption obtained by the cross-check dietary history method of the 37 men from SS Summer 1960 having participated in all three studies viz. 1960, 1961 and 1963 has been compared with the average consumption of the 12 men solely having participated in the 1960 and 1961 study and with the consumption of the 16 men solely having participated in the 1963 study. These averages appeared to correspond mutually very well. The same applies in comparison with the average consumption of foodstuffs of the total sub-sample SS Winter 1963, composed of the 37 men from 1960 and the 16 complementary men. A comparison of the results seems therefore justified.

Considering the figures it is clear that in 1963 the consumption of bread, milk and buttermilk, fat meat, bacon and butter and fruit obtained by both the cross-check dietary history method and one week recording was lower and the consumption of lean meat higher than the consumption in 1961 obtained by one week recording.

Nutritive value of the diet

The nutritive value of the food consumed by the sub-sample SS Summer 1960 of 49 men (A[1]) and that consumed by the sample S Summer 1960 (A), differ with regard to animal protein. From table 4 it is clear that the intake of animal protein in the sub-sample is 10 % higher than that in the sample. With regard to the nutritive value of the food consumed, it can be considered ample sufficient in every respect, particularly as regards proteins and fats (table 5). For standard deviations see annexe 3.

However, the nutritive value of the food consumed in 1963 is generally somewhat lower than that consumed in 1960 and 1961. The percentage of calories supplied by the various energy producing elements shows hardly any change; the calories supplied by vegetable proteins are chiefly derived from bread and to a lower degree from potatoes (table 7). As sources of vegetable proteins other products are of minor importance. The percentage of calories derived from fats has shown a tendency to fall, but it still amounts to approximately 40 %. It has been caused mainly by a decrease in the consumption of fat and bacon.

Margarine, fat meat, bacon and dairy products are the most important sources of fat in the diets of both sample S Summer 1960 and sub-samples SS Summer 1960, SS Winter 1961 and SS Winter 1963 (table 7). The percentage of calories supplied by sugar shows a decrease since 1960; however, the total percentage of calories supplied by carbohydrates has somewhat risen (table 8).

In how far these slight modifications in the food consumed are only temporary fluctuations will have to be proved by future studies.

Table 7. Percentage of calories supplied by various fat-sources in 1960, 1961 and 1963

Date and group	Method	Number of men	Percentage of calories supplied by: total fat	dairy prod.	margarine	fat fish	fat meat, bacon	cooking fat
S Summer 1960	A dietary history	852	43.1	10.5	17.0	0.9	12.2	0.2
SS Summer 1960	A[1] dietary history	49	43.4	10.9	18.0	1.3	10.5	0.2
	B one week record	49	42.0	12.0	17.0	0.9	10.1	—
SS Winter 1961	D one week record	49	43.2	11.2	15.4	0.9	13.1	—
SS Winter 1963	E dietary history	50	42.7	12.1	14.8	0.2	7.9	0.2
	F one week record	50	39.3	10.1	16.0	0.1	8.7	—

Table 8. Percentage of calories supplied by various protein and carbohydrate sources in 1960, 1961 and 1963

Date and group	Method	Number of men	% cal. veg. protein of total calories: bread	potatoes	pulse	other prod.	total	% cal. an. prot. of total calories	% cal. tot. prot. of total calories	% cal. supplied by sugar, sweet bread spread	% cal. carboh. of total calories
S Summer 1960	A dietary history	852	2.8	0.9	0.3	1.2	5.2	6.9	12.1	10.5	44.7
SS Summer 1960	A[1] dietary history	49	2.8	0.8	0.4	1.3	5.3	6.9	12.2	9.9	44.0
	B one week record	49	3.3	0.7	0.1	1.3	5.4	6.7	12.1	11.3	45.9
SS Winter 1961	D one week record	49	3.0	0.7	0.2	1.4	5.3	6.5	11.8	10.5	44.9
SS Winter 1963	E dietary history	50	2.6	0.6	0.5	1.2	4.9	7.1	12.0	9.0	45.3
	F one week record	50	3.0	0.8	0.4	1.3	5.5	7.0	12.5	9.1	48.2

Relationship between diet and serum-cholesterol level

In 1960 investigations were made as to the relation between the diet and the quantity of calories consumed by the sample (S Summer 1960, A), determined by means of the cross-check dietary history method, and the serum-cholesterol content (table 9). In the group with the highest serum-cholesterol values the consumption of calories, originating from animal protein and of fat from dairy products expressed in percentages of the total consumption of calories, is significancy **higher** ($P = 0.02$) and that of calories of carbohydrates, also expressed in percentages of the total consumption of calories, significantly **lower** ($P < 0.0001$) in relation to the group with the lowest serum-cholesterol values. The absolute figure for the total calorie consumption for the group with the highest cholesterol values (2818) was significantly lower as compared with the "lowest cholesterol group" (3070). The men who had had a myocardial infarction, angina pectoris or intermittent claudication had a significantly lower consumption of calories than the others ($P < 0.05$).

Table 9. Average percentage of calories obtained from various food sources 1960

	Classification according to serum-cholesterol content, mg %			
	0–209	210–249	250 and more	unknown
Numbers	254	313	289	50
Vegetable protein	5.3	5.3	5.1	5.3
Animal protein	6.8	7.0	7.4	6.7
Butterfat from dairy products	9.3	10.3	11.0	9.5
Total fat	42.4	42.8	43.3	43.5
Carbohydrates	45.5	45.0	44.2	44.6
Total calories	3070	2939	2818	3040

For persons, whose diet was chemically analysed, some data about the relation between the percentage of calories originating from protein, fat, saturated and poly-unsaturated fatty acids and the serum-cholesterol content are given in table 10a (1960, group C) and table 10b (1963, group C). To prepare this table, the persons were ordered according to various components of their food intake. For each of these orderings they were thereafter divided in two halves, viz. that of the persons with a low intake and the others (with a high intake); for both halves the mean value of the serum-cholesterol content was calculated. Those means are given in table 10a and 10b, together with the range of the intake and standard deviations.

Whereas the 1960 figures (table 10a) suggest some influence of the diet on the serum-cholesterol content of the blood, such influence cannot be found in the 1963 figures (table 10b). The correlation between the serum cholesterol values and the intake of fat, saturated fat (S), poly-unsaturated fat (P) and of 2 S—P (expressed as a percentage of total consumption of calories) was low and did not differ significantly from zero on the 5 % level. The correlation coefficient were all lower than 0.3.

Table 10a. Relationship between diet and serum-cholesterol [1]) Sub-sample, summer 1960, group C

Item	Number of men	% cal. from item range		% cal. from item mean	Serum-cholesterol mg% mean	s.d.	s.e.
Protein (N × 6.25)	26	8.4	12.1	10.7	238.2	56.85	11.15
„	27	12.2	18.1	13.8	222.3	30.36	5.84
Total fat	26	25.4	39.6	35.1	213.8	45.06	8.84
„	27	39.8	54.5	44.4	245.8	41.02	7.89
S	26	11.3	19.4	16.7	214.8	42.83	8.40
S	27	19.4	26.4	21.4	244.8	43.97	8.46
P	26	3.5	5.2	4.6	233.5	43.74	8.58
P	27	5.3	8.2	6.0	226.8	47.91	9.22
2 S - P	26	18.4	33.3	28.3	215.0	42.97	8.43
2 S - P	27	33.3	47.5	37.3	244.6	43.99	8.47

Table 10b. Relationship between diet and serum-cholesterol [1]) Sub-sample, winter 1963, group C

Item	Number of men	% cal. from item range		% cal. from item mean	Serum-cholesterol mg% mean	s.d.	s.e.
Protein (N × 6.25)	25	9.8	11.7	10.8	239.6	37.80	7.56
„	26	11.9	16.7	13.3	245.3	35.23	6.91
Total fat	25	29.9	40.4	36.8	239.8	39.33	7.87
„	26	40.6	49.2	44.1	245.0	33.63	6.60
S	25	14.1	19.7	17.8	247.4	41.50	8.30
S	26	19.8	24.2	21.7	237.8	30.49	5.98
P	25	3.1	4.8	4.2	238.7	32.10	6.42
P	26	4.8	7.6	5.8	246.2	40.16	7.88
2 S - P	25	22.9	33.8	30.5	249.7	39.94	7.99
2 S - P	26	33.9	45.0	38.4	235.6	31.58	6.19

[1]) Mean serum-cholesterol concentration of men in the upper and lower halves of the distribution of the percentage of calories in the diet provided by protein, total fat, saturated fatty-acids (S) and poly-unsaturated fatty-acids (P), and of the distribution of 2 S-P. Dietary data from direct chemical analysis.

Discussion and conclusion

It has become evident from these dietary studies, which formed part of a long-term epidemiological field survey on coronary heartdisease, that with regard to the survey-methods applied, the cross-check dietary history method generally produced higher values for the caloric and nutrient intake than the one-week recording method. This is in accord with the experiences in other surveys (10).

Discrepancies between the average food-consumption during a period of about one year and the average food intake during one week in a certain season can, naturally, be expected. However, it is not possible to explain in how far the higher values obtained by the cross-check dietary history method have been caused by overestimation when the dietary history method is applied, or by incomplete recording when using the one-week recording method.

By the third method applied, viz. the one-week sampling and chemical analysis method, higher values have also been obtained for the intake of calories, proteins and carbohydrates than by the one-week recording method.

From these studies it has become evident that in the Netherlands where

trained dietitians are available,

a food composition table with analyses of Dutch products can be used and the individuals to be surveyed generally do not live at a considerable distance from one another,

the cross-check dietary history method is far more preferable for this kind of longitudinal epidemiological survey than the one-week recording or one-week sampling and chemical analysis methods, and for various reasons:

a. In the Netherlands a great variety of foods and foodproducts are used in the various meals which makes sampling much more difficult than in areas where the food-pattern is rather monotonous.
b. By using the cross-check dietary analysis a great number of persons can be surveyed by a few dietitians within a short time, contrary to the one-week recording or sampling methods.
c. The cross-check dietary history method is hardly a burden to the person-surveyed and his family, for which reason this method offers a greater chance of long-term co-operation than the other two methods.

For a longitudinal survey such as this one, in which so many factors should be taken into consideration it is essential that nutritional data of a great group of individuals can be obtained for many consecutive years. This is still more important when, just as in this case, the group surveyed consists of aging people of whom in the course of years, a great many more will drop out owing to illness or decease than will be the case with groups of younger people.

With regard to the nutritive value of the diet of the sample and sub-sample surveyed it is clear that in spite of some differences in the results of the various survey-methods, it can be considered amply sufficient in every respect, particularly as regards total protein, fat, and carbohydrates derived from sugar. The intake of fat is even excessive.

The food-consumption pattern of the sample and the sub-samples shows a picture very much similar to the one obtained by the average food consumption per gram per day per capita in the Netherlands (8). In 1960 significant relations were shown to exist between the percentages of calories derived from animal proteins, dietary fat and carbohydrates, the quantity of calories consumed by the sample (S Summer 1960 A) and the serum-cholesterol level. Moreover, in 1960 a slight tendency of a positive relation between the percentages of the calories derived from fat, poly-unsaturated and saturated fatty acids and the serum cholestrol content of the sub-sample (SS Summer 1960 C) could be observed. However, in winter 1963, a similar relation between the diet and the serum-cholesterol of the sub-sample (SS Winter 1963 G) was not demonstrated.

The authors realize that the intake of fat calories and poly-unsaturated and saturated fatty acids only constitute a very small part of the many dietary factors, which may have some influence on the serum-cholesterol level. It would be desirable to study the influence of other diet-factors including sugar, di- and mono-saccharides, pectines, proteins from cereals, pulse and vitamin D both separately and in interrelationship. As sufficient basic data on various factors are available for analyses it is intended to issue further publications on this matter in the near future.

Summary

In 1960 a dietary study was carried out at Zutphen among 1050 men of 40-59 years according to the cross-check dietary history method of BURKE. Of these 1050 men 906 were subjected to a medical examination in 1960 and came for partial re-examination in the summer months of the following years, viz. 1961, 1962 and 1963. In 1960 a sub-sample of these 906 men was selected. The diet of the men in the sub-sample was studied during one week by recording the weighed and estimated intake and by sampling of the food and chemical analysis. In 1961 and 1963 the dietary study was repeated with a sub-sample using various methods. In comparing these methods within the sub-sample very similar results were found, which, in 1960 were, moreover, little different from the results obtained by the cross-chek dietary history method in the rest of the sample of 906 men. Some seasonal differences were found. In 1963 the consumption of bread, fat meat, bacon, milk, buttermilk, butter and fruit showed a slight tendency to decrease, as well as the percentage of calories supplied by fat. Positive relations between the serum cholesterol content of the sample in 1960 (S Summer 1960 A) and the consumption of protein and animal fat appeared as well as an inverse relation with the carbohydrate consumption. The total amount of calories calculated and those obtained from analysis were inversely related with the serum-cholesterol levels.

In 1960 and in 1963 the serum-cholesterol levels of the sub-sample (SS Summer 1960 C) and the sub-sample (SS Winter 1963 G) respectively, were correlated with percentages of calories originating from protein, fat, saturated and poly-unsaturated fatty acids.

The 1960 figures suggest some influence of the diet on the serum-cholesterol contrary to the 1963 figures which do not show any relation at all.

Publications on the correlation between some other dietary factors and the serum-cholesterol will follow in due time.

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Annexe 1. Some characteristics of the subjects constituting the sub-samples

Roster no.	Serial no.	Date of birth		Skinfolds 0.1 mm	Serum-cholesterol, mg %			
		year	month	mean	1960	1961	1962	1963 (winter)
0019	749	1900	08	7	198	234	227	—
0026	388		08	14	292	280	284	271
0035	634		08	8	207	204	210	207
0053	450	1901	10	8	—	247	255	—
0054	560		03	12	249	216	216	234
0062	87		10	13	257	250	283	232
0074	681		11	10	265	261	—	293
0079	755		12	13	205	205	260	256

Annexe 1. (Continued)

Roster no.	Serial no.	Date of birth year	month	Skinfolds 0.1 mm mean	Serum-cholesterol, mg % 1960	1961	1962	1963 (winter)
0085	712		05	11	250	261	275	—
0104	674	1902	04	16	238	239	254	210
0107	672		05	6	235	233	267	270
0115	572		06	20	220	218	224	255
0130	185		07	12	311	325	307	333
0142	417		06	11	167	175	205	204
0150	423	1903	12	21	344	331	337	290
0160	253		09	15	—	189	183	219
0171	490		12	10	221	209	219	—
0189	410		11	8	184	184	203	204
0192	250		09	21	169	—	—	—
0217	3	1904	08	11	314	276	—	—
0235	481		01	15	234	214	196	225
0247	421		04	8	203	233	232	234
0262	603		06	8	204	250	275	264
0263	630		08	8	191	209	218	219
0265	119		02	13	237	233	249	204
0268	217	1905	05	19	249	236	231	260
0329	1	1906	10	7	273	273	290	—
0335	906		01	—	219	211	234	209
0343	12		10	12	176	195	242	215
0344	754	1905	09	13	233	212	199	—
0373	581	1907	06	16	226	214	226	243
0390	95		03	16	199	190	220	208
0399	742		08	21	313	250	271	275
0430	18	1908	04	15	206	241	231	207
0456	866		05	12	296	191	273	284
0485	689	1909	05	5	173	161	195	200
0497	782		12	9	213	—	272	—
0503	204		06	22	234	—	—	269
0513	724		06	7	244	244	296	283
0517	753		05	14	215	205	203	195
0527	736		11	13	226	223	240	272
0585	49	1910	09	20	226	263	267	241
0590	241		10	15	211	186	282	237
0599	716		06	11	257	221	199	181
0610	118	1911	07	14	217	233	214	238
0614	766		07	7	285	313	295	299
0646	243		10	13	261	275	256	265
0708	472	1913	10	15	147	189	195	210
0775	24	1914	08	11	—	207	260	206
0779	575		06	6	170	165	192	181
0797	265		10	6	280	313	319	290
0809	412		05	9	220	193	251	203
0852	260	1915	07	10	258	265	284	283
0861	319		10	14	320	313	369	287
0867	127		07	14	264	264	261	239
0892	21	1916	01	16	258	226	250	256
0897	821		06	14	287	297	273	298
0905	815		03	16	232	216	233	227
0921	113		06	6	206	—	—	—
0955	71	1917	03	10	260	246	252	283
0998*	465	1918	05	9	93	84	89	79
1022	437		10	18	188	190	182	182
1030	501		07	19	—	263	241	273
1038	757	1919	09	16	265	274	—	265
1049	756		05	11	211	217	239	—
1083	623		03	15	247	257	254	240

* This person has symptoms of acanthocytosis; his data were excluded afterwards.

