



Serum Lipoprotein and Cholesterol Concentrations

Comparison of Rural Costa Rican, Guatemalan, and United States Populations

By NEVIN S. SCRIMSHAW, PH.D., M.D., MARTHA TRULSON, D.Sc., CARLOS TEJADA, M.D.,
D. MARK HEGSTED, PH.D., AND FREDRICK J. STARE, M.D.

A previous study indicated that Guatemalan Indians, both male and female, tended to maintain low levels of serum cholesterol throughout adulthood but that beta-lipoprotein fractions increased with age, as is usually the case in populations in the United States. To investigate whether race was of importance in this unexpected dissociation between these lipid fractions, a similar study was done with a rural group in Costa Rica, which was comparable in the diet consumed and in many other environmental factors, but of pure European origin. At all ages the cholesterol values were slightly higher but the same type of dissociation was observed.

IN A previous report Mann, Munoz, and Scrimshaw¹ demonstrated that rural Guatemalans, subsisting on a diet low in both animal protein and calories from fat, had much lower serum cholesterol values than men and women from business and professional families living in urban areas in either Guatemala or the United States. The rural Guatemalans also showed β -lipoprotein fractions that were within the same levels for males, and higher levels for females, than upper income populations in urban Guatemala and the United States. This dissociation between serum cholesterol and certain β -lipoprotein fractions was quite unexpected and contrary to the findings of studies dealing exclusively with United States subjects.

These observations have important implica-

From the Institute of Nutrition of Central America and Panama (INCAP), Guatemala City, Guatemala, and the Department of Nutrition, Harvard School of Public Health, Boston, Mass. The Institute of Nutrition of Central America and Panama is a cooperative activity of Costa Rica, El Salvador, Guatemala, Honduras, Nicaragua, and Panama, administered by the Pan American Sanitary Bureau, Regional Office for the Americas of the World Health Organization. INCAP Scientific Publication I-66.

This study was supported in part by grants from the National Heart Institute (grant no. H-2200) of the National Institutes of Health, U. S. Public Health Service, Bethesda, Md.; the Nutrition Foundation, Inc., New York City; the Albert and Mary Lasker Foundation, New York City; the John A. Hartford Memorial Fund; and the Fund for Research and Teaching, Department of Nutrition, Harvard School of Public Health.

tions because autopsy findings indicate that the rural Guatemalan group has a much lower incidence of the complications of atherosclerosis than persons in the United States.² Similarly, clinical records show coronary heart disease to be common among business and professional persons in urban areas in both the United States and Guatemala and exceedingly rare among lower income persons residing in rural Guatemala.

These results suggested that environmental differences, among which diet might be the most important determining factor, were responsible for the relative freedom from coronary heart disease and other complications of atherosclerosis of the Guatemalan rural group. This group differed, however, in racial origin: they were known to be predominantly Mayan Indian by their physical appearance, way of life, and the distribution of blood group O, which varied from 80 to 96 per cent, depending on the village studied.^{3,4} In contrast, the United States population is known to have a major blood group frequency of approximately 45 per cent type O⁵, and the upper income Guatemalan population a frequency only slightly higher,³ which correlates well with the predominantly European origin of this segment of the Guatemalan population. Before accepting environmental variations as responsible for the differences encountered between rural Guatemalans and the other 2 groups it was necessary to be certain that differences in racial origin were not of major significance.

In the present study comparable information has been obtained on a group of persons living in rural Costa Rica, under conditions similar to those previously studied in rural Guatemala, except that the Costa Rican population is predominantly European by history, physical appearance, and distribution of the major blood groups.³

MATERIAL AND METHODS

The population of the rural area of Turrialba, Costa Rica, was selected because detailed social, economic, agricultural, dietary, and clinical studies had been carried out on this group.^{6, 7} This population is European in origin, with essentially no Negro or Indian admixture. In other respects it resembles the rural Guatemalan Indian population previously studied. The results of the clinical and laboratory examinations showed little difference between the rural Costa Ricans and Guatemalans, except for a greater frequency of hookworm anemia among the former. The rural Guatemalans and United States business and professional groups included for comparison in this paper are those previously studied,¹ plus additional determinations in the latter group. Each subject was examined by a physician, and blood pressure, height, and weight were recorded. For the purposes of the present report, subjects with evidences of acute or chronic disease or a blood pressure exceeding 140/90 were excluded from the tabulations.

