LABORATORY INVESTIGATION

Diet and Atherosclerosis

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One of the objectives of the International Atherosclerosis Project was to test current hypotheses of relationships among diet, serum cholesterol, and the extent and severity of atherosclerosis. In order to correlate diet and atherosclerotic lesions, however, it was necessary to obtain information on the diet and serum lipids of the populations from which the autopsied persons were drawn. This report describes the data that were gathered on the populations represented, and shows the correlations that exist among severity of atherosclerosis, selected dietary variables, and serum cholesterol.

RELATIONSHIP AMONG DIET, SERUM LIPIDS, AND ATHEROSCLEROSIS

The relationship of diet and serum lipids to atherosclerosis and coronary heart disease (CHD) has received much attention^{2, 4, 108, 188, 238, 242, 247, 250, 267, 416} since Keys and his colleagues^{238, 242, 247} emphasized the association among the percentage of calories from fat, the values for serum cholesterol, and the mortality rates from CHD. Other investigators^{95, 277, 481, 484} have argued that this correlation applies to other dietary changes incident to improved economic status, such as the greater consumption of protein and refined carbohydrate and the decreased use of cereal grains.

Regardless of the exact role of diet in determining levels of serum cholesterol, high average levels are reported to be associated with a greater severity of atherosclerosis 105, 175, 335, 338 and a higher risk of CHD, 46, 47, 51, 120, 225, 248, 383 Although serum lipoproteins 172, 174, 372 and triglycerides 5, 6 are also associated with higher risk of CHD, some investigators 173 believe that these measurements have no greater predictive value than serum cholesterol levels alone. Attempts have been made to correlate postmortem or terminal serum cholesterol values with atherosclerosis at autopsy. However, cholesterol levels in terminal illness or in the postmortem states are not representative of the cholesterol levels present in life and do not correlate with the severity of atherosclerosis, 270, 275, 206, 315

EFFECT OF CHOLESTEROL IN DIET ON SERUM CHOLESTEROL

The cholesterol content of the average diet in North America was thought at one time to have little or no effect on serum cholesterol level. 49, 244, 245, 261, 268 Under some experimental conditions, however, increased dietary cholesterol has resulted in higher scrum cholesterol levels in man. 39, 54, 80, 81, 132, 421 For this reason an effort was made to estimate consumption of eggs, a major source of cholesterol in many diets, from reported surveys.

RELATIONSHIP BETWEEN TYPE OF FAT AND SERUM CHOLESTEROL LEVEL

Some investigators^{4, 132, 335} consider the type of fat more important than the total amount in influencing serum cholesterol levels. Fat intake can be classified according to relative amounts of saturated, monounsaturated, and polyunsaturated fatty acids. In relatively sedentary adult United States males, an increased proportion of saturated fat raises serum cholesterol level, increased polyunsaturated fat lowers it, and increased monounsaturated fat has little effect.^{203, 253} Despite the large amount of epidemiologic and experimental evidence relating type and quantity of dietary fat to hypercholesterolemia, the relationships are imperfect, and other factors contribute to the association.

RELATIONSHIP BETWEEN CARBOHYDRATE INTAKE AND SERUM LIPIDS

Sources of dietary carbohydrate can influence lipid metabolism in man.^{9, 73, 184, 187, 203, 237, 243, 223, 262, 277, 484-486} Complex carbohydrates include the polysaccharides supplied by cereals, potatoes, flour, lenist, and other vegetables. Simple carbohydrates are the monosaccharides and disaccharides present in refined sugars, jams, jellies, syrups, milk, and fruits. As amounts of sucrose increase and the quantity of complex carbohydrate decreases, concentrations of serum cholesterol and especially triglycerides tend to rise. Nomen, however, appear to be able to metabolize large loads of sugar without subsequent rise in lipids. In general, with large quantities of complex carbohydrates and smaller amounts of sucrose, serum lipids decrease.

RELATIONSHIP BETWEEN PHYSICAL ACTIVITY AND SERUM LIPIDS

Physical activity apparently influences serum cholesterol levels. 62, 352 Even the effect of an extremely high fat diet can be counteracted experimentally by physical activity that prevents gain in weight. 269 Observed differences in serum cholesterol levels between active and sedentary populations may be explained by differences in activity. 415 However, reliable estimates of levels of physical activity among the populations studied in the Project were not available, and it was not pusible to test this association.