Annexe 2 – Form A. Questionnaire dietary survey at Zutphen

Family name : ....
Christian name : ....
Birth date : ....
Address : ....
Municipality : ....
Date of survey : ....
Number of family members : ....
Family composition : ....
Husband's occupation : ....

Diet pattern

Breakfast	Snacks
Midday meal	
Evening meal	

a. Is the subject on a diet? Yes / No
b. If so, what kind of diet? ....
c. What is the reason for this diet?
    - to gain weight Yes / No
    - to lose weight Yes / No
    - allergy Yes / No
    - peptic ulcer Yes / No
    - gall-stones Yes / No
    - diabetes Yes / No
    - other reasons ....

d. Was this diet prescribed by a doctor? Yes / No

Annexe 2 – Form B. Questionnaire dietary survey at Zutphen Name: ....

Per man inquired	Times per week	Average number of units per day [1])	Number of g per unit	Average number of g per day	Total
Bread 1. A. Number of slices of so called Government bread (80 % extraction) B. Number of slices of wholemealbread C. Number of slices of ryebread					
2. A. Number of slices of milkbread B. Number of fancy-rolls C. Number of slices of fancy-bread, currant-bread D. Week-end currant-bread E. Number of rusks					
Milk and milkproducts 3. A. Number of beakers or cups of milk, cocoa or some other milkdrink 2½ / 3¾ [2]) Number of beakers or cups of coffee [3]) 2½ / 3¾ Number of servings of porridge, custard or pudding [4]) 2½ / 3¾) B. Number of servings of Bulgarian milk or skim milkyoghurt [2])					
4. Number of beakers or cups of buttermilk Number of servings of buttermilk-porridge or pudding [4])					
5. Number of slices of 40+ cheese Number of slices of full-cream cheese Quantity of pot-cheese					
6. Number of ice-creams					
Meat 7. A. Quantity of meat in soup B. Quantity of lean meat at dinner Quantity of lean meat on bread					
C. Quantity of fat meat and meatproducts at dinner Quantity of fat meat and meatproducts on bread					
8. A. Quantity of pork at dinner B. Quantity of pork on bread					
Fish 9. A. Quantity of lean fish at dinner Quantity of lean fish on bread					
B. Quantity of fat fish at dinner Quantity of fat fish on bread					
Eggs 10. Number of eggs					
Potatoes 11. Number of boiled potatoes					
Vegetables 12. Hotchpotch - number of times Quantity of cooked vegetables (.. sterilized, .. salted, .. dried, .. canned) Quantity of raw vegetables					

Per family		Per week or per day	
Bread 1. A. Government bread (80 % extraction) B. Wholemealbread C. Ryebread	 number of loaves of .. g number of loaves of .. g number of loaves of .. g		
2. A. Milkbread B. Fancy-rolls C. Fancy-bread, currant-bread D. Week-end currant-bread E. Rusks	number of loaves of .. g number number of loaves of .. g number of loaves number of rolls of .. each		
Milk and milkproducts 3. A. Milk 3¾ B. Milk 2½ C. Yogurt D. Bottled custard and porridge 4. Buttermilk 5. Cheese 6. Ice-creams	 number of litres number of litres number of litres number of litres number of litres quantity in g number		
Meat 7. A. Meat B. Meatproducts and sausage	 quantity in g quantity in g		
8. Fat bacon	quantity in g		
Fish 9.	 quantity in g at dinner quantity in g on bread		
Eggs 10.	 number of eggs		
Potatoes 11.	 quantity in g		
Vegetables 12.	 number of g		

Per man inquired	Times per week	Average number of units per day [1])	Number of g per unit	Average number of g per day	Total
Pulse (dried) 13. Quantity of pulse cooked as vegetables Quantity of pulse in soups					
14. Number of oranges, lemons, grapefruits 15. Number of other fruits Quantity of apple-sauce or stewed-pears					
16. A. Number of slices of bread with butter Quantity of butter in or with hot meal					
B. Number of slices of bread with margarine Quantity of margarine in or with hot meal					
17. A. Number of slices of bread with fat B. Quantity of lard or beef suet in or at warm meal C. Quantity of oil in or at warm meal D. Quantity of shortening in or at warm meal					
Cereals, cereal products and flour 18. Quantity of flour in g Quantity of Brinta (pre-cooked wheat) Quantity of oatmeal					
Sugar 19. Quantity of sugar (in tea, coffee etc. or on bread or in porridge etc.)					
Sweet breadspreads, jam and sweets 20. A. Number of slices of bread with jam B. Number of slices of bread with treacle C. Number of slices of bread with granulated chocolate or granulated sweets D. Number of slices of bread with apple-treacle E. Number of bonbons, chocolate-drops, toffees etc.					
21. Number of slices of bread with peanut butter					
Cookies, pastry etc. 22. A. Number of sweet biscuits and biscuits B. Number of fancy-cakes C. Quantity of breakfast-gingerbread					
Vitamins and calciumpreparations 23. Trade-name of vitamins and calciumpreparations Number of tablets / drops [5])					
24. Trade-name codliver-oil					
25. Further particulars					
Special drinks 26. Number of bottles of coca-cola					
27. Number of bottles of beer Number of bottles of lemonade					

[1]) unit: slice, beakerful, cupful, serving, piece, tablespoonful, teaspoonful, etc.
[2]) delete what is not applicable (% fat content)
[3]) only the quantity of milk used should be indicated here

Per family		Per week or per day	
Pulse (dried) 13.	number of g		
Fruits 14. Oranges, lemons, grapefruits 15. Other fruits	number number of g and / or piece		
16. A. Butter B. Margarine	packets of butter of 250 g or .. g packets of butter of 250 g or .. g		
17. Fats and oil, shortening	quantity in g		
Cereals, cereal products and flour 18.	total quantity of Brinta (precooked wheat) in g total quantity of oatmeal in g		
Sugar 19.	total quantity of granulated sugar and brown sugar in g		
Sweet breadspreads, jam and sweets 20. A. Jam B. Treacle C. Granulated sweets or granulated chocolate D. Apple-treacle E. Bonbons, choc.-drops and toffees etc. per day	number of pots of 450 g each number of pots of .. g each number of g number of pots of .. g each number of g		
21. Peanut butter	number of pots of ± 370 g each		
Cookies, pastry etc. 22. A. Sweet biscuits and biscuits B. Fancy-cakes C. Breakfast-gingerbread	quantity in g number of .. g each		
Vitamins and calciumpreparations 23.			
24. Codliver-oil			
25. Further particulars			
Special drinks 26. Number of bottles of coca-cola			
27. Further particulars			

[4]) only the quantity of milk used should be indicated; the thickening agent used should be indicated under the item: cereals, cereal products and flour

[5]) cross out if not applicable

Annexe 3. Food intake and nutritive value, 1960, measured by cross-check dietary history method Standard deviations

	$S_{\bar{x}}$	
F o o d s t u f f s	n = 850	n = 50
Brown bread	4.6	19
White bread	2.1*	9*
Coffeemilk	0.9*	4*
Cream	not determined	not determined
Milk and yogurt	11.5	47
Buttermilk	0.6*	2*
Cheese	0.7	3
Icecream	not determined	not determined
Lean meat	1.0	4
Fat meat	1.0	4
Bacon	0.4*	2*
Lean fish	0.5*	2*
Fat fish	0.3	1
Egg	0.7	3
Potatoes	5.5	23
Vegetables	2.1	9
Pulse (cooked)	0.9*	4*
Citrus fruit	1.7*	7*
Other fruits	1.7	7
Butter	0.6*	2*
Margarine	1.2	5
Fat	0.3*	1
Oil	0.1*	0.4
Frying fat	not determined	not determined
Cereal, cereal products, flour	0.5*	2*
Sugar	1.2	5
Sweets	0.5*	2*
Peanuts	not determined	not determined
Cookies and pastry	0.5*	2
N u t r i e n t s		
Vegetable proteins, all items	0.4	2
Animal protein	0.6	2
Fat from: dairy products	0.7	3
margarine, cookies, pastry	1.0	4
fish, oil, peanuts	0.1*	0.4*
all items	1.2	5
Carbohydrates from all items	2.9	12
Calories	22.2	91
‰ o f t o t a l c a l o r i e s d e r i v e d f r o m :		
Vegetable proteins	0.3	1
Animal proteins	0.8	2
Fat: dairy products	2.0	8
margarine, cookies, pastry	2.6	11
fish, oil, peanuts	0.3*	1*
all items	1.9	8
Carbohydrates from: sugar	1.5*	6
all items	2.0	8
Vitamin B_1, per 1000 calories derived from carbohydrates	0.01	0.05

x has been calculated from the difference between the 15.9 % and 84.1 % item.

If the 15.9 % item was 0, the standard deviation concerned has been indicated with an asterisk.

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DIETS OF RURAL FAMILIES AND HEADS OF FAMILIES IN TWO REGIONS OF YUGOSLAVIA [1])

by R. BUZINA, E. FERBER, A. KEYS [2]), A. BRODAREC, B. AGNELETTO and A. HORVAT

Introduction

Controlled experiments on man have shown that the amount and kind of fat in the diet have powerful effects on the concentration of cholesterol and associated lipids in blood serum. Under controlled experiments of limited duration the average serum cholesterol response to given changes in the proportion of total diet calories supplied by glycerides of saturated, monoene and polyene fatty acids is predictable (4, 7, 8). Studies on free-living populations have indicated some general correspondence with the results of controlled experiments (5), but more detailed data on the chemical composition of the diet in such field studies are needed. These questions are important because of the theory that the development of atherosclerosis and coronary heart disease is influenced by the diet and the serum cholesterol level in man as well as in experimental animals (2, 6, 9, 10). In 1953 a trial survey of serum cholesterol values was made on three samples of the population in Croatia (Yugoslavia), known to subsist on different types of diet (1). Two of the three samples were closely similar in the total amount of fat, estimated at 90-110 g/day, but differed sharply in the kind of fats used. In the „animal-fat area" over 90 per cent of the fat intake was of animal origin and was consumed in the form of lard, fat pork meat, smoked bacon and ham, and dairy products. In the „olive-oil area" this vegetable oil covered a similarly high proportion of total fat consumption. The third population sample was in the „low fat area" where the total of both animal and vegetable fat was estimated to be less than half that consumed in the other two regions. Serum cholesterol determinations showed significantly higher values in the „animal-fat area" than in the „low-fat area" or in the „olive-oil area".

On this basis, and from indications that the frequency of coronary heart disease differs in the various regions of Croatia, a study was organized to direct specific attention to dietary questions that arise in connection with the hypothesis that the diet influences the development of atherosclerosis and its complications in human populations. The dietary findings reported below are a part of the follow-up study on heart disease and related questions among substantially all men aged 40-59 who, in 1958, were living in two rural areas, one on the Dalmatian coast (669 men) and one in Slavonia in the plain near the Hungarian frontier (694 men). The total programme in Croatia is, in turn, part of a larger operation comprising parallel programme in eight other areas in Finland, Italy, the Nether-

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lands and Greece, using identical methods with men of the same age. Other aspects of the work in Dalmatia and Slavonia will be reported separately.

Sampling of subjects

Six villages in the region of Makarska on the southern coast of Dalmatia and the large village of Dalj with two adjacent villages near Osijek in Slavonia were the two areas of study. In each area a complete roster was obtained of all men aged 40-49 in the autumn of 1958 and more than 95 per cent of the men were subjected to medical examination. From among the 1363 men examined, excepting the few individuals who were not heads of families or who were ill or had family circumstances incompatible with the dietary study, names were drawn at random to provide four families to be studied in each of the six villages in Dalmatia and 25 families to be studied at Dalj in Slavonia.

The dietary studies started in October, 1958, in Dalmatia and immediately thereafter in Slavonia, ending on November 30th. One experienced dietitian was assigned to each unit of four families in Dalmatia and the same team similarly carried out the work in Slavonia. The surveys were repeated in April, 1960, August, 1961, January, 1962 (in Slavonia only), and in January, 1963, so as to cover all four seasons. In 1958 only the food consumption of the whole family was recorded but in the later surveys the consumption of the heads of the families was recorded separately, with each dietitian covering only two families at a time.

Illness, temporary absences of the men, etc., caused some changes in the samples of families. Out of the 24 original families in Dalmatia, 14 were studied on all four occasions, 18 in three and 24 in two surveys. In Slavonia, out of the 25 originally families, 16 were studied in all five surveys, 17 in four, 18 in three and 24 in two surveys.

Methods

Food comsumption was recorded for seven successive days in each survey. All foods were weighed, and the volume of all beverages except water was measured, and detailed menus and recipes were recorded on prescribed forms covering all details. From the records of food and beverage consumption, nutrients were estimated with the food composition tables used in our laboratory at Zagreb; these are based on the tables of the Food and Agriculture Organization (3), the Schall tables (11), and the results of our own analysis of certain foods typical or special to Croatia.

In the 1958, 1960 and 1961 studies, at the end of the week the totals of each foodstuff were computed for each village and corresponding proportions of those foods were obtained locally to make up an equivalent composite aliquot. These equivalent composites were homogenized, lyophilized, sealed under nitrogen and sent for analysis to central laboratories at the Universities of Minnesota and Naples. In the 1963 study for 18 family heads in Slavonia and 24 in Dalmatia identical portions of each food as served at each meal were taken for chemical analysis. The results of these special analysis, including fatty acids estimated by gas-liquid chromatography, will be reported separately.

Results

Family diets in autumn, 1958

The make-up of the families is given in table 1; in this respect the samples were similar in the two regions so that a simple comparison of the average family diets in the two areas is permissible. The family diets in the autumn of 1958 are summarized in table 2. At that time mean per capita intakes of total calories, proteins, and carbohydrate were significantly higher in Slavonia than in Dalmatia and alcohol was used more in Dalmatia. Thiamine, niacin and iron intakes tended to be significantly higher in Slavonia but other differences in nutrients per capita were not significant. Finally, the family diets in the two areas did not differ significantly in the distribution of calories supplied by the several classes of nutrients.