Dietary Data. Dietary histories were obtained from 140 representative families in the rural area of Turrialba by means of 7-day-diet records. The weekly purchasing pattern of this community facilitated the use of this procedure, which was carried out by a nutritionist familiar with the language, customs, and foods of the people. The nutrient intakes were estimated by means of a food composition table based on the analysis of Central American foods⁸ and their dietary allowances modified from those of the National Research Council (U. S.) to conform with local environmental conditions and mean body weights.⁹ The collection and calculation of the dietary data on the other 2 groups have been previously described.¹

Laboratory Data. The blood samples were collected in the field and immediately transported to a central laboratory for centrifugation and recovery of the sera. Serum samples, kept at 0 to 5 C. at all times, were placed in labeled tubes for air shipment to Boston. Lipoproteins were measured with the ultracentrifuge by the method of Gofman and co-workers.¹⁰ Although only the β -lipoprotein S_f 12-20 and S_f 35-100 fractions were measured in the previous study, the values for the S_f 0-12, S_f 12-20, and S_f 20-100 were determined in the Costa Rican sample. Our S_f 20-100 measurements are compa-

rable to the "standard" S_f 20-400 values of Delalla and Gofman.¹¹ Cholesterol was measured by the method of Abell and associates.¹²

Statistical Data. The laboratory data for each group were tabulated according to sex and decade of age. The tabulations differed from those presented in the previous paper in that no logarithmic transformations of the measurements were made. The degree of skewness of the distribution was sufficiently slight that this transformation was not considered necessary. The relative weights, ($100 \times$ observed weight) desirable weight) were computed for all subjects. For lack of more suitable reference data, the tables of Desirable Weights* of the Metropolitan Life Insurance Company^{13, 14} were used for this calculation. The *t* test was employed to evaluate the significance of difference between means.

RESULTS

A partial summary of the dietary findings in the 3 groups is given in table 1. When compared with the rural Guatemalans previously studied, the diets of the Turrialba rural area population are higher in calories, fat, and cholesterol. The percentage of total calories from fat, although slightly greater than in rural Guatemala, is still less than a third of the percentage given in the United States reports.¹⁵ The amount of protein and fat of animal as compared with vegetable origin, is nearly 3 times greater in the rural Costa Ricans than in the rural Guatemalans, but still much less than for the United States population. It is of interest that the small amount of free fat consumed in Guatemala came from lard, while in Turrialba, some butter and vegetable oil were also used. The cholesterol content of the diets of the 3 groups was estimated and found to be low in the rural Costa Ricans and still lower in the rural Guatemalans.

The levels of cholesterol and lipoproteins of the S_f 12-20 and S_f 20-100 classes for the 3 groups are shown in table 2. The data are grouped according to decade of age and the significance of each comparison is given. When the rural Costa Rican group is compared with the rural Guatemalan group, the serum cholesterol levels tend to be slightly higher, significantly so in 2 age groups for the males and 3 age groups for the females. On the other hand,

* Formerly referred to by the Metropolitan Life Insurance Company as "Ideal Weights."

TABLE 1.—*Approximate Dietary Intake of Study Groups*

	Calories	Cholesterol (mg./day)	Protein (Gm./day)			Fat (Gm./day)			Proportion of calories from fat (%)
			Animal	Veg.	Total	Animal	Veg.	Total	
Rural Costa Rica (Turrialba)	2705	150	17	56	73	25	12	37	12.0
Rural Guatemala (Highlands)	2283	50	6	61	67	9	14	23	7.5
U. S. business and professional workers*	3240	700	65	32	97	104	44	148	41.0

* Derived from available food of 1952¹⁵TABLE 2.—*Serum Lipoprotein and Cholesterol Levels of Rural Costa Ricans, Rural Guatemalans, and North Americans According to Age and Sex*

(Concentrations are mg. per 100 ml. serum; data not adjusted for differences in body weight.)