Effect of Modifying Dietary Fat on Serum Cholesterol Levels and CHD

The increased risk of CHD when serum lipids (notably cholesterol) are high has been substantiated by prospective studies. 188, 100, 101, 110, 111, 225, 316, 356 Further-

more, among groups of people with high risk of CHD who cooperate satisfactorily, programs which lower serum cholesterol levels by modifying dietary fat are reported to be successful.^{5a, 225, 418} Although it is too soon in most of these programs to judge possible effects on CHD, there are some indications that fewer than the expected number of coronary deaths have occurred in the study groups.⁶⁹

DIETARY HABITS AND CHOLESTEROL LEVELS IN THE POPULATIONS STUDIED

BRASIL

Dietary Habits. An unusually low average daily intake of protein per capita was observed in 1935, 105 and the situation had changed little by 1948, 102 In 1964 the daily caloric intake was 1600 to 2900 calories. 160 Fat, largely from cottonseed and soya oil, provided approximately 30 per cent of the calories; carbohydrates (mostly cereals) provided 50 to 60 per cent; and protein, 15 to 20 per cent. Forty per cent of the protein consumed came from animal sources. Other data 15 for 1964 showed a daily consumption of 40 gm, of meat and 0.17 gm, of milk per capita. Less than three eggs were available per person per week in the state of São Paulo. 14

Scrum Lipids. The information available^{329, 348} is not relevant to the autopsy sample of the present study.

CHILE

Dietary Habits. Dietary habits have been studied well in Chile^{68, 112, 262, 378, 433} as well as in one of its cities, Santiago.^{17-19, 371, 418}

Working class adults in Santiago in 1962 had an average daily intake of 2390 calories. Hat provided approximately 30 per cent, with 29 per cent from saturated fatty acids, 33 per cent from monosaturated, and 38 per cent from polyunsaturated fats. Only 15 per cent of calories came from protein, of which 52 per cent came from animal sources. Women consumed more fat and protein of animal origin, more saturated and less polyunsaturated fat, and more sugar and starches than men.

Working adults in Santiago in 1963 consumed, on the average, one-half of one egg per day. 448, 443

Serum Lipids. Two recent reports^{432, 448} indicated that serum cholesterol levels for adults residing in Santiago ranged from 98 to 330 mg. per 100 ml. For middle and upper age groups, serum cholesterol levels averaged more than 200 mg. per 100 ml., and values above 250 mg. were frequent. Women in all age groups tended to have higher serum cholesterol levels than men. Similar results were obtained in a study performed in 1960.³⁷¹

COLOMBIA

Dietary Habits. The first comprehensive survey of dietary habits was performed in 1962 and included

samples from Bogotá and Cali. The results indicated a low caloric intake (below 1600), and also a low fat intake which contributed only 16 to 25 per cent of the total calories. The egg consumption was negligible. A previous study in a poor district of Cali gave similar results.^{77, 134} Independently, a 1962 analysis of food availability for working class families²²¹ concluded that two-thirds of the working class population of Colombia consumed insufficient calories and that one-half was deficient in protein. In this report the situation in 1962 was considered worse than that in 1953.

Serum Lipids. A 1960 survey¹³⁴ based on 165 adults from 14 locations, which included Cali and Bogotá, showed little location variability. The over-all mean serum cholesterol level was 175 mg. per 100 ml. Similar results were obtained in 172 soldiers from all parts of the country.

Costa Rica and Guatemala

Dietary Habits, Surveys in Costa Rica from 1950 to 1954, and in Guatemala from 1950 to 1961 can be considered representative of the socioeconomic groups from which the autopsy samples of the Project were taken. 110-152, 304, 357, 358, 394, 430, 431 The data showed approximately the same caloric intake in both countries, 1800 to 2000 calories. Consumption figures changed little during the periods of the survey, and no important dietary changes occurred subsequently. Sci. 193 Fat intake was regularly low and furnished 10 to 15 per cent of the calories. Less than one-third of the fat consumed was from vegetable sources. Vegetable fat was derived principally from corn and cottonseed oil, both high in polyunsaturated fatty acids. Carbohydrate intake was high in the two countries, but Costa Ricans consumed larger amounts of sugar, mainly brown sugar in the form of panela. Protein intake was low; only 15 to 30 per cent of it came from animal sources. Egg consumption per capita in both countries was less than one-half of one egg per day.