Table 1. Composition of the population samples

Item	1958, Fall Dalmatia	1958, Fall Slavonia	1960, Spring Dalmatia	1960, Spring Slavonia	1961, Summer Dalmatia	1961, Summer Slavonia	1963, Winter Dalmatia	1963, Winter Slavonia
Households	24	25	24	25	24	25	24	25
Total persons	114	119	102	108	112	100	105	91
Children under 10	12	16	14	15	16	10	8	4
Children 10 to 18	24	19	14	13	16	14	17	12
Males aged 18 or over	35	38	31	38	37	33	42	38
Females aged 18 or over	43	46	43	42	43	43	38	37
Family heads, mean age	51.9	51.8	50.0	54.2	52.3	53.6	54.5	53.2

Table 2. Slavonia versus Dalmatia-Families, Fall 1958
Daily means and standard errors per capita Slavonia 25 families, Dalmatia 24 families
(Percentages of total Calories are given in brackets)

Item	Unit	Slavonia		Dalmatia		Δ	t
Calories		3331 ± 137		2869 ± 122		462	2.52
Protein	g	104 ± 4	(12.5)	82 ± 3	(11.4)	22	3.79
Fat	g	93 ± 7	(25.1)	97 ± 7	(30.4)	4	0.39
Carbohydrates	g	445 ± 27	(53.4)	353 ± 14	(49.2)	92	3.02
Alcohol	g	42 ± 2	(9.0)	36 ± 2	(8.9)	6	2.00
Vitamin A	I.U.	3152 ± 479		5180 ± 1057		2028	1.78
Thiamine	mg	1.7 ± 0.1		1.0 ± 0.1		0.7	5.38
Riboflavin	mg	1.4 ± 0.1		1.1 ± 0.1		0.3	1.86
Niacin	mg	24.0 ± 1.0		18.0 ± 1.0		6.0	2.96
Ascorbic acid	mg	135.0 ± 9.0		122.0 ± 18.0		13.0	0.83
Calcium	mg	626 ± 45		524 ± 63		102	1.32
Iron	mg	40.0 ± 5.0		25.0 ± 2.0		15.0	2.66

Heads of families vs. their families

As would be expected, in per capita terms the heads of the families expend more energy and eat more food than their families. The data on energy-yielding nutrients for two seasons in Dalmatia and three in Slavonia are summarized in table 3. The least difference between family heads and their families tends to be in carbohydrate intake; the greatest difference is in total calories.

Table 3. Energy-yielding nutrients in the diets of families and heads of families in Dalmatia (N=24) and in Slavonia (N=25)
Daily means and standard errors

Period	Item		Dalmatia Family	Dalmatia Head	t	Slavonia Family	Slavonia Head	t
1960, Spring	Calories		2639 ± 100	3754 ± 157	5.99	3047 ± 100	3687 ± 169	3.26
	Protein	g	80 ± 4	109 ± 6	4.02	109 ± 4	129 ± 7	2.48
	Fat	g	94 ± 6	126 ± 8	3.20	119 ± 5	150 ± 7	4.30
	Carbohydrates	g	289 ± 13	367 ± 21	3.16	370 ± 16	398 ± 25	0.94
	Alcohol	g	45 ± 5	103 ± 10	5.19	9 ± 2	25 ± 6	2.53
1961, Summer	Calories		2516 ± 103	3420 ± 149	4.92	2979 ± 115	3412 ± 122	2.58
	Protein	g	76 ± 5	99 ± 6	2.96	102 ± 4	117 ± 4	2.62
	Fat	g	89 ± 7	117 ± 11	2.24	109 ± 6	120 ± 6	1.29
	Carbohydrates	g	289 ± 11	326 ± 18	1.78	382 ± 17	421 ± 17	1.63
	Alcohol	g	36 ± 4	94 ± 17	3.27	10 ± 2	25 ± 10	1.40
1962, Winter	Calories		—	—		2928 ± 92	3312 ± 123	2.50
	Protein	g	—	—		109 ± 5	121 ± 7	1.46
	Fat	g	—	—		94 ± 6	105 ± 8	1.16
	Carbohydrates	g	—	—		365 ± 13	384 ± 19	0.84
	Alcohol	g	—	—		26 ± 5	49 ± 10	2.15

Table 4 shows that these differences in total food intake are reflected to some extent in the intakes of vitamins and minerals but in these nutrients the differences

Table 4. Head of family versus family

Period	Item		Dalmatia Family	Dalmatia Head	t	Slavonia Family	Slavonia Head	t
1960, Spring	Vitamin A	I.U.	4591 ± 672	6081 ± 766	1.49	4093 ± 316	4957 ± 663	1.18
	Thiamine	mg	0.9 ± 0.1	1.2 ± 0.1	2.13	1.4 ± 0.1	1.9 ± 0.2	2.17
	Riboflavin	mg	1 ± 0.1	1.4 ± 0.1	2.86	1.3 ± 0.1	1.6 ± 0.1	2.13
	Niacin	mg	14 ± 0.7	20 ± 0.1	4.92	17 ± 1	19 ± 1	1.42
	Ascorbid acid	mg	83 ± 8	103 ± 8	1.77	46.3 ± 3	50 ± 3	0.94
	Calcium	mg	480 ± 43	671 ± 76	2.19	479 ± 40	549 ± 50	1.09
	Iron	mg	16 ± 1	20 ± 1	2.84	19 ± 1	26 ± 1	4.97
1961, Summer	Vitamin A	I.U.	3388 ± 440	4718 ± 697	1.62	4861 ± 544	4702 ± 395	0.39
	Thiamine	mg	0.9 ± 0.1	1.1 ± 0.1	1.64	1.7 ± 0.1	1.7 ± 0.1	0.08
	Riboflavin	mg	1.3 ± 0.1	1.5 ± 0.1	1.28	1.7 ± 0.1	1.7 ± 0.1	0.12
	Niacin	mg	16.7 ± 0.7	19.6 ± 1.3	1.97	23.7 ± 1.3	22.6 ± 1.1	0.27
	Ascorbid acid	mg	116.6 ± 10.4	119.7 ± 11.3	0.20	104.3 ±.7 5	114 ± 9.6	0.79
	Calcium	mg	561 ± 53	632 ± 68	0.82	512 ± 47	761 ± 81	2.18
	Iron	mg	16.1 ± 1.0	18.4 ± 0.4	2.14	24.9 ± 1.1	25.6 ± 1.3	0.41
1962, Winter	Vitamin A	I.E.	—	—		5276 ± 720	4182 ± 387	1.34
	Thiamine	mg	—	—		1.7 ± 0.1	1.7 ± 0.1	0.00
	Riboflavin	mg	—	—		2.1 ± 0.2	1.4 ± 0.1	3.13
	Niacin	mg	—	—		24.8 ± 1.4	24.6 ± 1.5	0.78
	Ascorbid acid	mg	—	—		75.4 ± 10	70 ± 7.8	0.42
	Calcium	mg	—	—		440.5 ± 37.3	455.1 ± 59.8	0.35
	Iron	mg	—	—		23.3 ± 0.9	25.7 ± 2.4	0.94

between families and their heads are relatively small and often insignificant. In general, both for families and their heads the vitamin and mineral intakes listed in table 3 seem to be relatively satisfactory but, as in 1958, thiamine intakes tend to be low in Dalmatia.

In spite of the differences between families and their heads in regard to the absolute amounts of the several sources of energy in the diet, the percentage distribution tends to be much the same. For the heads of families in Slavonia protein and fats contribute respectively 14.1 and 32.8 per cent of total calories; the corresponding averages for their families are 14.3 and 32.3 per cent. In Dalmatia the heads of families averaged 11.6 per cent of calories from proteins and 30.6 from fats; their families averaged 12.2 and 31.3, respectively. The only major difference between the families and their heads in regard to sources of energy is in alcohol. For the spring of 1960 and the summer of 1961 the family heads in Dalmatia averaged 19.4 per cent of total calories from alcohol while their families averaged 11.1 per cent. In Slavonia, alcohol is a less important source of calories but the heads of families tend to consume at least double the family average.

Heads of families - winter 1963

In the winter of 1963 the dietary studies were concentrated on the heads of the families and foods were collected for chemical analysis of individual diets. The principal results of current interest are summarized in table 5. The average diets of the heads of families in these two regions differ significantly in respect to proteins and alcohol but not in total calories or total fat. Except in regard to fat, in the winter of 1963 the distribution of sources of calories is similar to that in the other surveys; protein is more abundantly represented in Slavonia than in Dalmatia, the reverse is true for alcohol, and total calories are similar in the two populations.

Table 5. Energy-yielding nutrient intakes of heads of families in Dalmatia (N=24) and in Slavonia (N=25) in the winter of 1963 (Means and standard errors)

Item	Unit	Dalmatia	Slavonia	t
Calories		2907 ± 151	3068 ± 152	0.71
Protein	g	73 ± 3	93 ± 4	3.98
Fat	g	86 ± 6	93 ± 7	0.75
Carbohydrates	g	293 ± 14	355 ± 24	2.22
Alcohol	g	97 ± 14	63 ± 15	2.74

Seasonal variation

The choice of foods in the diet is influenced by seasonal availability and variation in this respect is particularly marked in rural farming areas where almost all the food is locally produced. Seasonal differences are evident in table 6; fruit, eggs, milk and milk products and fish show particularly marked seasonal variations. In Slavonia wine is seasonal, also, because production is limited and each year's new supply tends to run out by the end of the winter, wine consumption in Dalmatia tends to be relatively constant all year, reflecting more abundant production and storage.

Table 6. Average daily consumption of foods (edible portion), in g (head of the family)

Item	Spring 1960		Summer 1961		Winter 1963	
	Slavonia	Dalmatia	Slavonia	Dalmatia	Slavonia	Dalmatia
Cereals and -products	584	498	581	320	455	356
Roots and tubers	137	151	105	220	119	199
Sugar	27	34	22	31	27	31
Pulses	30	8	26	0	28	26
Vegetables	235	201	293	278	185	119
Fruit	3	9	154	74	19	6
Meat and -products ..	193	115	162	113	176	81
Fish	33	88	30	91	11	27
Eggs	1	1	30	21	9	12
Milk and -products ..	289	177	295	244	103	17
Fat	63	16	52	35	32	14
Oil	8	72	5	53	2	37
Wine	73	905	54	733	430	741
Brandy	40	9	53	15	39	20

Simple weights of raw foods do not properly portray their contributions to metabolism. The food weight data in table 6 are converted into terms of percentages of total calories in table 7. The great importance of bread („cereals"), especially in Slavonia, is clear. It is notable, also, how small the calorie contribution of „vegetables" is in spite of the impressive mass consumed daily.

Table 7. Percentage of total calories supplied by various foods, including alcoholic beverages, in the diet of the heads of the families

Item	Spring 1960		Summer 1961		Winter 1963	
	Slavonia	Dalmatia	Slavonia	Dalmatia	Slavonia	Dalmatia
Cereal and -products	42.7	35.8	44.1	29.0	43.4	30.6
Roots and tubers	3.4	3.6	2.9	5.9	3.9	6.4
Sugars	2.3	3.6	2.5	3.5	3.7	4.2
Pulses	2.4	0.6	2.3	0.0	2.9	2.8
Vegetables	2.4	1.4	2.9	2.4	2.8	4.3
Fruit	0.1	0.5	2.0	1.5	1.0	0.4
Meat and -products ..	18.0	7.3	13.4	5.4	13.9	8.9
Fish	1.6	3.8	1.6	4.2	0.6	1.9
Eggs	0.1	0.2	1.2	0.8	0.5	0.6
Milk and -products ..	5.4	3.4	7.5	5.6	2.7	0.9
Fat	15.6	3.8	13.5	8.9	10.0	4.4
Oil	1.9	17.1	1.1	13.6	0.5	11.3
Wine	1.4	18.3	1.1	18.2	10.7	21.9
Brandy	2.8	0.6	4.0	1.1	3.5	1.8

Seasonal variation in the intake of vitamins and minerals is substantial for some items, as shown in table 8. Thiamine and niacin are relatively constant, though they tend to be lower in winter than at other times of the year. The low winter intakes of riboflavin and calcium reflect the small supply of milk at that time. Ascorbic acid intake holds up fairly well in the winter, especially in Slavonia, because of the use of potatoes and cabbage. The lack of leafy vegetables in the winter explains the low intake of vitamin A in that season.

Table 8. Seasonal variations - Heads of the families
Means and standard errors for Slavonia (N=25) and Dalmatia (N=24)

		Vitamin A I.U.	Thiamine mg	Riboflavin mg	Niacin mg	Ascorbic acid mg	Calcium mg	Iron mg
Slavonia								
Spring	1960	4957±663	1.9±0.2	1.6±0.1	19.0±1.0	50.0±3.0	549±50	26.0±1.0
Summer	1961	4703±395	1.7±0.1	1.7±0.1	22.6±1.1	114.0±9.6	761±81	26.0±1.3
Winter	1962	4182±387	1.7±0.1	1.4±0.1	26.4±1.5	70.0±7.8	465±60	25.7±2.4
Winter	1963	2755±393	1.6±0.1	1.0±0.1	20.0±0.1	73.6±8.5	351±59	25.7±1.4
Dalmatia								
Spring	1960	6081±766	1.2±0.1	1.4±0.1	20.0±1.0	103.0±8.0	671±76	20.0±1.0
Summer	1961	4718±697	1.1±0.1	1.5±0.1	19.6±1.3	119.7±11.3	632±68	18.4±0.4
Winter	1963	1140±316	0.9±0.1	0.7±0.1	14.2±0.7	42.4±4.3	226±18	19.3±2.6

Discussion

The 7-day food weighing method used in the present study is relatively expensive and time-consuming but seemed to be particularly appropriate for obtaining individual dietary data in farming populations. Comparisons of the data on the heads of the families with those on the whole families show the limitations of family data on food consumption. Not only does the head of the family have a higher per capita intake of most foods than that of his family; his diet also shows much more seasonal variation, his intake of energy yielding foodstuffs being highest in the spring and lowest in the winter.

In these populations, the percentage contributions of proteins and fats to the total calories are much the same in the family diet and in the diet of the head of the family, though this fact would not be recognized if alcohol in the diet would not be recorded. Alcohol represents an important source of energy for the heads of the families in these populations, providing up to almost one forth of their total calories in Dalmatia and an average of 15 per cent during the winter in Slavonia. Many of the men in Dalmatia regularly consume far more than a liter of wine daily, and so could be classed as heavy drinkers, but except in a few cases they should not be classed as alcoholics. During work in hot weather these men have high sweat losses which they compensate by drinking a mixture of wine and water. However, it is rare to see an intoxicated man in Dalmatia on working days. In contrast, in Slavonia the alcohol consumption tends to be concentrated after work and on holidays. It is interesting that enlargement and cirrhosis of the liver is seen much more often in Slavonia than in Dalmatia.

If alcohol is neglected in the dietary survey, it would be concluded that the heads of families in Slavonia have an average of some 400 more calories per day than their counterparts in Dalmatia. Since the relative weight and body fatness tends to be the same in these two regions, the conclusion might be that the men in Slavonia have more physical activity. But when alcohol is included in the total calories the men in the two areas do not differ and it would seem that the total energy output must be similar.

Allowance for alcohol also affects the computation of the proportion of total calories provided by fats in the diet. Ignoring wine in Dalmatia would lead to the estimate of about 37 per cent of calories from fats for the heads of the families and their diet would be classed as high in fat. When the proper calculation is made, including alcohol calories, the figure is 29 per cent fat calories, and the diet is properly classed as „moderate" in fat content.

The calorie intake data in these studies undoubtedly underestimate seasonal variations in energy expenditure. It is common for these farmers to lose a considerable amount of weight during the spring and summer and to regain this weight during the autumn and winter. When weighed in the same season (September, October) the heads of families dit not show any significant change in 1963 as compared with their first examination in 1958.

Compared with city dwellers and with men in general in the U.S.A., these men in rural Croatia tend to be muscular and thin. But abundant supplies of calorie-yielding foods are generally available to them and they cannot be considered to be calorically undernourished; it would be more reasonable to suggest that urban men tend to be overfat because of lack of exercise.

In general, vitamin and mineral intakes in these surveys fail to indicate serious deficiencies. Vitamin A intakes are low in the winter in both areas but this vitamin, well stored in the body, is abundantly supplied in the other seasons. In Dalmatia the intakes of thiamine and riboflavin are low in winter. If it is proposed that 0.6 mg of thiamine should be provided for each 1000 non-fat calories, then the winter diet in Dalmatia is deficient in thiamine.