Age (yr.)	Group	A			B			A compared with B p	C			A compared with C p	
		N	X	S D	N	X	S D.		N	X̄	S D		
Males													
20-29	S _f 12-20	17	21	12	13	24	10	↓	57	31	19	↓	0.05
	S _f 20-100		56	44		60	29	↓		66	56	↓	
	Cholesterol		145	31		132	21	↑		213	44	↓	
30-39	S _f 12-20	9	28	15	34	31	15	↓	296	37	20	↓	0.05
	S _f 20-100		68	28		67	37	→		81	59	↓	
	Cholesterol		178	38		140	21	↑		228	48	↓	
40-49	S _f 12-20	11	26	11	18	32	17	↓	685	39	21	↓	0.05
	S _f 20-100		65	37		83	40	↓		88	73	↓	
	Cholesterol		170	19		150	48	↑		241	49	↓	
50-59	S _f 12-20	13	21	10	16	32	18	↓	356	39	21	↓	0.05
	S _f 20-100		43	24		67	43	↓		82	59	↓	
	Cholesterol		162	23		134	32	↑		244	48	↓	
Females													
20-29	S _f 12-20	14	26	14	21	35	20	↓	37	24	14	→	0.05
	S _f 20-100		53	32		79	52	↓		29	19	↑	
	Cholesterol		159	30		144	30	↑		196	52	↓	
30-39	S _f 12-20	20	27	12	15	41	20	↓	56	27	17	→	0.05
	S _f 20-100		60	39		80	43	↓		41	34	↑	
	Cholesterol		179	39		147	24	↑		209	32	↓	
40-49	S _f 12-20	11	25	10	11	45	21	↓	208	29	15	↓	0.05
	S _f 20-100		52	24		89	45	↓		39	28	↑	
	Cholesterol		180	32		165	36	↑		225	40	↓	
50-59	S _f 12-20	4	35	8	10	43	19	↓	91	39	24	↓	0.05
	S _f 20-100		82	41		65	28	↑		60	59	↑	
	Cholesterol		191	28		147	33	↑		252	51	↓	

the S_f 12-20 fraction tended to be lower in rural Costa Ricans, significantly so in 1 age group for each sex. The S_f 20-100 fraction also tended to be somewhat lower in rural Costa Ricans than in the rural Guatemalans. This difference was significant in females, for the decades 30 to 39 and 40 to 49, with a reversal of this trend in the 50 to 59 age group,

probably due to the small number of cases in the latter. The direction of the changes tended to put the rural Costa Ricans closer to the values encountered in the urban United States population, but the magnitude of the differences between the rural Costa Rican and rural Guatemalan populations was small.