Serum Lipids. As a result of special interest on the part of Instituto de Nutricion de Centro America y Panama (INCAP), serum lipids have been studied more extensively in Central America than in other regions of the study, with the exception of the United States.

Early studies^{268, 262, 263, 267} showed similar serum cholesterol and lipoprotein levels for persons from business and professional environments in the United States and Guatemala. Rural Guatemalans had lower mean cholesterol values at all ages than the professional business subjects. Also, serum cholesterol levels did not vary with either age or sex in the Guatemalan group studied. An unexpected finding was that serum lipoprotein levels of rural Guatemalan males were only slightly lower than those of the professional and business subjects. Rural Guatemalan females had higher serum lipoprotein levels at all ages than more privileged women in either Guatemala, or the United States. Similar results, but with higher lipoprotein levels, were obtained from a parallel study in Costa Rica.⁵⁶⁵

More extensive studies of serum lipids, 196 as well as results obtained in the course of fat-feeding trials in

human subjects,291, 294, 295, 393 support the general findings described above.

JAMAICA

Dietary Habits. The autopsied persons in the Project came from the whole island, with more than one-half coming from rural areas. The general impression is that the diet has improved somewhat in the last 20 years. Surveys in rural areas from 1953 to 1960, however, indicate little change in total caloric intake, with estimates of approximately 1700 daily calories per capita. Low income suburban groups had a higher daily intake, approximately 2200 calories per capita. 156

Carbohydrates contributed 60 to 70 per cent of the calories, and protein and 1at furnished the remainder in essentially equal proportions. Fifty per cent of the fat came from animal sources. Coconut oil, butter, and ghee accounted for more than one-half of the total fat intake. Egg consumption was less than one egg per person per week. 93, 156

Serum Lipids. No published data have been found.

NORWAY

Dietary Habits. Approximately 90 per cent of the autopsy sample came from Oslo; the other autopsied persons came from all parts of the country. The data from surveys^{331, 332} between 1947 and 1954 and from those performed later^{325, 444} suggest a relatively stable diet. The daily intake per capita for industrial workers was estimated at 3260 calories, and for rural workers at 3270 calories.

For the country as a whole, calories derived from protein varied from 11 to 13 per cent, whereas fat calories varied from 32 to 41 per cent. Industrial workers consumed 10 per cent of the calories from fat, and agricultural workers 36 per cent. The consumption of sugar was higher in rural areas than that reported for industrial workers. Farmers consumed more than one egg per day, on the average. Industrial workers consumed approximately one-half of this amount. All groups had a fairly equal consumption of milk, cereals, margarine, and potatoes.

Serum Lipids. Studies on serum cholesterol levels in Oslo relate to patients with myocardial infarction in Oslo City Hospital²⁵⁶ and to men, aged 40 to 59 years, working in Oslo.⁴⁶² Cholesterol levels ranged mainly from 250 to 300 mg. per 100 ml. and varied from a low of 200 to a high of 550 mg. The general pattern was similar in a group of 6886 adult Oslo males, 4516 of whom had serum cholesterol levels of 250 mg. or more; only 6 per cent had serum cholesterol levels below 200 mg. per 100 mt.

PERU

Dietary Habits. A number of Peruvian surveys have been published. 75, 76, 199-201, 213, 463 Surveys of dietary habits 4 were made in 22 localities throughout the country from 1951 to 1955. One survey of a random sample of 50 families living in an urban area near the center of Lima indicated that these families received 9 per cent of their calories from sugar and 22

per cent from fat. Of the free fat, 55 per cent came from vegetable oil, 27 per cent from lard, 10 per cent from butter, and 8 per cent from margarine. Protein intake averaged 72 gm. per capita, with animal protein in a proportion of 40 per cent. A total of 18 per cent of the families consumed no eggs at all, and the remainder averaged less than one and one-half eggs per person per week.

Serum Lipids. A 1956 survey³¹⁹ was restricted to the armed forces in eight major areas, and only 8 per cent of the subjects were over 25 years of age. The mean cholesterol values for this group ranged from 131 to 170 mg. per 100 ml., and were lower than those for persons of comparable age in the United States. If the cholesterol values in this study are at all representative of the civilian population, the serum cholesterol levels of the population from which the autopsy sample was obtained were probably low.