The main objective in these studies was to obtain dietary data that might be relevant to the incidence of coronary heart disease and possible differences in this regard between the two areas. It was observed that when the heads of the families are compared, the diets in the two regions were similar in many regards, though there were some differences in protein, alcohol and fats.

If data from all seasons are considered, the average difference between the areas in percentage of total calories from total fats is of doubtful significance. About half of all the individual diets in both areas are in the narrow range of 25 through 34 per cent of calories from fats. However, there is a significant difference between the regions in the frequency of dietary extremes in regard to fat. Among heads of families in Dalmatia, 26.4 per cent of all the 72 diets recorded provided less than 25 per cent of total calories from fats; the corresponding figure for 75 diets in Slavonia is 12.0 per cent. On the high fat side of the distribution, 35 or more per cent of total calories from fat, the percentages of men represented are 22.2 per cent in Dalmatia and 40.0 per cent in Slavonia. The difference between these distributions in Dalmatia and Slavonia has a chi-square value of 6.37 which is significant at probability less than $p = 0,02$

However, in regard to the concentration of cholesterol in the blood serum, it is necessary to consider the kinds of fats and their fatty acid composition. In this respect the diets of the two areas differ markedly. Up to 15 per cent of total calories is provided by lard in Slavonia while lard is only a third as important in Dalmatia; olive oil provides less than 2 per cent of the calories in Slavonia but about 15 per cent in Dalmatia. Milk and milk products are more commonly used in Slavonia; fish in Dalmatia.

These differences in the kinds of fats in the diet produce a significant difference between the two regions in the dietary intake of saturated fats. Accordingly, it would be expected that the concentration of cholesterol in the blood serum should tend to be higher in Slavonia than in Dalmatia and this expectation is borne out in fact. On the occasion of the original examinations of all men in the autumn of 1958 the medians for men grouped by quinquennia of age in Slavonia averaged 197 mg cholesterol per 100 ml of serum while the corresponding figure for the men in Dalmatia was 185.

Though there is a significant difference between these regions of Croatia, in both regions the serum cholesterol values tend to be lower than in the U.S.A. or in northern and western Europe where, in general, the diets are higher in saturated fats. Details of the blood findings and their relationship to the diet will be presented elsewhere.

Individual variability

Complete 7-day dietary data were obtained on each of three occasions from the same 14 heads of families in Dalmatia and from Slavonia there are four sets of such data for each of the same 17 men. These materials provide material for a detailed analysis of variability; the results of such an analysis are relevant to the question of further analyses of relationships between diets and other characteristics of the individuals concerned.

Table 9 gives average inter- and intra-individual standard deviations, as well as group means, for the percentages of total calories supplied by protein and by fat in the diet. For both areas combined, in any given survey about two-thirds of the values for protein for all individuals will fall in the range of about 12.1 $\pm$ 2.7, i.e. from 9.4 to 14.8 per cent of total calories supplied by protein. The corresponding figures for dietary fat are from 24.0 to 38.6 per cent of total calories. The values for variability within the individual are of much the same order of magnitude; the average intra-individual standard deviation, both areas combined, is 2.4 for protein and 7.7 for fat as percentage of total calories.

The values in table 9 do not indicate any extreme variability; a spread of about $\pm$ 20 per cent covers two-thirds of the variability in percentage protein calories and the variability in fat proportion is scarcely greater. However, on closer inspection it seems that from such data it would be very difficult to distinguish individuals. Tabel 10 summarizes the results of analysis of variance. The components of total variance contributed by consistent differences among years and

Table 9. Inter- and intra-variability in the percentage of Calories provided by protein and by fat in the diets of 14 heads of families studied three times in Dalmatia and 17 heads studies four times in Slavonia

Sample	Protein % Cal.			Fat % Cal.		
		Average S.D.			Average S.D.	
	Mean	Inter-	Intra-	Mean	Inter-	Intra-
Dalmatia	10.7	2.3	2.1	29.5	7.5	7.1
Slavonia	13.5	1.9	2.1	31.9	6.8	8.1
Both areas	12.1	2.7	2.4	31.3	7.3	7.7

even among individuals turn out to be much less important than the variability of the individuals. For protein, 57 per cent of the total variance is the result of variability within the individuals, unaccounted for by any trend among all the individuals. The corresponding figure for fat percentage of the total calories is much greater, 77.9 per cent.

Table 10. Source of variability in percentage of Calories from proteins and fats in the diets of family heads in Dalmatia and Slavonia
Tabulated values are percentages of total variance

Variability source	Dalmatia		Slavonia		Both areas	
	Protein	Fat	Protein	Fat	Protein	Fat
Year	17.7	6.3	13.7	–2.7	12.7	6.0
Inter-variability	32.4	15.6	–5.7	28.7	30.3	16.1
Intra-variability	49.9	78.1	92.0	74.0	57.0	77.9
Total	100.0	100.0	100.0	100.0	100.0	100.0

It may be argued that the findings in table 10 reflect relative constancy of the regional dietary pattern, with only small consistent differences between individuals, and that the intra-individual variability is not unduly great in absolute terms. But in any case the implication is plain: attempts to discover and evaluate relationship between these dietary variables and other characteristics of the individuals in such study populations will almost certainly lead to frustration or error or both. It makes no difference whether the variability observed within the individuals is true variability of the individual or simply a result of errors in recording food amounts and in the food composition tables used in computation. Unless interindividual variability is consistently large in comparison with intra-individual variability there is little justification in attempting to analyse relationship among individual characteristics. The data in table 10 clearly indicate that any fundamental relationship that may exist, for example, between serum cholesterol level and the proportion of fat or of protein calories to total calories cannot be effectively analysed on the basis of such dietary data as characterize these population samples.

Summary

Random samples of all men aged 40-59 in two defined rural areas in Dalmatia and Slavonia were selected for 7-day measurements of all food and beverages consumed in each of four seasons. In the first survey family diets were

measured but in subsequent surveys the diets of the heads of families were recorded separately. Nutrient intakes were computed from tables of food composition including data from local analyses and from chemical analysis of local food composites. The data show the need to record alcohol in such surveys.

Heads of families consumed more nutrients per capita than their families but the diets were similar in nutreint composition. Seasonal variations were small except for the family heads whose caloric intakes reflected seasonal farm work. Intake of protein was lower and that of alcohol was higher in Dalmatia than in Slavonia. The two areas differed little in percentage of total calories from fats but olive oil was dominate in Dalmatia while lard was the main fat in Slavonia so fatty acid patterns in the diets were different. Intra-individual variability accounted for most of the total variance in percentage of total calories from proteins and fats.

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DIETARY SURVEYS IN RURAL YUGOSLAVIA

II. Chemical analyses of diets in Dalmatia and Slavonia [1]

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Introduction

The theory that the diet is important in the etiology of coronary heart disease (C.H.D.), originally suggested by experimental findings on animals, has received indirect support from research on man himself that points to a connection between the diet and C.H.D. through cholesterol level in the blood serum. Follow-up studies consistently show that the risk of developing C.H.D. is directly related to the concentration of cholesterol in the serum (5, 6, 15, 17), while controlled dietary experiments on man show that the serum cholesterol level is strongly influenced by the lipids in the diet (1, 10, 16). Much epidemiological evidence indicates that populations subsisting on diets differing in fat content tend to differ in the average serum cholesterol level and in their susceptibility to C.H.D. However, the dietary data from these surveys are generally deficient in regard to details on the fatty acids which, in experimental studies, have the main effect on the blood lipids (14). It was against this background that the surveys reported here were carried out.

In connection with studies on the epidemiology of C.H.D., substantially all men aged 40-59 were examined in two rural areas of Yugoslavia, one in Dalmatia and one in Slavonia (3). The results confirmed indications from vital statistics and clinical impressions that C.H.D. is less prevalent in Dalmatia than in Slavonia, though even in the latter area this disease is less common than in some other areas of Europe and in the United States. Dietary surveys, carried out on random samples of the men examined in these epidemiological studies, showed that the diets in these two regions differed little in the percentage of calories provided by total proteins and total fats but as expected from previous studies in Yugoslavia (2) there were notable differences in the kinds of fats used in the diets. The present paper reports the fatty acid patterns of these diets as found by direct chemical analysis.

Sample and methods

The dietary surveys reported here, carried out in the winter of 1963, covered random sub-samples of men in the C.H.D. epidemiological study. The food intakes of 25 family heads in Slavonia and 24 in Dalmatia were weighed for seven consecutive days and the volume of all beverages except water was measured. At the end of the week the totals of each foodstuff were computed for each subject

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and corresponding proportions of those foods were obtained locally to make up an equivalent composite aliquot for each man. These equivalent composites were cooked in a sealed glass preserving jar, homogenized, lyophilized, sealed under nitrogen and sent for analysis to the Laboratory of Physiological Hygiene of the University of Minnesota.

Water was determined by heating to constant weight in vacuum at 60 °C and ash by ignition at 600 °C. Protein was determined by digesting a 4 g sample with H_2SO_4 and a catalyst in a 500 ml Kjeldahl flask followed by distillation of ammonia from an aliquot in a micro-Kjeldahl still and titration of the ammonia collected. The extraction of lipids was made by a liquid—liquid extraction procedure in which the pulverized mixed foods are suspended in 0.15 N HCl in a mixture of one part water and four parts ethanol and this mixture is extracted with an organic solvent mixture of equal volumes of ethyl ether and petroleum ether. The extracted lipids are purified by evaporating to dryness and redissolving in petroleum ether, the insoluble matter being considered non-lipid.

The fatty acids of the diet were converted to methyl esters by heating about 1 g of homogenate with 10 ml of methanol and 0.5 ml of sulfuric acid in a sealed container for 15 hours. Ten ml of water was added, the methyl esters were extracted into petroleum ether and the solvent was evaporated by passing ntrogen through the container. About 0.2 microliter portions were analyzed by gas liquid chromatography. The columns, stainless steel tubes 10 feet long and ⅛ inch outside diameter, contained ethylene glycol adipate polyester (stabilized, Analabs type C1) 5 to 35 per cent coated on Anakron AS (60 to 80 mesh) solid support. The column temperature was between 200 and 230 °C; temperature and helium flow being adjusted to give a retention time of 9 min. for methyl oleate. A thermal conductivity detector was used and the peak areas were evaluated from the tracing of a disc integrator. A standard methyl ester mixture which contained nearly equal amounts of stearate and oleate was analyzed daily to control reproducibility. The efficiency of separation of components was controlled by requiring that the notch between stearate and oleate peaks was always less than 10 per cent of the peak heights. The square root of molecular weight of each methyl ester was used as weighting factor in converting peak area into weight per cent. Components with retention times shorter than that of methyl decenoate were neglected in evaluating the tracings.

Results and discussion

The results of the chemical analysis of food equivalent composites are summarized in table 1. They show an average total calorie intake, as well as intake of proteins, fats and carbohydrates higher among these men in Slavonia as compared with their counterparts in Dalmatia. Except for the total calorie intake, this is in agreement with previously published data obtained by calculation of nutrient intake from food composition tables (3). The quantitative data on the daily consumption of energy-yielding nutrients per capita vary to a certain extent from the results obtained from food tables; these differences are discussed in more detail in a separate paper dealing with the comparison of various methods used in our dietary surveys (4).

The difference in the total calorie intake between the two areas as shown in table 1 does not indicate the complete picture since alcohol consumption, which is higher among the heads of families in Dalmatia and makes up to a large extent for the difference in calorie intake of these two groups as already reported (3), was not allowed for in the chemical analysis of equivalent composites. The results in table 1 do, however, confirm that the averages proportions of daily calories supplied by proteins and by total fats do not differ in these areas.

Table 1. Intake of calories and energy-yielding nutrients (grams per person a day), mean ± standard error and their percentage contribution to total energy. Percentages of total fatty acids are given in parentheses. Results of chemical analysis (laboratory prepared samples)

		Slavonia (N = 25)	Dalmatia (N = 24)
Total calories		2976 ± 154.3	2482 ± 107.6
Carbohydrates	g	399 ± 25.1	333 ± 15.5
Proteins	g	101 ± 5.3	87 ± 6.0
Total lipids	g	109 ± 6.7	89 ± 6.7
All saturated fatty acids	g	44.8 ± 3.0 (44.8 %)	25.5 ± 2.5 (31.4 %)
Saturated fatty acids 12 : 0 – 17 : 0	g	30.7 ± 2.1 (30.8 %)	17.8 ± 1.6 (22.1 %)
Saturated fatty acids 18 : 0	g	13.2 ± 1.0 (13.1 %)	7.4 ± 1.0 (8.9 %)
Monoene	g	43.7 ± 2.8 (43.7 %)	37.3 ± 2.8 (45.8 %)
All poly-unsaturated fatty acids	g	11.2 ± 0.9 (11.4 %)	18.8 ± 2.3 (22.7 %)
Linoleic acid	g	7.4 ± 0.9 (7.6 %)	15.5 ± 2.3 (18.5 %)
Percentage of total calories			
Carbohydrates		53.3	54.0
Proteins		13.8	14.0
Total lipids		32.9	32.0
All saturated fatty acids		13.6	9.1
Saturated fatty acids 12 : 0 – 17 : 0		9.3	6.3
Saturated fatty acids 18 : 0		4.0	2.6
Monoene		13.3	13.4
All poly-unsaterated fatty acids		3.4	6.9
Linoleic acid		2.2	5.7

However, there is an important difference in the chemical composition of the dietary lipids. Saturated fats, whether expressed as intake in grams per day or as percentage of total daily calories, are consumed more in Slavonia than in Dalmatia, whereas the reverse holds for the intake of poly-unsaturated fatty acids. There is a much smaller difference in the intake of mono-enic fatty acids. These differences are primarily the result of a higher consumption of lard, meat (particularly pork) and milk in Slavonia and of olive oil and fish in Dalmatia. Lard furnishes 10 per cent of daily calories in Slavonia and 4.4 per cent in Dalmatia whereas vegetable oil furnishes 11.3 per cent of daily calories in Dalmatia as against only 0.5 per cent in Slavonia (3).

In controlled dietary experiments on man, fats rich in saturated fatty acids raise the serum cholesterol concentration while fats high in poly-unsaturated fatty acids have the opposite effect (10,14). Roughly, the serum cholesterol effect of the mixed fats in many ordinary diets is related to the iodine number of the fats.

More detailed analysis of the effect of an isocaloric exchange of fats of known fatty acid composition in the diet, and of exchange of fats for equivalent calories in the form of carbohydrates, shows that about two grams of poly-unsaturated fatty acid counteracts the cholesterol-raising effect of one gram of saturated fatty acid while ordinary mono-enes (oleic and erucic acids) have substantially no effect or are practically the equivalent of equal calories of starch in the diet.

Recently it has been found that even this more detailed formulation of the effects of dietary fats is an over-simplification because all saturated fatty acids are not equivalent in this respect. Saturated fatty acids with fewer than 12 or with 18 or more carbons in the chain have little or no effect on the serum cholesterol level (14). This means that the cholesterol-raising effect of fats in the diet is dependent on the saturated fatty acids with 12 to 17 carbons and, in essence, the main culprits are lauric, myristic and palmitic acids. On the average, the effect on the serum cholesterol level of changing fats in an isocaloric diet which is constant in other respects is predicted:

$$\triangle \text{Chol.} = 1.3\,(2\triangle S - \triangle P),$$

where $\triangle$Chol. is the change in serum cholesterol, in mg per 100 ml, and S and P are, respectively, percentages of calories supplied by glycerides of saturated fatty acids with 12 to 17 carbons in the chain and of poly-unsaturated fatty acids (14).