When the rural Costa Rican was compared

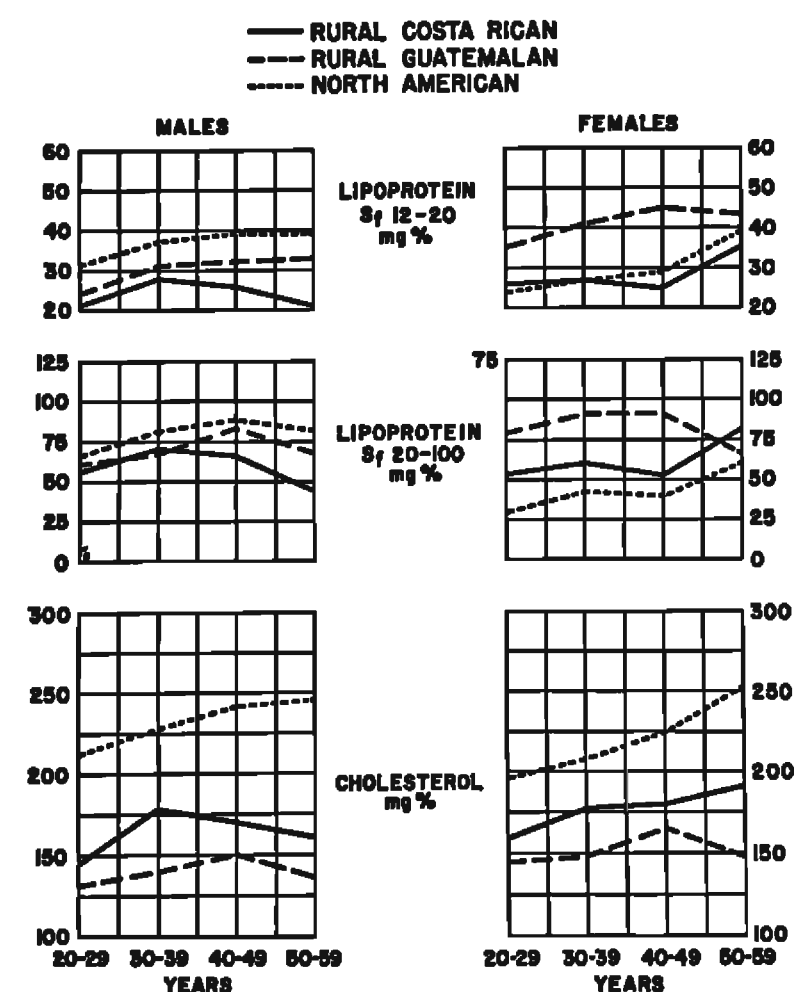


FIG. 1. A comparison of the levels of serum cholesterol and 2 classes of lipoproteins among subjects consuming different diets.

with the urban United States population, the serum cholesterol levels were significantly lower in every decade. However, the trend of the lipoprotein values differed in males and females. Costa Rican males tended to have lower S_f 12-20 and 20-100 values than males of similar age in the United States, although the differences were significant in only about half. On the other hand, a dissociation between cholesterol and lipoprotein was noted in rural Costa Rican women. The S_f 20-100 fraction tended to be significantly higher in the decade 20 to 29. The S_f 12-20 fraction was not significantly different between United States and Costa Rican women.

These trends are shown graphically in figure 1, where the Costa Rican males are intermediate in cholesterol, but tend to be lower in S_f 12-20 and 20-100 levels than the other 2 groups. For the Costa Rican females, the cholesterol and S_f 20-100 levels are seen to be intermediate between the rural Guatemalan and United States groups, but the S_f 12-20 fractions were almost identical in rural Costa Rica and United States.

In the previous study, the levels of the S_f

TABLE 3.—Levels of S_f 0-12 Lipoprotein Fractions in Rural Costa Rican and United States Populations

Age (yr.)	Group						A compared with C	
	A Rural Costa Rican			C United States*				
	N	\bar{X}	S.D.	N	\bar{X}	S.D.	<i>p</i>	
Males								
20-29	17	197	53	75	302	81	↓	.01
30-39	9	239	48	358	340	88	↓	.01
40-49	11	247	42	313	364	84	↓	.01
50-59	13	220	40	228	367	89	↓	.01
Females								
20-29	14	233	35	86	283	72	↓	.01
30-39	20	268	61	452	304	81	↓	.01
40-49	11	233	41	399	346	81	↓	.01
50-59	4	235	26	269	363	78	↓	.01

* "Standard" S_f 0-12 values¹⁶; these are slightly lower than "observed" figures as measured on the Costa Ricans.

0-12 lipoprotein fractions were not determined. Table 3 gives the S_f 0-12 concentrations for the rural Costa Ricans and a United States sample done in a different laboratory. The latter is reasonably comparable economically and socially to the United States sample studied in this laboratory. It will be seen that in both sexes and in every decade the S_f 0-12 fractions for the rural Costa Ricans were significantly lower than in the United States population.

The rural Costa Ricans showed the same relative weight as the rural Guatemalans for the earlier decades and this remained unchanged through the 50-59 age group, at which time the relative weight of the rural Guatemalan males showed a significant drop. As shown in table 4, the rural Costa Rican females began at a relative weight below that of the rural Guatemalans, and unlike them, did not show any further decrease in relative weight with increasing age. The rural Costa Ricans showed much lower relative weights than the United States subjects. Analysis of covariance had shown in the previous comparison among rural Guatemalans, urban Guatemalans, and a United States urban population sample that relative weight was of

TABLE 4.—*Relative Weights by Age and Sex for Rural Costa Ricans, Rural Guatemalans, and United States Subjects*

Age (yr.)	A Rural Costa Rican		B Rural Guatemalan		A compared with B <i>p</i>	C United States		A compared with C <i>p</i>
	\bar{X}	SE \bar{X}	\bar{X}	SE \bar{X}		\bar{X}	SE \bar{X}	
Males								
20-29	94 ± 1.09		96 ± 2.77		↓	107 ± 1.82		↓ .05
30-39	90 ± 2.17		96 ± 1.54		↓ .01	107 ± 0.73		↓ .05
40-49	97 ± 1.62		93 ± 1.89		↑	110 ± 0.45		↓ .05
50-59	94 ± 1.76		76 ± 2.00		↑ .01	111 ± 0.88		↓ .05
Females								
20-29	89 ± 4.00		100 ± 3.41		↓ .01	103 ± 2.47		↓ .05
30-39	91 ± 3.02		93 ± 2.58		↓	107 ± 2.55		↓ .05
40-49	95 ± 5.05		94 ± 3.92		↑ .01	106 ± 1.55		↓ .05
50-59	90 ± 4.26		83 ± 3.33		↑	106 ± 2.53		↓ .05

minor importance in influencing serum lipid levels. This analysis was repeated for the comparison between the rural Costa Rican and United States group, with the same results.