PHILIPPINES

Dietary Habits. There have been several studies of dietary habits of this population. 78, 197, 343, 379-381 An extensive nutrition survey 354, 355 in Manila in 1959 showed an average daily intake of 1727 calories per capita, 66 per cent of which came from carbohydrates, 11 per cent from protein, and 23 per cent from fat. Animal sources provided 46 per cent of the protein. Of total fat, 15 per cent came from animals, 84 per cent from coconut oil, and the rest from other vegetable oils. The calorie contribution of fats was approximately 15 per cent higher in Manila than in rural areas. Conversely, the calories derived from carbohydrate were 16 per cent lower. Egg consumption averaged approximately six eggs per month per person in Manila, twice as much as in rural areas.

Scrum Lipids. Serum cholesterol levels for men in the armed forces, aged 19 to 58 years, averaged 170 mg. per 100 ml. Unpublished data for Manila show mean values between 175 and 323 mg. per 100 ml. for groups over 16 years of age. In the groups over 40 years of age, the mean serum cholesterol level was 212 ± 53 mg. per 100 ml. for males and 223 ± 51 for females.

Puerto Rico

Dietary Habits. Recent industrialization has brought profound changes in dietary patterns. Consumption of eggs trebled, and consumption of milk and milk products increased four-fold from 1939 to 1960. The total food intake per capita also increased steadily. A recent survey confirmed these trends and showed that adult males (45 to 64 years of age) consumed approximately 2400 calories per day. A total of 15 per cent of the calories was derived from proteins and 30 per cent from fat. Urban men consumed more fat and more vegetable fat than rural men who obtained 90 per cent of their fat from animal sources. The ratio of polyunsaturated to saturated fat was greater for the urban group, but was still well below 1.0.42

Urban and rural groups consumed roughly the same amount of protein although the rural men consumed more fish protein.⁴² Rural residents obtained 90 per

cent and urban residents obtained 65 to 75 per cent of their carbohydrates from starch. Percentage of calories from refined sugar was low (8 to 11 per cent) for the urban population, but was still twice the proportion of calories from refined sugar for rural groups.

Serum Lipids. Serum cholesterol level data were not available for the city of San Juan, where most of the autopsied persons originated. The mean levels for two of three small rural communities studied,³⁵¹ however, ranged from 140 mg. per 100 ml. at ages 15 to 19 years to 186 mg. per 100 ml. at ages 30 to 59 years. The third community showed mean values ranging from 181 to 221 mg. per 100 ml., which are still low by North American and European standards, but which are somewhat high for lower income persons in Latin America.

SOUTH AFRICA

Dictary Habits. The general dietary pattern of the urban South African Bantu has changed little through the years. The daily caloric intake per capita was approximately 2750 in 1953; 11 per cent of these calories came from protein and 21 per cent from fat. Nearly three-quarters of the fat consumed was from animal sources.

A random sample of 100 Bantu families studied in 1951⁴⁵⁶ showed a relatively high daily calorie intake per capita (2950 calories) and a very low fat intake. The proportion of calories from fat was only 14 per cent, and polyunsaturated fatty acids furnished 7 per cent of the calories. In 1960, Bantu families with children of school age in Pretoria had a similarly low fat intake; eggs were available to only a few of the families.³³⁷ The protein and fat intakes for the Bantu were much lower than for white university students.⁴⁵⁷

Serum Lipids. No data on serum lipids were available for the Durban area. Mean cholesterol values for clinically healthy men in Capetown, 40 to 58 years old, showed a progressive increase from relatively low values for the Bantu to relatively high values for Europeans, with the "colored" or mulatto group in an intermediate position. Serum cholesterol levels paralleled differences in total dietary fat and in fat of animal origin.⁵¹ Studies in Bantu populations in Johannesburg ¹⁵⁰ gave similar results. Serum lipoproteins were 50 to 65 per cent lower than those in New York and showed little change with age;³⁷⁴ serum triglyceride levels of urban Bantu in Johannesburg were also low.¹³

Durban Indian. The above information pertains to the Bantu. No reliable survey of the diet of the Indian population of Durban could be found.