It is interesting to apply this information to the data from the present study. The arithmetic mean values of the men aged 40-59 in Slavonia and Dalmatia were, respectively, 197 and 186 mg cholesterol per 100 ml of serum and the difference, 11 mg per 100 ml, is statistically significant. Now if the men in these two samples are metabolically alike and the two sets of observations may be considered to be similar to an experiment in which the same men change from one diet to another. we may write, using the data in table 1, above:

$$\triangle \text{Chol. predicted} = 1.3\,[2\,(9.3 - 6.3) - (3.4 - 6.9)] = 12.4.$$

The agreement between observation and prediction is satisfactory and it appears that the average difference in serum cholesterol level between the men in these two areas is explained by the difference in fats in their diets.

Discussion

The average serum cholesterol values reported here are in reasonable agreement, when adjustment is made for differences in methods, with data reported earlier in parts of Yugoslavia where the diets are similar (2). However, it must be emphasized that though average diets and serum cholesterol values in a given population may be reasonably constant, much intra-individual variability in both variables is found when measurements are repeated on the same individuals (9, 10).

Group averages for serum cholesterol seem to be reasonably closely related to the average diets of the populations concerned, inter-individual differences in serum cholesterol values are only poorly correlated with individual differences in the diet. This fact is evident in all of the dietary studies in a cooperative international program of which the present study is a part. Specifically, reference may be made to previously published data from Finland (18), the Netherlands (8) and Italy (7).

Errors in the methods, both in the dietary and blood analyses, may account for some of the discrepancies between observation and theory when individuals are considered. But controlled dietary experiments show that there are intrinsic differences between individuals in their average serum cholesterol level on the same diet, as well as spontaneous variations from time to time (10). Though such variability is unexplained, it is not entirely capricious; when it is carefully examined it appears that there are consistent differences between individuals in regard to their intrinsic cholesterol metabolism (11, 13).

Besides fatty acids in the diet, account should be taken of the dietary cholesterol in an exact analysis of the relationship between the diet and the serum cholesterol level. Precise data on the dietary cholesterol intake are not available from the surveys reported here, though it seems almost certain that the diet provided more cholesterol in Slavonia than in Dalmatia. However, from the results in controlled dietary experiments (12) the expected effect on the serum level would be so small that it may be disregarded in the present analysis. In the diets eaten in these regions of Croatia it may be estimated that a difference of 100 mg of cholesterol in the average daily diet would produce an average serum cholesterol concentration of less than 2 mg per 100 ml (12).

Summary

Seven-day dietary studies were made on random samples of middle-aged men (heads of families) in rural Dalmatia (N = 24) and rural Slavonia (N = 25). Replicate composites of all foods eaten by each man were chemically analyzed in a central laboratory. Average values for calories, carbohydrates, proteins and lipids consumed were somewhat higher in Slavonia than in Dalmatia (20 per cent for calories, 16 per cent for proteins, 22 per cent for lipids) but the percentages of calories from the several nutrients were not statistically different in the two areas. The fatty acid compositions of the diets differed, corresponding to the different sources of fats dominant in the two regions, the Slavonian diets being substantially higher in saturates and lower in poly-unsaturates than the Dalmatian diets. Average serum cholesterol values differed between the two regions and the differences corresponded to expectations from controlled dietary experiments.

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DIETARY SURVEYS IN RURAL YUGOSLAVIA

III. Comparison of three methods [1]

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Introduction

Various methods may be used in dietary surveys of free-living individuals; all have limitations (7). Dietary interviews and recall of food habits are useful for some purposes. However, if the aim is to obtain quantitative estimates of actual nutrients consumed during the period of the survey, contemporary **measurements** of the foodstuffs eaten are essential. Within this general methodological requirement several distinctly different procedures are to be considered. Most commonly, the amounts of all foodstuffs are recorded, preferably by actual weighing, as they are served, corrections are applied for plate waste and nutrient intakes are then **calculated** by the use of tables of food composition. Presuming that the food intake records are good, the accuracy of the result depends on the exact applicability of the food tables to the particular local foods eaten. A much more laborious but obviously potentially more accurate method is to collect duplicates, identical in amount and kind, of all foods as eaten and subsequently to analyze aliquots of the **food composites**. A somewhat simpler method is to record all foods as eaten and then, at the end of the dietary survey period, to prepare an equivalent composite from the corresponding local foodstuffs which is then analyzed chemically.

These three methods — estimation of nutrient intakes by calculation from tables of food composition, by analysis of identical food composites and by analysis of „equivalent composites" — have been simultaneously applied in a seven-day survey in rural Croatia. The results are reported here.

Subjects

The subjects were 25 middle-aged men, heads of families, in rural Croatia, drawn at random from the roster of all men of prescribed age (40-59 in 1958) who had been medically examined in detail previously and who were being followed in regard to health and possible development of disease (3). The group from which they were drawn represented over 95 per cent of all men of the prescribed age in the designated geographical area. From repeated earlier contacts they, and their wives, were well acquainted with those in charge of the dietary survey.

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Methods

The men, and their wives, were carefully instructed to follow their normal dietary customs during the seven-day period of the survey and efforts were made to persuade them against altering the amount, kind, or quality of the foods eaten. Dietitans, trained in such field work, made or supervised all measurements, and food collections in the home, taking account of between-meal snacks as well as the regular meals and of recipes for the various dishes (1).

All foods eaten by the individual men were weighed and the volumes of all beverages except water were measured for seven consecutive days. Quantitative duplicates of everything eaten except alcoholic beverages and water were collected as they were consumed to make a seven-day **food composite** for chemical analysis; we term this Method C. At the end of the week the totals of each foodstuff were computed and corresponding proportions of those foods were obtained locally to provide an **equivalent composite** for separate chemical analysis; this is Method A. Finally, tables of food composition (5, 10) were used to **calculate** the nutrient intakes from the records of the various foods eaten; this is Method B.

The composites were homogenized and aliquots were prepared and chemically analyzed in central laboratories as described previously (2).

Results

The arithmetic means, standard deviations and coefficients of variation for daily intakes of calories, proteins, fats, and carbohydrates are given in table 1 Though for each of the items the means from the three methods are grossly similar, for every item Method A gave considerably higher values than either Method B or Method C; there is relatively little difference between the means from Methods B and C. The probability values in table 1 („p") show no significant differences between Methods B and C but, except for fats, the differences between Methods A and B are significant; the differences between Methods A and C are significant for calories and proteins.

All three methods indicate similar inter-individual variation; the differences between the coefficients of variation for any one item in table 1 are not significant. Among the four items considered, all three methods indicate the greatest inter-individual variability in fats and the least in calories. Taking Method A as an example, the data indicate that about one-third of the men will have average daily fat intakes outside the range from 69 to 125 g daily; one third of the men will have calorie intakes outside the range 2144 to 2954 daily.

Since all men were studied with all three methods simultaneously, the best analysis for most purposes is summarized in table 2 in which the differences between method results for each man are examined. The mean differences in table 2 are, of course, identical with those indicated in table 1, but in general the significance of those differences is much greater than indicated in table 1.

Table 1. Results from chemical analysis of equivalent composites made from local foods to match dietary records (Method A); of calculation from tables of food composition (Method B), and of chemical analysis of composites directly matching every meal (Method C)
Data from 7-day studies on 25 heads of families. S.D. = standard deviation; C.V. = coefficient of variation; p = probability of chance explanation of the difference between means

Item	Method A	Method B	Method C	p Value of Difference A—B	A—C	B—C
Calories						
Mean	2549	2235	2287	<0.01	<0.03	>0.10
S.D.	405	420	398	—	—	—
C.V.	16%	19%	17%	—	—	—
Proteins, g						
Mean	89	75	67	<0.02	<0.001	0.06
S.D.	22	15	15	—	—	—
C.V.	24%	20%	22%	—	—	—
Fat, g						
Mean	97	83	89	0.09	>0.10	>0.10
S.D.	28	29	23	—	—	—
C.V.	29%	35%	26%	—	—	—
Carbohydrate, g						
Mean	331	297	306	0.05	>0.10	>0.10
S.D.	63	58	58	—	—	—
C.V.	19%	20%	19%	—	—	—

For all items Method A gave significantly higher values than obtained with Method B or with Method C; the differences are highly significant for all comparisons except for fats with Method A versus Method C. The differences between results from Method B versus Method C are insignificant except for proteins.

Table 3 gives the coefficients of correlation for each of the nutrient items for each of the three pairs of method comparisons. All of the values for r are statistically significant but those for the correlation between Method A and Method B are highest while those for Method A versus Method C are lowest.

Table 2. Comparison of results with three methods, calculated from intra-individual differences $(SEM)^2 = (\Sigma\Delta^2)/2N$, where N, here, = 25 CHO = carbohydrates

Item	Method A—Method B Mean Δ	SEM	p	Method A—Method C Mean Δ	SEM	p	Method B—Method C Mean Δ	SEM	p
Calories	314	267.7	<0.001	262	291.3	<0.001	−52	208.6	>0.10
Protein, g	14	14.6	<0.001	22	21.1	<0.001	8	10.2	<0.005
Fats, g	14	18.0	<0.005	8	15.3	<0.050	− 6	15.5	>0.10
CHO, g	34	28.1	<0.001	25	31.2	<0.005	− 9	20.5	>0.10

Probability, p was estimated from "Student" 's t values which may be obtained from SEM and mean Δ in table 2: $t^2 = (N-1)/(2C^2-1)$, where $C = (SEM)/\Delta$.

The correlation coefficients for any one nutrient are not significantly different in any of the three method comparisons in table 3.

Table 3. Correlation between values obtained from three methods for estimating nutrient intake

Tabular values are for r = coefficient of correlation N = 25

Item	A vs. B	A vs. C	B vs. C
Calories	0.864	0.674	0.737
Proteins	0.721	0.455	0.648
Fats	0.716	0.698	0.689
Carbohydrates	0.929	0.812	0.888

It is interesting that, though the mean values of Methods B and C agree most closely, the correlations between Method B and Method C values are not significantly higher than for A vs. C and are lower in every case than for A vs. B. The explanation may be in the fact that the starting point in both Methods A and B is the same; it is the record of weights of the several foods eaten during 7 days. If the food composition tables and the chemical analyses were both free from error, the correlation should be r= 1.00, of course.

There remains the question as to why Method A systematically yielded higher values for all nutrients than either of the other methods. The explanation may have to do with the way the foods were handled. In Method A, the food items were not prepared for the dinner table before analysis, as in Method C; the foodstuffs, locally obtained, were assembled in proportion to their amounts recorded for the week, sealed in glass jars and cooked as a mass before analysis. If the food composition tables used in Method B actually correspond to „foods as eaten", they should correspond more to Method C than to Method A.

Discussion

The data and their analysis here indicate that, in general, for the nutrients considered here, the values obtained by calculations from tables of food composition are not significantly different from those obtained by the chemical analysis of replicate meals collected during the same 7-day period. And they indicate that the method of chemical analysis of equivalent composites, made up of local foods in proportion to the amounts weighed during the week, gives significantly higher values than the other method.

However, the correlation analysis indicates that the method of analysis of equivalent composites could be made fully comparable to the other methods by introducing correction constants. If Method C were taken as the reference, the corrections to be applied to Method A data would be multiplication factors of 0.90 for calories, 0.75 for protein, 0.92 for fat and 0.92 for carbohydrate. Actually, since it cannot be shown that the percentage discrepancies between Method A and Method C results differ significantly for the several nutrients, it would be justifiable to apply the same multiplication factor 0.9 to the Method A data for all four nutrient items.

Different relationships may hold for the several methods when other nutrients are concerned. For example, food composition tables for dietary fatty acids as yet lack validation comparable to that for the nutrient items considered in this paper so it would be dangerous to assume that Methods B and C would agree for specific fatty acids as well as they do for calories, total proteins, total fats, or carbohydrates.

Other groups of investigators in these cooperative investigations on the diet and heart disease have also used in parallel several methods of dietary surveys. It is interesting to examine the experiences in Finland and in the Netherlands.

In Finland, ROINE, PEKKARINEN and KARVONEN (9) compared Methods A and B, as designated here, on 20 men in a 7-day study. Excellent agreement for averages of the 20 men was obtained, the calculated (Method B) and analysis of equivalent composites (Method A) mean values, g per day, being, respectively, 121.3 and 121.2 for protein, 162.8 and 166.3 for total fats. The work in Finland used special tables of food composition developed for Finnish foods, which might explain the better agreement between Methods A and B than found in the present data from Yugoslavia. But if the tables of food composition used in Yugoslavia are less strictly applicable to the local Yugoslavian foods, it is difficult to explain the agreement observed here between Methods B and C.

In the Netherlands, DEN HARTOG et al. (6) applied Methods B and C in a 7-day study on 49 men and obtained excellent agreement between the averages from the two methods for calories, proteins, total fats, and carbohydrates, the differences, as per cent of the average of both methods, being only 2.4 for calories, 3.5 for proteins, 0.8 for fats, 4.9 for carbohydrate. On the same subjects in the Netherlands a dietary history interview and „cross check" method was used to get estimates of average food consumption over some months. Compared with Method B, this method yielded higher averages, the discrepancies being 9 per cent for calories, 12 per cent for protein, 12 per cent for fats and 2 per cent for carbohydrate.

The results mentioned above, together with the present results in Yugoslavia, by no means resolve questions as to what dietary survey method is most accurate or should be the method of choice when both accuracy and cost are considered. In regard to cost, there is increasing evidence that for many purposes 3 days of measurements may serve as well as a 7-day survey. One of us (F.F.) has obtained evidence on this point (4). In India a study on 237 families failed to show any significant advantage of 7-day as compared to 3-day surveys (8). For some purposes it may be suggested that a hybrid method, less laborious than either Methods A or C, would be satisfactory. For example, suppose it were desired to obtain reliable data on the four food items considered here and on the percentage of total calories provided by specified fatty acids. The records of food weights would be obtained and from these the calorie, protein, fat and carbohydrate values would be calculated from tables of food composition. An equivalent composite would be prepared as in Method A but the analysis would be limited to obtaining a sample of lipid extract, not necessarily a quantitative aliquot of all the lipid, and this would be analyzed for fatty acids by gas-liquid chromatography.

The present study did not compare any of these three methods with methods in which only recall or interview data are obtained. Here it is enough to note that it would be difficult to organize a study that would make a strictly valid comparison of this sort. Ideally, we demand that both methods be applied to the same individuals at the same time. But if the records are kept of the actual weights of foods concerned, the very fact of keeping those records would certainly affect the subsequent recall. The result would not be applicable to the situation in which only recall or interview is the method.

One alternative, in which all the foods are weighed and recorded as eaten but without any knowledge of this on the part of the subject, could be attempted in some very special situations, for example in a prison, but even so the results might be applicable only to that special situation and not to ordinary life at home. The other alternative would be to study a large group, subdivided into two carefully matched groups each then being studied simultaneously but independently by the two methods. The resulting statistical comparison might be valid for the means and such items as the standard deviation about the means, etc., but nothing would be learned about the distribution of intra-individual variations between methods.

It is beyond the scope of this paper to examine the question of the validity of any of these methods in providing data on the long-time, spontaneously eaten diets unaffected by any influence of the dietitians and the necessary arrangements to measure food intake. With the precautions used here, there is no evidence that the data obtained are not truly representative for the individuals concerned.

Speculation is easy but not necessarily useful.

Summary

Three methods were applied to a seven-day dietary survey of 25 heads of families drawn at random from a designated rural area in Croatia, Yugoslavia: A. all foods were weighted at all meals, at the end of the week for each subject a proportional composite replicate was prepared from local foods and this was analyzed chemically; B. calories, proteins, fats and carbohydrates were calculated from the food records using tables of food composition; C. a quantitative duplicate of each meal eaten was collected at the time it was eaten; this 7-day food composite was homogenized, sampled and analyzed chemically.