DISCUSSION

These observations indicate that the differences previously encountered between the rural Guatemalan population and the urban groups in Guatemala and the United States were not due to racial factors. Furthermore, the differences in serum lipids between the rural Costa Ricans and rural Guatemalans are in general small and not statistically significant. It is of interest that these differences are almost always in the direction of the urban business and professional population in the United States and Guatemala, so that the Costa Ricans can be said to have gone a very small part of the distance toward the type of serum cholesterol and lipoprotein relationships found in the United States.

Table 1 shows that urban Costa Ricans do have slightly more animal protein, slightly more fat, a higher percentage of calories from fat, and more cholesterol in their diets than do the rural Guatemalans. The evidence that dietary cholesterol has no relation to serum cholesterol levels within the range of cholesterol intake under consideration is convincing.^{17, 18} If the difference in serum cholesterol can be attributed to the difference in fat in-

take, as has been suggested by Keys,^{19, 20} it may be somewhat discouraging from the point of view of prevention to note that differences as small as those existing between these 2 rural populations may have a detectable effect on cholesterol levels. However, assuming serum cholesterol levels correlate with the occurrence of atherosclerosis, the upper limits beyond which the development of atheromata are accelerated are still unknown.

Attention has also been called to the similarity between the predominantly vegetable diet consumed by persons in rural Central America and the "rice diet" regimen for the treatment of essential hypertension. In the latter, Hatch's group¹⁷ has reported a lowering of cholesterol values in most cases together with a 9 per cent increase in S_f 12-20 and a 99 per cent rise in S_f 20-100 values. This was associated with an increase in serum neutral fat.

The suggestion has been made that some factor in vegetable protein diets, apart from the major nutrients, might be responsible for a decrease in serum cholesterol levels in persons consuming low-fat diets. Sitosterol has been studied as an example of such a substance.²¹ However, it has been difficult to conceive of a factor that would be common to low-fat diets in all of the various parts of the world in which low serum cholesterol levels have been encountered. Malmros and Wigand²²

have reported that a fall in serum cholesterol levels accompanied the institution of a diet rich in corn oil in 8 cases of familial hypercholesterolemia. They suggested that these effects might be due to the high essential fatty acid content of corn oil, which contains 57 per cent linoleic acid. This suggestion is supported by the recent observations of Lever and Waddell,²³ Kinsell and co-workers,²⁴ Bronte-Stewart,²⁵ and Ahrens.²⁶ However, most of these studies have been on few patients and for short periods of observation. When 1000 calories of an unsaturated vegetable oil (peanut) with 22 per cent linoleic acid were incorporated into the daily diets of 25 men for a period of 4 weeks, we observed a marked drop in cholesterol after 1 or 2 weeks, but by the fourth week the cholesterol values were mostly back at starting levels.²⁷ Thus, the decrease in serum cholesterol reported to follow the generous administration of some oil rich in essential fatty acids may turn out to be only transient when added to the daily diet. Nevertheless, it is of interest that the high-corn diets of Central America, although low in total fat, do at least provide a generous proportion of the essential fatty acid, linoleic acid.

The results again indicate that under the conditions prevailing in Central America, particularly in females, a definite dissociation between the levels of serum lipoprotein and cholesterol occurs. The Guatemalan females can no longer be said to be unique in combining low cholesterol levels with high lipoprotein levels when compared with age-matched North American females, for the Costa Rican females also show this phenomenon in the S_r 20–100 lipoprotein fractions. This discrepancy raises the question as to which fractions in the lipoprotein complex system are involved in the lower levels of cholesterol in the blood stream under the environmental influences prevailing in rural Guatemala and Costa Rica. The cholesterol associated with the various lipoprotein fractions accounts for at least 95 per cent of the total.²⁸ The cholesterol content of the S_r 0–12 fraction alone has been given as approximately 33 per cent.^{27–30} From the results of the present study it appears that most of the

difference in cholesterol levels can be accounted for by the low levels of the S_r 0–12 fraction.