UNITED STATES

Dictary Habits. The population of the United States has experienced profound alterations in patterns of diet and in dietary practices during the present century. 12, 155, 276, 533, 475 These can be summarized as follows:

1. A slight decrease has occurred in the amount of taily total calories consumed per capita; associated with this is an increased proportion of fat calories, consisting of approximately 30 to 50 per cent of the total calories.

2. There has been a considerable decrease in complex

carbohydrate consumption, principally due to a decreased use of cereals, cereal flour, and potatoes. Consumption of simple sugars and syrups has increased greatly.

3. A slight increase in total fat consumption has been noted, and is in association with greater utilization of polyunsaturated fatty acids.

The general situation of the data above applies to New Orleans, Louisiana, although regional differences exist in levels of food intake and food preferences. 155, 255, 310, 311, 403

Serum Lipids. Serum cholesterol levels and β -lipoprotein values in the United States population are higher than in most other populations included in the Project. Many groups in the United States have been studied for levels of serum cholesterol, triglycerides, and lipoproteins, and the serum triglyceride values as well as serum cholesterol values tend to be high.

VENEZUELA

Dietary Habits. The diet of this population has been extensively studied.31-34, 180 Surveys of working class residents of Caracas^{178, 170, 451-453} from 1953 to 1961 suggested that the daily intake of calories per capita has decreased from approximately 2500 to 2000. A survey in 1963 confirmed these results and estimated consumption of 1840 calories per capita per day. The proportional contribution of protein and fat to total calories also has decreased and represents 10 and 15 per cent, respectively. Daily fat intake in Caracas from 1960 to 1961 averaged 58 gm. per capita. 454 Milk and milk products supplied 25 per cent; sesame oil, 18 per cent; other oils and fats, 12 per cent; corn and other cereals, 19 per cent; mean, 8 per cent; and vegetables, 8 per cent. The ratio of polyunsaturated fat to saturated fat was 0.68. Of the protein intake, 60 per cent came from vegetable sources. Beef, milk, and milk products supplied 29 per cent of animal protein; fish, 8 per cent; and eggs, approximately 3 per cent. Cereal grains contributed 41 per cent of dietary carbohydrate; sugar supplied 16 per cent; and starchy roots (principally yucca), other vegetables and fruits, roughly 38 per cent.

Serum Lipids. Only one published report⁴⁴ of serum lipoproteins in Venezuela could be located, and it concerned a population that was not pertinent to the present study.

RANKING POPULATIONS BY DIET COM-PONENTS, SERUM CHOLESTEROL, AND ATHEROSCLEROSIS

RANKING POPULATIONS BY DIET AND SERUM CHOLESTEROL LEVEL

After reviewing the surveys which have been cited, one of the authors (N. S. S.) ranked the 19 geographic and ethnic groups in the Project according to percentage of calories derived from fat, percentage of fat derived from animal fat, amount of eggs consumed, and amount of sucrose consumed. The author ranking these groups took into account the quality of the various surveys, and relied to some degree on personal experience and

familiarity with research work which is not given in the list of references. He did not, however, know the results of the prior arterial gradings and rankings of the populations by severity of atherosclerosis. Knowledge of these results would have made these rankings susceptible to unconscious bias.

A similar procedure was followed for the ranking of the groups, in order, by mean serum cholesterol concentration. Less subjective judgment was involved in these rankings because specific and comparable data were available for most groups. Although the precise position of groups ranking close together is not certain, it is believed that the rankings are realistic and that the general order is reliable.

RANKING POPULATIONS BY SEVERITY OF ATHEROSCLEROSIS

In the principal analysis of Project data, the 19 location-race groups were ranked in order by the mean percentage of intimal surface involved with raised atherosclerotic lesions (the sum of percentage of intimal surface involved with fibrous plaques, complicated lesions, and calcified lesions). This rank order was termed reference rank, and for convenience the 19 groups were placed in this order in most presentations of the data.

CORRELATION OF RANKINGS BY DIFFERENT MEASURES

The results of the various rankings are presented in Table 1. We next determined the Spearman rank correlation coefficient of each listed variable, with the reference rank based on advanced atherosclerotic lesions. The results are presented in Table 2.