The results from the three methods, as calories, proteins, fats and carbohydrates daily were correlated best for carbohydrates ($r = 0.81$ tot 0.92), poorest for protein ($r = 0.46$ to 0.72). For all nutrients Method A gave significantly higher mean values than Methods B and C, these latter being similar to each other. By all three methods inter-individual differences were smallest for calories, largest for fats.

With a correction factor applied to Method A, all three methods, as applied in these studies, seem to be equally reliable for the nutrients considered here. The significance of these findings for dietary survey work is discussed.

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DIETARY STUDIES IN CONNECTION WITH EPIDEMIOLOGY OF HEART DISEASES: RESULTS OF SURVEYS IN CZECHOSLOVAKIA *)

by K. OŠANCOVA and S. HEJDA

The mortality and morbidity from cardiovascular diseases and their rising trend in Czechoslovakia (table 1) are, similarly as in other countries, of great concern to health workers. Therefore in recent years attention was paid to differences in the incidence of cardiovascular affections in different parts of the country, in relation to environmental factors which may have a bearing on the development of these conditions.

Table 1. Mortality from cardiovascular diseases in Czechoslovakia per 100,000 population

Year	1937	1946	1960	1961	1962
	247.5	271.4	306.5	313.0	354.5

In the present paper we wish to report the results of dietary studies from three rural areas — a mountainous area, a hilly area and a prosperous village in the lowlands (fig. 1) which according to the results of clinical investigations (3) differ considerably as regards the incidence of atherosclerosis. The highest incidence of atherosclerosis and some indicators usually accepted to suggest the development of atherosclerosis, were most frequent in the population of the lowlands, least frequent in the mountainous area, the hilly area being intermediate. In these areas detailed nutrition and dietary surveys were conducted in order to reveal possible differences in dietary habits.

Methods and material

In the three areas nutrition surveys were carried out covering the entire population. In the present paper we shall, however, submit only the results pertaining to matched groups of adults performing "medium heavy" physical work according to our classification. We are, of course, aware of the fact, that the population in the mountainous area is subjected to a greater physical strain imposed by the mountainous countryside and a less dense network of transport facilities.

The total number of subjects was 428, the greater part being women.

The average age was 41-44 years, the differences in the three groups not being statistically significant. The groups under investigation were subjected to clinical examination and their heights and weights were assessed. The food consumption of the investigated groups was assessed by means of the inventory method in the modification used at our Institute (1). The field worker visits the selected families

*) These surveys were not strictly a part of the program represented by the other papers and precise comparability cannot be assured on that account.

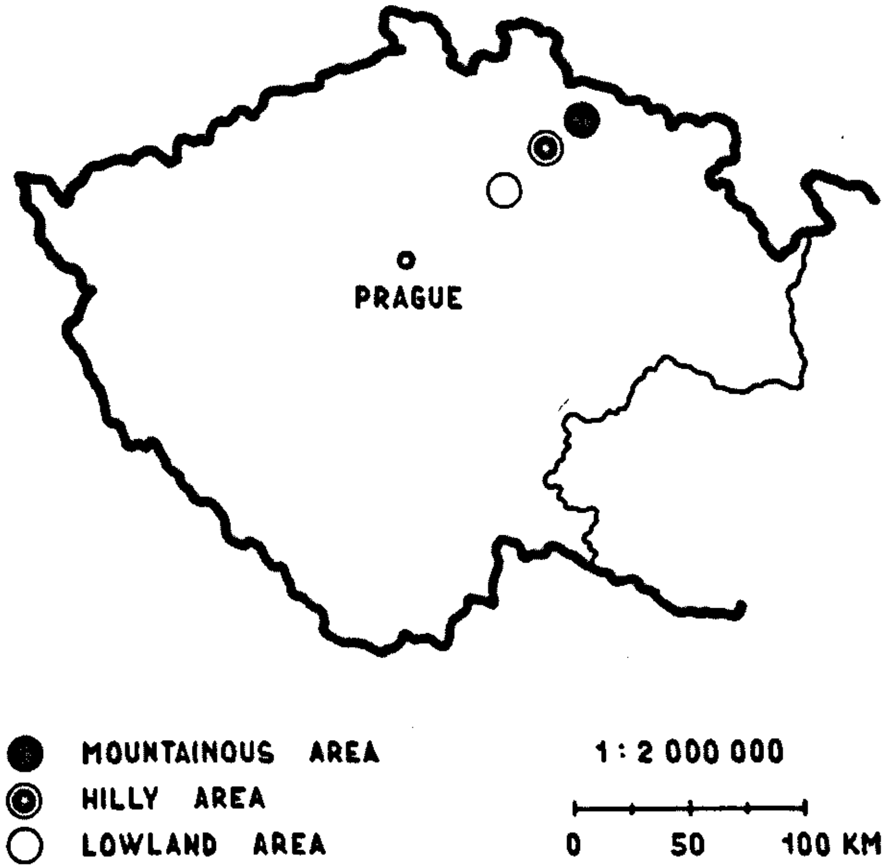

Fig. 1. Map of Bohemia with surveyed areas

and for a period of 10-14 days she records the food consumption and amount of prepared dishes and their distribution among individual members of the family. At the same time she keeps records of food eaten outside the home and of the amount of plate waste. From the above data the energy and nutrient intake are calculated from food tables (6).

Results

The highest calorie and nutrient intakes were found in the lowlands, the lowest in the mountainous area. The caloric intake, total protein and fat consumption are compared in figures 2, 3 and 4. When comparing the two extreme areas — lowlands and mountains — it is typical that the greatest difference is in the fat intake (40 and 45 % resp. in men and women), while the difference in caloric intake is only 21 and 15 % and in protein 31 % in men and only 12 % in women.

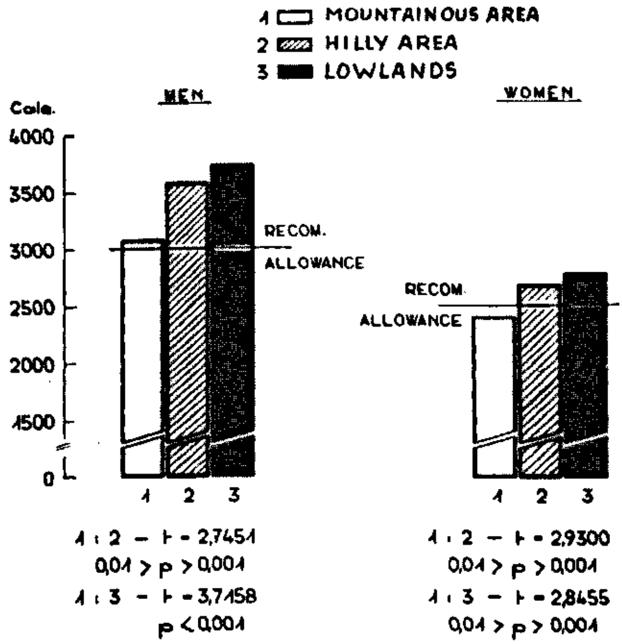

Fig. 2. Caloric intake in investigated areas

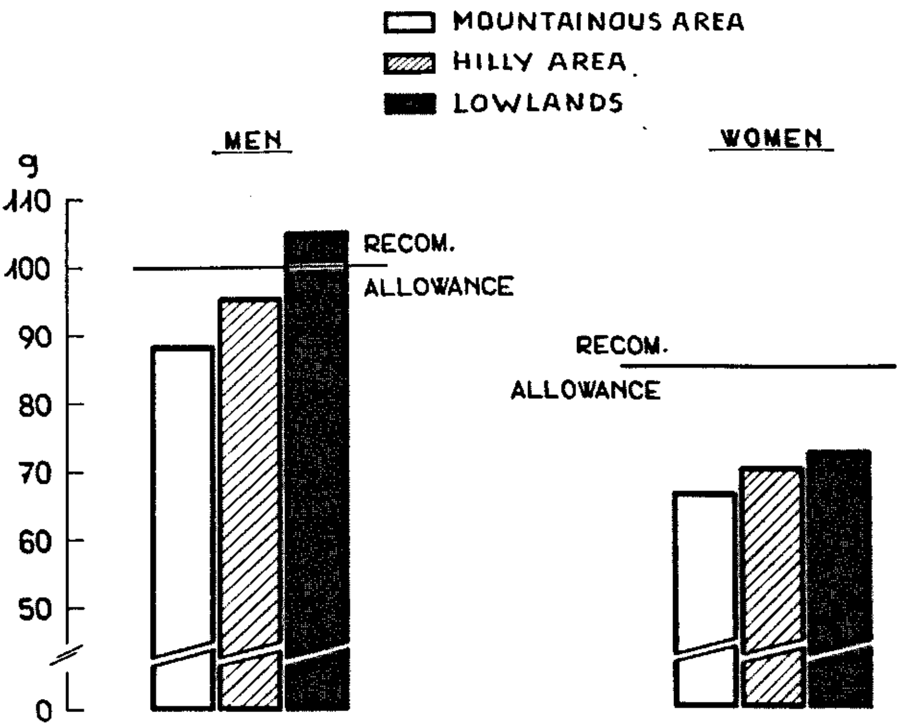

Fig. 3. Total protein consumption in investigated areas

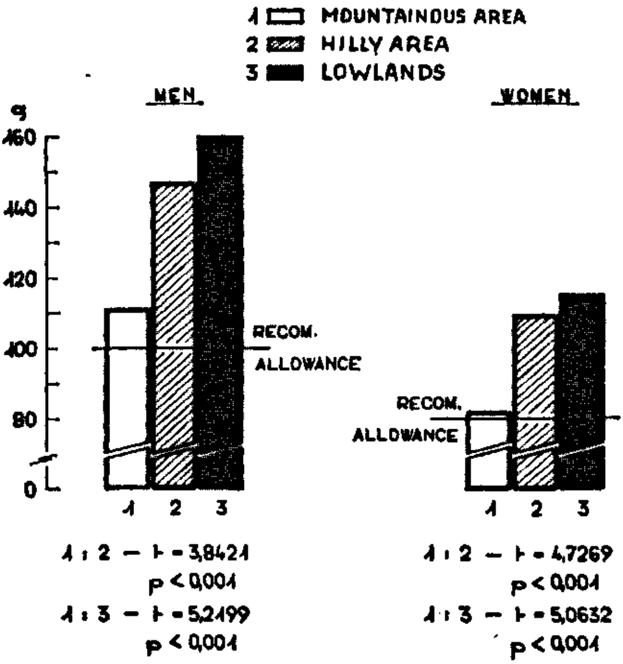

Fig. 4. Total fat intake in investigated areas

The differences in the caloric and fat intakes in both sexes are statistically significant if we compare the population from the mountains with the two other groups. The high consumption of fats in the lowlands is the most typical feature by which the diet differs from that in the two other areas, as apparent from tables 2, 3, 4. The figures for the fat intake in the lowlands are very high for this country and are usually encountered only among miners or other population groups performing very hard labour.

Table 2. Calories and nutrients of the diet in investigated population groups, per head per day

		MEN (Medium work)		
		Lowlands n=63	Hilly Area n=66	Mountains n=65
Calories		3742	3593	3083
Total protein	g	105.1	95.5	88.5
Animal protein	g	55.7	46.4	42.2
Total fat	g	160.3	147.1	111.7
Animal fat	g	127.9	130.3	91.3
Carbohydrates	g	473.6	475.6	434.3
Calcium	mg	650.0	783.0	726.0
Iron	mg	14.75	12.38	12.49
Vitamin B_1	mg	1.57	1.54	1.48
Vitamin B_2	mg	1.38	1.31	1.28
Niacin	mg	22.97	17.61	17.06

Table 3. Calories and nutrients of the diet in investigated population groups, per head per day

		WOMEN (Medium work)		
		Lowlands n=82	*Hilly Area* n=72	*Mountains* n=79
Calories		2771	2720	2395
Total protein	g	72.6	70.7	66.4
Animal protein	g	35.9	34.1	32.0
Total fat	g	115.1	110.7	81.7
Animal fat	g	89.5	96.1	64.3
Carbohydrates	g	368.4	365.1	352.6
Calcium	mg	596.0	643.0	647.0
Iron	mg	11.05	9.13	8.58
Vitamin B_1	mg	1.19	1.15	1.10
Vitamin B_2	mg	1.10	1.05	1.03
Niacin	mg	16.03	12.80	12.28

Table 4. Percentages of Calories derived from protein, fat and carbohydrates

	Mountains		Hilly Area		Lowlands	
	Men	Women	Men	Women	Men	Women
Protein	11.4	11.0	10.6	10.4	11.2	10.4
Fat	32.5	30.5	36.8	36.6	38.4	37.0
Carbohydrates .	56.1	58.5	52.6	53.0	50.4	52.6

Percentages of animal protein from total protein intake

	Mountains	Hilly Area	Lowlands
Men	47.7	48.7	53.0
Women	48.2	48.2	49.5

The structure of the animal protein consumed in the three regions is illustrated in figure 5. From the figure the predominance of meat protein in the lowlands is apparent at the expense of milk protein, while in the mountains the position is reverse. Important seems also the difference in the consumption of visible fats as apparent from figure 6.

The plainer character of the diet in the mountains is also reflected in the higher ratio of calories derived cereals (table 5).

Table 5. Percentage of Calories derived from cereals

	Mountains	Lowlands
Men	44.9	42.2
Women	44.0	39.8

Among environmental factors which according to some authors have a bearing on the incidence of cardiovascular diseases is the hardness of drinking water (2, 4, 5). In order to test whether this also holds in the areas investigated, some 30 samples of drinking water were collected in each area and the calcium content was estimated. The results are listed in table 6. From the data it is apparent that there are marked differences in the three areas.

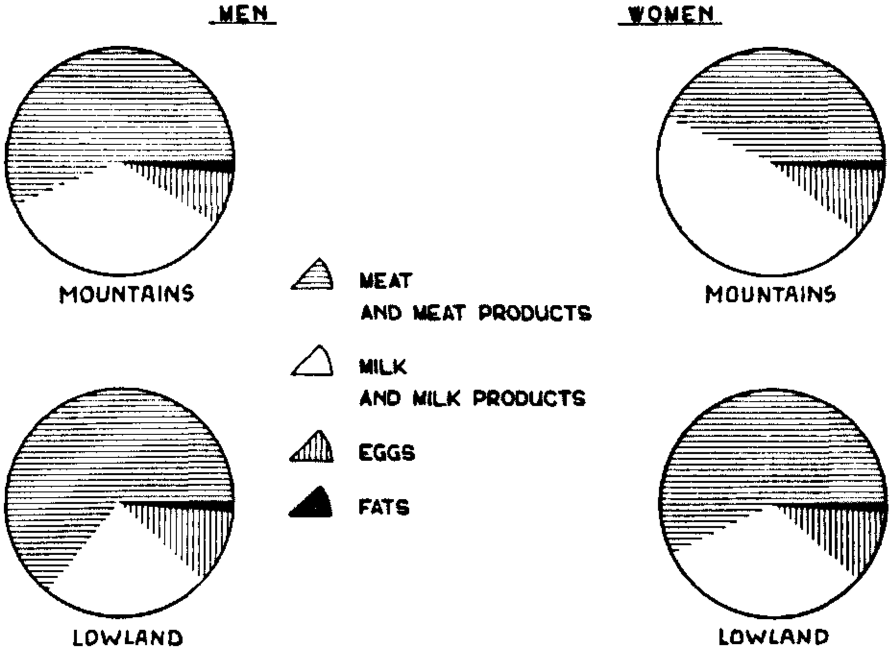

Fig. 5. Structure of animal protein intake in the mountainous area and lowland area in %

Table 6. Calcium content of drinking water, mg/l
(average figures from ca. 30 samples)

Mountains	Hilly Area	Lowlands
n=24	n=28	n=23
31.6	105.8	179.4

However, contrary to the results reported by MORRIS and SCHROEDER, the calcium content is highest in the lowland area with the comparatively highest rate of cardiovascular diseases and lowest in the mountains. Some preliminary tests were also made to assess the calcium content of milk from the three areas where the differences of Ca content were, however, slight (table 7)) but in reverse order. Further investigations will be needed before an attempt can be made at evaluating these results.