The question may be raised as to whether the rural Costa Rican population is as free from the complications of atherosclerosis as the rural Guatemalan population. Preliminary information being collected by one of us (C. T.) in cooperation with Dr. Rodolfo Céspedes in Costa Rica indicates that the Costa Rican rural population does, in fact, share in this relative immunity from coronary heart disease and other complications of atherosclerosis. If this is true, our data on both the Guatemalans and Costa Ricans, indicating rather minor differences in the serum concentrations of the lipoproteins, S_r 12–100, between these groups and the United States population, but large and significant differences in the S_r 0–12 and cholesterol concentrations between the Costa Rican and United States groups, suggest that these latter 2 fractions may be more intimately related to the development of atherosclerosis than the S_r 12–100 fractions.

There is no doubt that the rural and poor urban populations of Central America consume much less fat than most persons in the United States, derive a far smaller percentage of their total calories from this class of nutrient, and utilize different kinds of fats. Other known differences in the dietary data include the amount of animal protein, the number of calories from refined carbohydrates, and the percentage of crude fiber. In addition, vitamin A and riboflavin intakes are lower and the mineral composition of the diets differ. It is also conceivable that there are other specific factors in the Central American diets that can serve to depress the serum cholesterol. Furthermore, the relative body weights of the rural population are less and they are more active physically. For these reasons, it would be premature to assert which specific environmental factors are the ones responsible for the lower serum cholesterol and freedom from the complications of atherosclerosis of the lower income groups of Central America. Fortunately, the Central American area offers a very favorable opportunity for the continuing investigation of this problem.

SUMMARY

The total cholesterol and major beta-lipoprotein fractions were determined in a group of 99 men and women living in the rural zone of Turrialba, Costa Rica, and subsisting on diets low in fat and animal protein. These measurements were compared with North Americans and rural Guatemalans previously investigated.

At all ages studied the Costa Rican subjects, both male and female, were shown to have mean cholesterol values that were slightly higher than the rural Guatemalans, but much lower than the North Americans. There was little evidence among the Costa Ricans of the tendency for serum cholesterol to increase with age. The S_f 12-20 and 20-100 levels in the rural Costa Rican males were only slightly lower and in the females were frequently higher than in the North Americans. They tended to be lower, however, than in the rural Guatemalans previously studied. The S_f 0-12 levels were lower in the rural Costa Ricans than in the North Americans and this difference was sufficient to account for most of the differences in cholesterol levels.

The greater leanness of the rural Costa Rican was shown not to be a significant factor in the lower cholesterol values. Since the rural Costa Rican population was of European origin in contrast to the predominantly Mayan Indian of the rural Guatemalan group previously studied, the similarity of the results in both populations would seem to eliminate racial factors from serious consideration and to confirm the hypothesis that environmental factors, most likely nutritional, are responsible for the differences observed between rural Central Americans and North Americans.

In view of the supposed freedom of Central American rural and low income urban populations from the complications of atherosclerosis it is important that the dietary and other environmental factors be identified that may be responsible for these differences.

Since there are only minor differences in the concentration of the S_f 12-100 lipoproteins in the United States and Central American groups, but large differences in the serum cholesterol and S_f 0-12 lipoproteins, the latter 2

fractions may be more closely related to the development of atherosclerosis than the lipoproteins with high flotation rates.

ACKNOWLEDGMENT

The authors wish to acknowledge the assistance of Dr. Eleanor Y. Lawry and Miss Mary McCann in carrying out the lipoprotein measurements and assistance in the preparation of the manuscript. Dr. Carlos Díaz Amador, Chief of the Nutrition Section of the Public Health Department of Costa Rica, assisted in the collection of blood samples and clinical data. Dietary data on the Costa Rican population were furnished by Margaret Fiester.

SUMMARIO IN INTERLINGUA

Le cholesterol total e le major fractiones de lipoproteina beta esseva determinate in un gruppo de 99 homines e feminas habitante le zona rural de Turrialba in Costa Rica e vivente con dietas a basse contento de grassia e proteina animal. Iste mesurationes esseva comparate con datos obtenite in previe investigationes de nord-americanos e guatemalanos.