The correlation of reference rank with serum cholesterol levels was relatively high and statistically significant. The correlation of percentage of calories from fat with reference rank and serum cholesterol levels was nearly as great. The correlations of reference rank with proportion of dietary fat supplied as animal fat and also with the amount of sugar consumed were small and not statistically significant.

INTERPRETATION OF RESULTS

The results in Table 2 support the vast experimental and epidemiologic evidence which relates atherosclerosis to serum cholesterol levels and to the proportion of calories derived from total fat. The present findings cast no doubt on experimental studies which show that serum cholesterol levels are altered by change in sources of fat and carbohydrate in the diet. The findings do, however, suggest that in most populations sources of fat and of carbohydrate are not the primary factors in determining severity of atherosclerotic lesions.

OHER CORRELATIONS

No valid method could be found to evaluate the contribution of physical activity to these correlations. Evidence cited at the beginning of this paper suggests that decreased physical activity in most groups of the populations in countries consuming relatively high amounts of fat could have contributed to correlations of reference rank with both scrum cholesterol levels

TABLE 1. RANKING OF 12 LOGATIONS BY RAISED ATHLROSCLEROTIC LESIONS (RL), TOTAL SERUM CHOLESTEROL, AND SELECTED DIET COMPONENTS^a

	Reference rank, based on RL	Rank by				
Location-race group		Serum choles- terol	c; calories from fat	animal fut in total fat	Amount of sugar consumed	
Bogotá	13	9	11	15	7	
Caracas	5		9	13	2	
Cali	8	8	14	14	11	
Durban Bantu	15	7	10	; 3	14	
Guatemala	14	12	12	6	12	
Jamaica	6		7	9	1 10	
Lima	10	10	6	11	3	
Manila	4	6	8	7	13	
New Orleans white	1	1	2	ા છ	1 8	
New Orleans Negro	3	3	5	10	5	
Oslo	2	2	1	5	4	
Puerto Rico	7	4	4	1	9	
San José	! 11	11	15	2	' 1	
São Paulo white	9		3	12	, 6	
Santiago	12	5	13	4	15	

^a Data from Tables 1 and 2 were derived from the International Atherosclerosis Project, 1960 to 1965.

Table 2. Correlation Coefficients among Rankings in 12
Locations According to Five Criteria

Rankings	Serum cholesterol	calories from fat	total fat from animal fat	Amount of sugar consumed
Atherosclerosis reference	0.755	0.668	0.070	0.315
Scrum cholesterol Per cent calories from fat Per cent total fat from animal fat		0.741	0.074 -0.006	-0.070 0.265 -0.248

[•] Statistically significant, p < 0.01.

and fat calories. Countries were also ranked for animal protein consumption, and correlations with other variables were calculated. Inasmuch as these corresponded closely to the ranking by percentage of fat calories, and, consequently, gave no new information, they are not listed in Table 2. On the basis of experimental and epidemiologic evidence similar to that cited earlier in this report, the correlation between reference rank and animal protein intake was interpreted as comparable to the correlation with various indicators of economic and social development, such as telephones and automobiles, rather than of primary etiologic significance. Data on egg consumption were too fragmentary to permit reliable ranking, but the rank order tended to follow both fat and protein calories.

SUMMARY

Information on dietary habits and serum cholesterol levels was sought for the population groups included

^b No data were available for São Paulo, Jamaica, and Caracas.

in the International Atherosclerosis Project. As many populations as the data permitted were ranked in order of percentage of calories from lat, percentage of total fat of animal origin, consumption of sucrose, and serum cholesterol levels. The rank order correlation coefficient of this ranking with that for the reference rank based on advanced atherosclerotic lesions gave a highly significant r_s of 0.755 with percentage of calories from fat and r_s of 0.688 with serum cholesterol. The rank correlation of fat calories with serum cholesterol was 0.741. The rank correlations of athero-

sclerosis reference rank with the other dietary variables were small and without significance. A strong positive correlation of animal protein consumption with reference rank was observed because this dietary variable closely paralleled the fat calories; it was not considered etiologically important because of the strong supporting evidence for a primary role of fat, rather than protein, in determining levels of serum cholesterol, severity of atherosclerosis, and incidence of CHD. Egg consumption generally paralleled protein and fat intake, but the data were too fragmentary for reliable ranking.