Table 7. Calcium content of milk, mg/l

Mountains	Hilly Area	Lowlands	Dairy Milk, Prague
135.6	115.7	89.2	123.6
125.6	124.4	109.3	
		106.0	

Discussion

To elucidate the possible effect of dietary factors on the incidence of cardiovascular affections accurate records of food intake are an essential prerequisite. This is why for the present investigation a detailed survey in a smaller population sample was preferred to a more extensive investigation where less accurate methods would have to be applied. The inventory method which has been used for many years in this Institute gives, as has been proved, sufficiently accurate results for this work. As regards the dietary intake of the groups investigated its adequacy is compared with recommended nutrient allowances adopted for Czechoslovakia (table 8).

Table 8. Recommended nutrient allowances. Institute of Human Nutrition, Prague

		Proteins					Vitamins				
	Cal.	Total g	Anim. g	Fat g	Ca mg	Fe mg	A mg	B_1 mg	B_2 mg	Niacin mg	C mg
Men											
Sedentary work	2600	90	45	85	800	14	5000	1.30	1.80	13.0	75
Hard work	3000	100	50	100	800	14	5000	1.50	2.00	15.0	75
Very hard work	3500	120	50	115	800	14	5000	1.75	2.20	17.5	75
Miners	4000	120	50	170	800	18	6000	2.00	2.20	17.5	100
Above 65 years	2450	80	40	70	800	14	5000	1.25	1.60	12.5	60
Women											
Sedentary work	2300	75	35	70	800	14	5000	1.15	1.50	12.0	75
Hard work	2500	85	45	80	800	14	5000	1.25	1.60	12.5	75
Very hard work	3000	100	45	100	800	14	5000	1.50	2.00	15.0	75
Pregnancy	2800	100	50	90	1500	14	6000	1.70	2.00	16.0	100
Lactation	3000	110	60	100	2000	14	8000	1.80	2.20	17.0	150
Above 65 years	2000	65	35	55	800	14	5000	1.00	1.40	10.0	60
Children and adolescents											
0–1 years	1000	35	30	35	800	6	1500	0.60	0.80	6.0	30
1–3 years	1300	50	30	45	1000	8	2000	0.65	1.00	6.5	35
3–7 years	1700	65	45	60	1000	10	2500	0.85	1.25	8.5	50
7–11 years	2200	85	55	80	1200	12	4000	1.10	1.65	11.0	60
11–15 years:											
boys	3000	115	65	115	1300	15	5000	1.50	2.25	15.0	70
girls	2600	100	60	85	1200	15	5000	1.30	1.95	13.0	80
15–18 years:											
boys	3600	135	75	125	1300	15	5000	1.80	2.70	18.0	80
girls	2400	90	50	80	1200	15	5000	1.20	1.80	12.0	80

From a comparison with the recommended allowances it becomes apparent that in men the caloric intake is somewhat excessive, while the recommended allowance of animal protein is not met by all groups; the same applies to the calcium and riboflavin intake. The fat intake is higher than recommended in all three groups of men. In women the caloric intake is above the recommended level in two groups, but somewhat below the recommended standard in the mountainous area, the same being true of fats. On the other hand, the calcium and riboflavin intake are lower than recommended. The different energy value of the ingested diet and moreover the assumed higher energy output in the

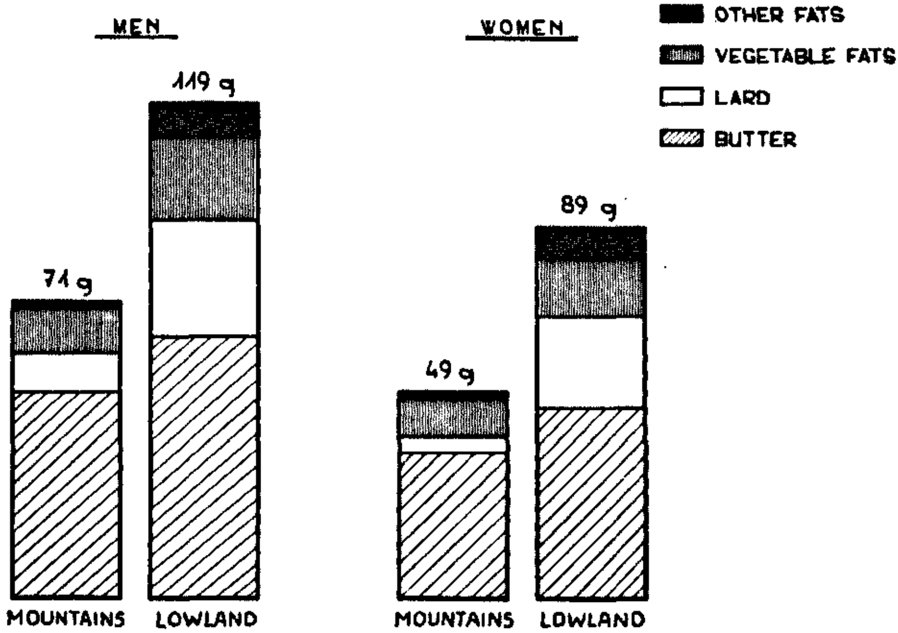

Fig. 6. Composition of visible fat intake in the mountainous and lowland area

mountains is reflected by the incidence of obesity in the three areas (3) listed in table 9, the criterion taken being 10 % above the "ideal weight" according to Broca's formula.

Table 9. Incidence of overweight in investigated areas
Percentage of population above 110 % of "ideal weight"

	Mountains	Hilly Area	Lowlands
Men	3	37	42
Women	20	39	52

It deserves mention that in our country we find in general only very slight differences in the food consumption of different population groups and in different areas, and it is thus usually difficult to correlate the dietary intake with indicators of the health status. Despite the fact that the investigated three areas are only a few tens of miles apart, the differences in the energy value and composition of the diet are significant and we may thus assume that the diet may be one of the factors which participate in the process of atherogenesis in these population groups.

Summary

In the present paper the authors compare the dietary intake of three fairly close

areas in Czechoslovakia with a different incidence of atherosclerosis. Detailed dietary surveys revealed:

1. significant differences in the energy value and fat content of the investigated areas, the highest incidence being in the lowlands with the highest energy and fat content of the diet;
2. a different structure of the animal protein intake, the main difference being a higher intake of milk protein in the mountains and a lower intake of meat protein as compared with the lowlands;
3. the amount and composition of the visible fat intake differs, the greatest difference being the total amount of fat consumed and the much larger proportion of lard consumed in the lowlands;
4. investigations of the calcium content of water which according to some authors has an influence on the incidence of atherosclerosis revealed the highest Ca content in the lowlands and the lowest in the mountains, and require further exploration before any conclusions can be made.

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DIETARY STUDIES IN CONNECTION WITH EPIDEMIOLOGY OF HEART DISEASES: RESULTS IN SERBIA *)

by B. DJORDJEVIC, B. SIMIC **), A. SIMIC, T. STRASER, V. JOSIPOVIC, V. MACAROL, L. KLINC, S. NEDELJKOVIC, with the help of the statistician P. TODOROVIC

Introduction

In the course of studies of the epidemiology and aetiology of degenerative diseases of the cardiovascular system carried out with national teams of Italy, the Netherlands, Finland, Greece, S.R. Croatia and in collaboration with KEYS who unifies the whole work, and in addition to our previous research work on this problem in Serbia (8, 9), we have undertaken another wide longitudinal study in 1962/64.
This research was carried out in Sumadija, Vojvodina and in Beograd with the aim to examine the health state of three groups of the population. In the first plan was the assessment of correlation between nutrition and the incidence of degenerative diseases of the cardiovascular system. In this paper we shall present only the data connected with nutrition, anthropometric characteristics and the serum cholesterol level as obtained during this first examination. The data regarding the prevalence of cardiovascular diseases, and other data showing the state of health of the examined groups, are presented in a separate article (4). Thus, these reports connected with nutrition, anthropometric characteristics and serum cholesterol level are a part of an international survey of the epidemiology of degenerative diseases of the cardiovascular system.

The population studied

The study comprises only men from 25 to 65 years of age; 706 from Velika Krsna (Sumadija), 720 from Zrenjanin (Vojvodina), and 654 from Beograd — 2080 persons in all. The random sampling method was not applied as all the male inhabitants of the village Velika Krsna were examined, at Zrenjanin all the male workers of the factory "Servo Mihalj" and in Beograd from the total number of University teaching staff only men over 40 were examined.
Of the inhabitants examined in the village Velika Krsna over 90 % were peasants whose average caloric requirements, according to the Yugoslav Commission for Nutrition, are about 4200 calories, and the average caloric requirements of the workers at Zrenjanin, with regard to their way of life and work, are 3600 calories, while the caloric requirements of the university professors, because of their mental work mainly in a sitting position, according to the recommendations of the same Commission are not more than 2800 calories (7).
Bearing in mind the different requirements of these three groups of the population and their different living and social conditions, we have planned to follow the

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*) These surveys were not strictly a part of the program represented by the other papers and precise comparability cannot be assured on that account.
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general state of health, and particularly the incidences of degenerative diseases of the cardiovascular system in these groups of the population.

At the first examination of the 2080 persons, clinical, electro-balistrocardiographic, anthropometric and laboratory examinations (cholesterol, glucose in blood and urine) were carried out by the same method as set by the plan for international collaboration on the research of epidemiology of cardiovascular diseases (2).

A survey of the nutrition was also carried out at Velika Krsna in October 1962 and in May 1963; at Zrenjanin in September and November 1963; and in Beograd in January and February 1964. The survey was carried out by senior medical students supervised by medical nutritionists.

Method

Food analysis

A dietary survey of the selected groups was carried out at the same time as the medical examination. Namely, from the total number of examined men 41 peasants were chosen by random method at Velika Krsna, 44 industrial workers at Zrenjanin, and 41 university professors in Beograd. Each survey lasted for seven consecutive days and was carried out by the precise weighing method of the food consumed by each of the persons under survey. If any of the persons had taken a meal out of his home the quantity of food was recorded and taken into account when the total food intake was calculated. Only the edible parts of raw food were weighed. According to the data obtained by weighing the food consumed in the course of seven days the average value of the daily intake of each kind of food was calculated separately. After that, the quantities corresponding to each kind of food for each person were taken from the corresponding households, homogenized and freeze dried.

In the food samples obtained in this way the following were determined: the dry residue, ash, proteins, fats, glycerides, unsoponifable matters, phospholipids and caloric value of the total daily food intake (the analyses were made in the Institute for Public Health of SR Serbia) (5). In the lyophilized samples of food the content of fatty acids was determined by applying gas liquid chromatography. This was done at the Institute of Physiology of the University of Naples (Prof. F. Fidanza) and in the Laboratory of Physiological Hygiene, University of Minnesota, USA (Prof. A. Keys).

As the analyses of fatty acids are still being made, on this occasion we shall present only the other results of chemical analyses of food samples.

Anthropometric assessment

The body weight was taken by a medical decimal weighing machine, and the body height by an anthropometer. The persons whose body weight was taken had only their underwear on. The relative body weight is shown as a percentage of the ideal body weight calculated according to the formula of DEMOLE (3). The skinfold measurements were taken in the middle of the right upper arm (triceps) and the subscapular. According to the triceps value the content of body fat was assessed by the formula KEYS — BROZEK (1). The thickness of skinfold was taken by the large skinfold Caliper at the pressure of 10 g/1 m^2.

Blood assessment

Total cholesterol serum level was determined by a modified Abell method (6). Serum samples were put on filter paper, dried and later analysed in the laboratory. In order to check the results one part of the samples was analysed in the Laboratory of Physiological Hygiene, University of Minnesota, USA (Prof. Anderson).

Results

Velika Krsna

From the data in table 1 it can be seen that the daily energy intake was 3420 ± 92.8 in October and 3560 ± 122.0 calories in May, which means that there were no great seasonal differences in calories. But there were great differences in the daily energy intake among the individuals (min. 1800, max. 5750 calories).

Table 1. Nutritive value of daily food intake in Velika Krsna
Chemical analysis

October 1962	n.	Min.	Max.	Mean	SD	SE
Calories	41	1806	5754	3421	594	92.8
Total dry residue	41	398	1186.7	803.3	179.6	28.1
Ash g	41	18.3	102.1	44.5	16.4	2.6
Proteins g	41	61.4	189.3	111.8	29.9	4.6
Carbohydrates g	41	255.9	833.8	576.2	126	19.7
Fat g	41	15.5	249.7	70.6	42.3	6.6
Glycerides g	—	—	—	—	—	—
Unsaponifable fraction	—	—	—	—	—	—
Phospholipids	—	—	—	—	—	—
May 1963						
Calories	39	1872	5079	3563	762	122.1
Total dry residue	39	409.5	1100.9	731.6	172.8	27.6
Ash g	39	15.4	51.8	31.6	8.6	1.4
Proteins g	39	77.3	175.9	121.9	27.9	4.5
Carbohydrates g	39	226.1	755.2	489.5	89	14.3
Fat g	39	37.2	214.9	113.4	46.8	7.5
Glycerides g	39	34.2	209.9	108.8	53.7	8.6
Unsaponifable fraction	39	0.27	2.53	1.04	0.51	0.08
Phospholipids	39	0.93	8.35	3.42	1.6	0.2

The daily protein intake was 111 g ± 4.6 and 121 g ± 4.5 which exceeds the recommended values. However, the composition of the daily meals shows that proteins of animal origin represent only a small percentage. The total proteins form 13 % and 14 % of the daily energy intake (table 4).
The daily fat intake was 70 g ± 6.6 in October and 113 g ± 7.5 in May, which

is 19 % and 30 % of the total daily energy intake. This difference may be explained by the fact that peasant households produce for their requirements pork fat and in October their reserves are usually exhausted.
From the data obtained by the analysis of glycerides by gas liquid chromatography (Fidanza) it can be seen that 42 % are saturated fatty acids, 37 % monoethenoid and 21 % are polyethenoid fatty acids.

Zrenjanin

From the data in table 2 it can be seen that the daily energy intake was 3490 ± 138.5 in September and 3368 ± 149.1 calories in November, which means that there was no great difference in the nutrition of the examined group in these months, but there was a great difference in the daily energy intake of individuals (min. 1770, max. 5880 calories).

Table 2. Nutritive value of daily food intake in Zrenjanin
Chemical analysis

September 1963		n.	Min.	Max.	Mean	SD	SE
Calories		44	1770	5370	3490	917	138.5
Total dry residue		44	360.5	1153.3	713.7	192.4	29
Ash	g	44	13.4	55.3	31.2	6.9	1
Proteins	g	44	59.4	198.6	114	37.8	5.7
Carbohydrates	g	44	191.5	764.9	428.4	126.2	19
Fat	g	44	52	227	139.8	47.9	7.2
Glycerides	g	44	49.2	217.3	134.7	46.1	6.9
Unsaponifable fraction		44	0.18	3.36	1.17	0.54	0.08
Phospholipids		44	0.44	8.29	2.52	1.62	0.24
November 1963							
Calories		40	2028	5882	3368	943	149.1
Total dry residue		40	432	1223	686	159.3	25.1
Ash	g	40	16.8	65.2	31.3	9.8	1.54
Proteins	g	40	62.5	142.3	109.6	20.1	3.18
Carbohydrates	g	40	254.4	616.8	404.9	108.5	17.1
Fat	g	40	63.9	255.5	135.3	75.1	11.9
Glycerides	g	40	39.1	252.8	132.5	52.1	8.2
Unsaponifable fraction		40	0.42	2.25	0.946	0.38	0.06
Phospholipids		40	0.07	0.5	0.197	0.11	0.02

The daily total protein intake was 114 g ± 5.6 and 190 g ± 3.2 which forms 13 % and 14 % of the total calories daily intake and exceeds the recommended values (table 4). The compositon of the daily meals shows that the greater part of proteins are of vegetable origin.
The daily fat intake was 139 g ± 7.2 and 135 g ± 11.9, which in both cases is 37 % of the total calories. The content of fat in the meal of the Vojvodina group is much higher than in the meals of the Sumadija group. According to the kind of foodstuffs of which the daily meal is composed it can be concluded that the fats were mainly of animal origin. The composition of glycerides and the content of fatty acids are not yet known.