Esseva constatate que le costaricanos de ambe sexos e de omne etates representate in le studio habeva valores medie de cholesterol que esseva levemente plus alte que illos del guatemalanos rural sed multo plus basse que illos del nord-americanos. In le costaricanos le tendentia de cholesterol seral de augmentar se con le etate del subjectos esseva paucamente evidente. Le nivellos S_f 12-20 e 20-100 in masculos rural de Costa Rica esseva solmente paucamente plus basse que in nord-americanos; in feminas illos esseva frequentemente plus alte. Tamen, iste valores tendeva a esser plus basse que in le previeamente studiate guatemalanos rural. Le nivellos S_f 0-12 esseva plus basse in le costaricanos rural que in le nord-americanos, e iste differentia sufficeva a coperir le plus grande parte del differentias in le nivellos de cholesterol.

Esseva monstrate que le plus grande magressa del costaricano rural non esseva un factor significative in le causation del plus basse valores de cholesterol. Proque le subjectos rural in Costa Rica esseva de origine europeica durante que le previeamente studiate gruppo de guatemalanos rural esseva predominantemente indianos mayan, le similaritate del resultatos obtenite in le duo populationes pare

eliminar le possibilitate que factores racial debe esser prendite in consideration e confirma le hypothese que factores ambiental, probabilissimamente nutritional, es responsabile pro le differentias observate inter centro- e nord-americanos rural.

Viste le observation que le populationes rural a basse revenitos in America Central es libere del complicationes de atherosclerosis, il es importante identificar le factores dietari e alteremente ambiental que es responsabile pro iste differentias.

Proque il existe solmente minor differentias in le concentrationes del lipoproteinas a S_f 12-100 inter le gruppos statounitese e centro-american sed grande differentias in le cholesterol seral e le lipoproteinas a S_f 0-12, iste ultime fractiones es forsan plus directemente connectite con le disveloppamento de atherosclerosis que le lipoproteinas a S_f 12-100.

REFERENCES

- ¹ MANN, G. V., MUÑOZ, J. A., AND SCRIMSHAW, N. S.: The serum lipoprotein and cholesterol concentrations of Central and North Americans with different dietary habits. *Am. J. Med.* **19**: 25, 1955.
- ² TEJADA, C. E., AND GORE, I.: Unpublished data, 1956. Comparison of atherosclerosis in Guatemala and New Orleans. Submitted for publication.
- ³ INSTITUTO DE NUTRICION DE CENTRO AMERICA Y PANAMA. Unpublished data, 1956.
- ⁴ AGUIRRE, F., TANDON, O. B., AND SCRIMSHAW, N. S.: Distribution of blood groups in Guatemalan Indians. *Rec. Genet. Soc. Am.* **22**: 63, 1953.
- ⁵ BOYD, W. C.: *Genetics and the Races of Man*. Boston, Little, Brown and Company, 1950.
- ⁶ SCRIMSHAW, N. S., MORALES, J. O., SALAZAR B. A., AND LOOMIS, C. P.: Health aspects of the community development project, rural area, Turrialba, Costa Rica. *Am. J. Trop. Med. & Hyg.* **2**: 583, 1953.
- ⁷ LOOMIS, C. P., MORALES, J. O., CLIFFORD, R. A., AND LEONARD, O. E.: *Turrialba. Social Systems and the Introduction of Change*. Glencoe, Ill., The Free Press, 1953.
- ⁸ INSTITUTO DE NUTRICION DE CENTRO AMERICA Y PANAMA: Tercera edición de la tabla de composición de alimentos de Centro América y Panamá. *Bol. Ofic. san. panam., Suppl.* **1**: 129, 1953.
- ⁹ —: Recomendaciones nutricionales para las poblaciones de Centro América y Panamá. *Bol. Ofic. san. panam., Suppl.* **1**: 119, 1953.
- ¹⁰ GOFMAN, J. W., LINDGREN, F., ELLIOTT, H. A., MANTZ, W., HEWITT, J., STRISOWER, B., AND HERRING, V.: The role of lipids and lipoproteins in atherosclerosis. *Science* **11**: 166, 1950.
- ¹¹ DELALLA, O. F., AND GOFMAN, J. W.: Ultracentrifugal analysis of serum lipoproteins. In *Methods of Biochemical Analysis* vol. 1. Edited by Glick, D. New York, Interscience Publishers, Inc., 1954, p. 459-478.
- ¹² ABELL, L. L., LEVY, B. B., BRODIE, B. B., AND KENDALL, F. E.: A simplified method for estimation of total cholesterol in serum and demonstration of its specificity. *J. Biol. & Chem.* **195**: 357, 1952.
- ¹³ Ideal Weights for Women. *Statistical Bulletin. Metropolitan Life Insurance Company*, **23**: 6, 1942.
- ¹⁴ Ideal Weights for Men. *Statistical Bulletin. Metropolitan Life Insurance Company*, **24**: 6, 1943.
- ¹⁵ UNITED STATES DEPARTMENT OF AGRICULTURE: Consumption of food in the United States, 1909-1952. U.S.D.A. Misc. Public. No. 62, 1953.
- ¹⁶ GLAZIER, F. W., TAMPLIN, A. R., STRISOWER, B., DELALLA, O. F., GOFMAN, J. W., DAWBER, T. R., AND PHILLIPS, E.: Human serum lipoprotein concentrations. *J. Gerontol.* **9**: 395, 1954.
- ¹⁷ HATCH, F. T., ABELL, L. L., AND KENDALL, F. E.: Effects of restriction of dietary fat and cholesterol upon serum lipids and lipoproteins in patients with hypertension. *Am. J. Med.* **19**: 48, 1955.
- ¹⁸ KEYS, A., ANDERSON, J. T., MICKELSEN, O., ADELSON, S. F., AND FIDANZA, F.: Diet and serum cholesterol in man: Lack of effect of dietary cholesterol. *J. Nutrition* **59**: 39, 1956.
- ¹⁹ —: Mode of life and the prevalence of coronary heart disease. Arteriosclerosis. A Symposium presented by the Minnesota Heart Association and the University of Minnesota. September 7-9, 1955. University of Minnesota, 1956, p. 28.
- ²⁰ —: Human atherosclerosis and diet. *Circulation* **5**: 115, 1952.
- ²¹ BEST, M. M., DUNCAN, C. H., VAN LOON, E. J., AND WATHEN, J. D.: The effects of sitosterol on serum lipids. *Am. J. Med.* **19**: 61, 1956.
- ²² MALMROS, H., AND WIGAND, G.: Treatment of hypercholesteremia. Arteriosclerosis. A Symposium presented by the Minnesota Heart Association and the University of Minnesota. September 7-9, 1955. University of Minnesota, 1956, p. 114.
- ²³ LEVER, W. F., AND WADDELL, W. R.: Idiopathic hyperlipemia and primary hypocholesteremic xanthomatosis. VII. Effect of intravenously administered fat on the serum lipids. *J. Invest. Dermat.* **25**: 233, 1955.

- ²⁴ KINSELL, L. W., FRIEKEY, R. W., MICHAELS, G. D., AND BROWN, F. R.: Effect of a synthetic triglyceride on lipid metabolism. *J. Clin. Nutrition* **4**: 285, 1956.
- ²⁵ BRONTE-STEWART, B., ANTONIS, A., EALES, L., AND BROCK, J. F.: Effects of feeding different fats on serum-cholesterol level. *Lancet* **1**: 521, 1956.
- ²⁶ AHRENS, E. H., JR., TSALTAS, T. T., HIRSCH, J., AND INSULL, W., JR.: Effects of dietary fats on the serum lipids of human subjects. *J. Clin. Invest.* **34**: 918, 1955.
- ²⁷ DEPARTMENT OF NUTRITION: Harvard School of Public Health. Unpublished data, 1956.
- ²⁸ LINDGREN, F. T., ELLIOTT, H. A., AND GOFMAN, J. W.: The ultracentrifugal characterization and isolation of human blood lipids and lipoproteins with applications to the study of atherosclerosis. *J. Phys. Colloid Chem.* **55**: 80, 1951.
- ²⁹ HAVEL, R. J., EDER, H. A., AND BRAGDON, J. H.: The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum, *J. Clin. Invest.* **34**: 1345, 1955.
- ³⁰ HILLYARD, L. A., ENTENMAN, C., FEINBERG, H., AND CHAIKOFF, I. L.: Lipid and protein composition of four fractions accounting for total serum lipoproteins. *J. Biol. Chem.* **214**: 79, 1955.