Beograd

The data in table 3 show that the daily energy intake of the university professors was 3035 ± 109 calories, i.e. far lower than the energy intake of peasants and industrial workers. Although only one survey was made, with regard to their living conditions, it can be assumed that there are no great seasonal variations in the nutrition of this group. However, there are considerable differences in the daily caloric intake among individuals (min. 1970, max. 5570 calories).

Table 3. Nutritive value of daily food intake in Beograd
Chemical analysis

January/February 1964		N.	Min.	Max.	Mean	SD	SE
Calories		41	1969	5571	3035	703	109
Total dry residue		41	367.7	1089.2	595.9	139.5	21.8
Ash	g	41	14.2	45.3	26	17	26.5
Proteins	g	41	67.7	147.4	106.3	24.7	3.85
Carbohydrates	g	41	189.8	772.2	373.3	132.4	20.68
Fat	g	41	60.3	234.2	113.1	59.2	9.24
Glycerides	g	41	59.6	232.3	112.1	32.8	5.11
Unsoponifable fraction		41	0.25	1.78	0.87	0.27	0.04
Phospholipids		41	0.04	0.96	0.138	0.47	0.07

The daily total protein intake was 106 g ± 3.8, which is 14 % of the total daily energy intake. The daily intake of fat was 113 g ± 9.2, which is 35 % of the total caloric value of the daily meal (table 4).

Table 4. Proteins, fats and carbohydrates as percentage of total daily caloric intake

			Proteins	Fats	Carbohydrates
Velika Krsna	October	1962	13	19	68
	May	1963	14	30	56
Zrenjanin	September	1963	13	37	50
	November	1963	14	37	49
Beograd	January	1964	14	35	51

The composition of glycerides is not known yet as the analyses of fatty acids have not been completed.

Anthropometric characteristics

Velika Krsna

From the data in table 5 it can be seen that the average body weight of the examined peasants increases with age, reaching the maximum value of 64.8 kg ± 9.47 in the group of 45 to 49 years, and then decreases.

If these values are compared with the average body weight of the 40 peasants

Table 5. Anthropometric characteristics of men investigated in Velika Krsna

Age group	N		Body weight kg	Body height cm	Relative B.W.	Thickness of skin-fold mm	Fat as % of B.W.
		Mean	63.54	170.81	93.84	5.15	7.05
< 39	155	SD	7.01	6.30	8.30	2.10	1.48
		SE	0.53	0.51	0.67	0.17	0.10
		Mean	64.44	169.96	91.47	6.03	8.81
40–44	136	SD	8.98	3.59	10.10	2.55	2.02
		SE	0.77	0.31	0.87	0.22	0.17
		Mean	64.78	169.93	90.27	6.59	7.93
45–49	82	SD	9.47	5.81	12.00	3.60	2.60
		SE	1.04	0.64	1.32	0.40	0.29
		Mean	62.32	167.51	86.76	6.09	7.65
50–54	138	SD	9.57	6.09	10.97	2.63	2.06
		SE	0.81	0.52	0.93	0.22	0.17
		Mean	61.52	168.39	83.72	5.86	7.52
55–59	162	SD	8.69	6.30	9.93	2.77	1.96
		SE	0.68	0.49	0.78	0.22	0.15
		Mean	61.40	165.99	87.07	7.01	8.42
60 and over	32	SD	9.92	5.72	14.48	3.59	2.51
		SE	1.75	1.01	2.56	0.63	0.44

whose food intake was examined and which was 63.7 kg ± 1.65 (table 8), it can be seen that the chosen group is representative enough. The relative body weight (table 5) shows that not a single subgroup has the ideal body weight of which the data correspond to the average relative body weight of the group of peasants whose nutrition was examined (89.7 ± 1.8). This means that the body weight of peasants was for about 10 % less than the ideal body weight.

The upper arm skinfold was 5 to 7 mm and the content of body fats from 7 % to 8.8 %.

The average body fat content in the group whose food intake was examined was 9.2 ± 0.43 (table 8).

From the data calculated on the basis of the Davenport-Kaup index in table 8 it can be seen that the number of thin persons was 40 % and the number of obese persons 15 % in the group whose food intake was examined.

Zrenjanin

From the data in table 6 it can be seen that the body weight of the examined industrial workers increases with age and reaches its maximum in the 44 age group (71.5 kg ± 0.96) and then decreases. The average body weight of all the examined industrial workers corresponds to the body weight of workers whose food intake was examined (72.4 kg ± 1.3). It is obvious that the average body weight of workers is far greater than the body weight of the examined peasants. The relative body weight shows that all the subgroups, except the persons over 60 years of age, have the ideal body weight.

Table 6. Anthropometric characteristics of men investigated in Zrenjanin

Age group	N		Body weight kg	Body height cm	Relative B.W.	Thickness of skin-fold mm	Fat as % of B.W.
< 39	197	Mean	68.21	169.46	100.59	7.90	9.03
		SD	10.54	5.94	12.84	4.56	3.52
		SE	0.74	0.42	0.92	0.32	0.25
40–44	147	Mean	71.46	167.66	103.78	9.77	10.63
		SD	11.60	5.64	16.17	5.57	4.26
		SE	0.96	0.46	1.38	0.46	0.35
45–49	99	Mean	69.90	166.59	101.46	10.10	10.04
		SD	11.74	6.86	15.54	5.93	3.35
		SE	1.18	0.69	1.56	0.59	0.34
50–54	166	Mean	68.10	164.76	98.15	9.13	9.96
		SD	10.60	6.17	12.08	4.64	3.38
		SE	0.82	0.48	0.94	0.36	0.26
55–59	99	Mean	68.89	165.86	96.47	9.80	10.73
		SD	12.41	5.79	13.87	5.31	4.01
		SE	1.25	0.58	1.40	0.53	0.40
60 and over	8	Mean	62.21	164.75	87.58	6.81	8.24
		SD	8.86	1.71	12.80	2.59	1.89
		SE	3.35	0.65	4.85	0.98	0.72

Table 7. Anthropometric characteristics of men investigated in Beograd

Age group	N		Body weight kg	Body height cm	Relative B.W.	Thickness of skin-fold mm	Fat as % of B.W.
< 39	85	Mean	78.48	177.37	105.60	13.53	13.38
		SD	10.28	5.12	11.44	4.80	3.87
		SE	1.11	0.55	1.24	0.52	0.42
40–44	250	Mean	80.92	175.03	108.05	15.81	12.91
		SD	9.54	8.97	22.94	5.18	4.38
		SE	0.60	0.57	1.45	0.33	0.28
45–49	88	Mean	81.14	174.98	105.16	14.64	14.11
		SD	11.39	6.18	11.24	4.93	3.56
		SE	1.21	0.67	1.22	0.53	0.38
50–54	131	Mean	80.12	174.57	104.28	14.58	14.57
		SD	9.66	6.03	12.98	5.25	4.59
		SE	0.85	0.53	1.13	0.46	0.40
55–59	77	Mean	79.72	173.49	102.49	14.66	14.21
		SD	9.83	6.29	11.52	5.35	4.588
		SE	1.12	0.72	1.34	0.61	0.52
60 and over	22	Mean	83.86	176.00	105.18	16.52	15.60
		SD	12.70	6.59	17.05	5.09	4.20
		SE	2.77	1.43	3.72	1.11	0.92

Table 8. Anthropometric characteristics of men over 40 years of age *)

Group	Average age	No of cases		Body weight kg	Ideal B.W. kg	Body height cm	Relative B.W.	Fat as % of B.W.	Davenport - Kaup Index Mean	$\leqq 2.14$ in %	$\geqq 2.57$ in %
			Min.	44.00	61.70	157.00	69.93	5.70	1.75		
Velika Krsna			Max.	87.70	83.40	181.50	120.19	20.00	3.10		
Peasants	51.3	40	Mean	63.72	70.85	167.90	89.72	9.25	2.25	40 %	15 %
			SD	10.46	4.92	11.04	10.66	2.71	0.31		
			SE	1.65	0.66	1.84	1.68	0.43	0.05		
			Min.	54.50	63.30	154.50	75.27	6.50	1.91		
Zrenjanin			Max.	91.70	80.30	181.50	135.17	31.50	3.37		
Industrial workers	50.2	43	Mean	72.43	70.49	168.01	103.05	16.40	2.57	14 %	54 %
			SD	9.04	30.28	6.53	13.50	9.26	0.24		
			SE	1.37	4.61	0.99	2.06	1.41	0.01		
			Min.	52.00	65.00	164.00	69.98	7.90	1.69		
Beograd			Max.	116.00	86.00	188.00	144.27	38.20	3.56		
University professors	49.4	37	Mean	80.89	76.88	176.70	105.28	19.61	2.58	11 %	62 %
			SD	11.70	4.82	8.01	6.70	6.33	0.31		
			SE	1.92	0.78	1.31	1.10	1.04	0.04		

*) Results obtained for the persons of which by chemical analysis nutrition was estimated.

Table 9. Total serum cholesterol mg per 100 ml

Age group	Velika Krsna No of samples	Mean	SD	SE	Zrenjanin No of samples	Mean	SD	SE	Beograd No of samples	Mean	SD	SE
< 39	155	146.0	25.6	2.05	197	155.5	40.0	2.85	85	190.5	43.6	4.73
40–44	136	155.8	27.1	2.32	148	164.7	24.5	2.01	250	206.0	40.6	2.56
45–49	82	164.1	25.2	2.78	101	170.9	28.3	2.81	88	210.0	42.4	4.52
50–54	138	160.8	18.0	1.53	167	172.0	24.5	1.89	131	214.3	49.0	4.28
55–59	162	160.3	26.7	2.09	98	163.2	28.3	2.84	78	218.7	46.9	5.30
60 and over	32	165.1	33.9	5.98	8	168.6	44.7	16.9	22	167.5	27.5	6.00

The upper arm thickness of skinfold is 7 to 10 mm and the fat content is 8 % to 11 % of the total body weight. However, in the group whose food intake was examined, the average content of body fat was 16 %, and the number of obese persons 54 %, thus far more than was the case with the group of peasants (table 8).

Beograd

From the data on table 7 it can be seen that the body weight of the university professors increases with age, and that, as distinguished from the previous two groups, it reaches its maximum in the age subgroup of over 60 (83.8 kg ± 2.7). The average body weight of university professors whose food intake was examined was 0.9 kg ± 1.9. The relative body weight in all subgroups exceeds the value of the ideal body weight for about 5 % as well as in the group whose food intake was examined.

The upper arm thickness of skinfold was 13.5 to 16.5 mm and the body fat content 12.9 % to 15.6 % while in the group whose food intake was examined it was 19.6 % (table 8). These data confirm the fact that the number of obese persons in the group of university professors whose food intake was examined was 62 %, thus far more than in the groups of peasants and industrial workers.

Total serum cholesterol

From the data in table 9 it can be seen that the total serum cholesterol increased with age in all examined groups and that the values of cholesterol level were lowest in the group of peasants, but they were significantly higher in the group of university professors.

From table 10, showing the serum cholesterol levels in persons whose food intake was examined. it can be seen that the peasants have far less serum cholesterol (159.4 ± 4.6 mg %) than industrial workers (170.4 ± 4.5 mg %) and university professors (165.8 ± 6.1 mg %).

Table 10. Serum cholesterol level in men over 40 years of age*)

Group	No of cases	Mean ± SE
Velika Krsna		
Peasants	39	159.4 ± 4.61
Zrenjanin		
Industrial workers	43	170.7 ± 4.54
Beograd		
University professors	37	165.8 ± 6.16

*) Results obtained for the persons of which by chemical analyses nutrition was estimated.

Discussion

Although the main task of our study was to follow the incidences of degenerative diseases of the cardiovascular system in a group of peasants, industrial workers and university professors throughout five to ten years — the data collected at the beginning of our longitudinal study, including the data on nutrition, enable us to compare these three groups of the population.

As the nutritional status is the result of the nutritive value of food intake and the energy expenditure, the correlation between serum cholesterol and nutrition may be also sought indirectly on the basis of anthropometric characteristics.
From the average daily intake it can be seen that the group of peasants consumed more food (about 3500 calories), but in spite of that they were lacking about 700 calories, while the industrial workers were lacking 200 calories, and the university professors consuming about 3000 calories per day had a relative excess of about 200 calories. One should also bear in mind that the daily fat intake, expressed in percentage of the total calories, is far greater in university professors and industrial workers than in peasants. Such an energy balance can explain the existing anthropometric characteristics of the examined population groups. Namely, the average relative body weight in the group of peasants is 10 % lower than the ideal body weight. On the other hand in the group of workers the relative body weight corresponds to the ideal body weight, while in the group of university professors it exceeds for about 5 % the idal body weight. Bearing in mind the positive correlationship between physical characteristics and serum cholesterol level, it is natural to expect that the level of the total serum cholesterol of peasants should be lower than in the group of industrial workers and university professors, particularly. Namely, according to the existing correlationship of the parameters taken it can be concluded that we may expect a higher serum cholesterol level in the overnourished groups.
It seems that it will be possible to evaluate the significance of the correlation between the daily intake of fatty acids and the serum cholesterol level for the aetiology of degenerative diseases of the cardiovascular system when the analyses of glycerides from lyophilized samples of food are completed and after we have finished our longitudinal survey.

Summary
In order to study the correlation between nutrition and the degenerative diseases of the cardiovascular system in Serbia, 706 peasants were examined at Velika Krsna, 720 industrial workers at Zrenjanin, and 654 university professors in Beograd. By the method of random sampling 41 peasants, 44 industrial workers, and 41 university professors were selected and the food consumed by each person in the course of seven days was weighed. Dietary surveys of peasants and workers were made twice, while for the university professors only once. The nutritive value of the meals was assessed by chemical analysis of equivalent composites of the uncooked food corresponding to the food consumption of each examined person.
Apart from the dietary surveys and anthropometric characteristics the serum cholesterol level was also assessed.
The daily caloric intake in the group of peasants was 3420 $\pm$ 92.8 and 3560 $\pm$ 122 calories, in the group of industrial workers 3490 $\pm$ 138.5 and 3368 $\pm$ 149.1 calories, and in the group of university professors 3035 $\pm$ 109 calories. The daily total fat intake in the group of peasants was 25 %, in the group of industrial

workers 37 %, and in the group of university professors 35 % of the total calories intake.

According to the recommendations of the Commission for Nutrition of the Federal Institute for Public Health it can be concluded that the group of peasants was lacking about 700 calories, the group of industrial workers about 200 calories, and the group of university professors had an excess of 200 calories.

Such an energy balance had corresponding repercussions on physical characteristics of the people examined — so that the relative body weight of peasants was 10 % below the ideal body weight, in the group of industrial workers it corresponded to the ideal body weight, and in the group of university professors the relative body weight was on the average 5 % above the ideal body weight.

Further study of the prevalence and incidence of degenerative diseases of the cardiovascular system in these groups of the population in the following period of 10 years will show in what correlation they are with nutrition, particularly with the energy balance, the quantity and the kind of fat in the daily meal.

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