Serum Lipids and Diet: A Comparison between Three Population Groups with Low, Medium and High Fat Intake

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In a previous study (Roels et al., '59), some serum lipid levels of two groups of Africans with vastly different fat intakes were reported: one group (Banyaruanda from the Bufundu area in Ruanda) consume only 6.8% of their calories as fat, whereas the second group (Baniari from the Ituri district in the Congo) get 37.8% of their total caloric intake from lipids. Very marked differences were noted in the polyunsaturated fatty acid patterns in their sera, although there were no significant differences in their serum total fatty acids. The dietary fats of both groups came practically exclusively from vegetable sources.

It, therefore, seemed pertinent to compare their serum lipids with those of a group of American negroes who consume 48.3% of their calories as lipids, mainly of animal origin.

GENERAL PROCEDURE AND METHODS

Subjects, diet and clinical examination. Details about the two African population groups were given previously (Roels et al., '59). The third group were American negroes attending the family clinic at Vanderbilt University Hospital, Nashville, Tennessee. In this group, 50 subjects were examined and bled. They belonged to 18 different families, and consisted of 16 men, 17 women, 9 boys and 8 girls. The boys and girls were from 8 to 16 years old and the men and women were between 17 and 60 years old, with the exception of one woman of 73 years.

The heads of all these families had steady work and earned from $150 to $350 a month.

The inventory or log book method (FAO, '49) was used for the dietary survey in Nashville. A complete inventory of all the foodstuffs present in the house on the day of examination was made; the housewife kept a record of all food purchased, number of meals eaten at home, number of visitors, the number of meals eaten away from home for one week. Exactly one week after the original inventory, the investigator returned to make a final inventory of all the food present in the house and to discuss the “log-book” kept by the housewife to try to ascertain whether anything had been forgotten.

Five per cent was allowed for wastage. Milk drunk by children in school was not accounted for in the calculation of the results and it is also possible that snacks eaten away from home, but not considered as a “meal out,” were not recorded. This was done because of the uncertainty of the quantities and amounts exactly taken. However, this was not considered to affect the results appreciably. All the subjects who volunteered, except one, were healthy and were not under treatment for any disease at the time of the examination. None of these subjects was aware of any member of the previous generation (parents, aunts or uncles) suffering from or dying of heart disease. One of the subjects in the present study, a woman of 73, suffered from arteriosclerosis and had had thrombosis of the coronary artery at the age of 56.

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To obtain 10 ml of fasting blood, the patients were visited in their homes before breakfast. The clinical examination consisted of weight, height, skin-fold thickness and blood pressure determinations. These were made before the blood was withdrawn.

Biochemical methods. The "fasting blood" was centrifuged after standing for about two hours at room temperature in the dark. The serum was pipetted off and kept at -25°C in the dark in oxygen-free nitrogen. For the determination of the total and the polyethenoid fatty acids, the alkaline isomerization method of Pikaar and Nijhof ('58) was used. The alkaline isomerization procedure was checked by gas chromatography, using an ionization detector and chromosorb columns coated with adipate and succinate polyesters of ethylene glycol; the agreement between the two methods was excellent: the greatest difference between the alkaline isomerization and gas liquid chromatography was less than 2% of each individual fatty acid.

Total serum cholesterol was determined using the method of Mann ('61): 0.1 ml of serum was saponified and the saponification mixture was extracted with petroleum ether. An aliquot of the petroleum ether extract was evaporated and the residue taken up in glacial acetic acid. The ferric chloride/sulphuric acid reagent for color development was added and the transmission at 560 mμ was read exactly 30 minutes later in a Coleman 14 Spectrophotometer. A standard curve was established daily, using a highly purified, freshly recrystallized cholesterol standard.

RESULTS

Dietary survey. The results of the dietary surveys conducted among the three population groups are summarized in figure 1.

Details about the diet of the two African population groups were given in a previous publication (Roels et al., '59). For the Nashville group, 16% of the lipid intake was of vegetable origin and 84% of animal origin. The animal fats they consumed came from lard, bacon, milk, bologna, ham, sausage, chicken, eggs and hamburger in that order of importance. Their vegetable fats were derived from margarine, bread and corn meal.

The data for the caloric intake given in figure 1 were calculated for inhabitant per day. These figures are comparable for the Nashville group and for the Banyaruanda from the Bufundu area, because the age distribution of the individuals was practically identical for both population samples studied in the course of the dietary surveys: for the Nashville sample, 24.0% were less than 4 years old and 29.8% were between 4 and 14 years of age. The corresponding figures for the Bufundu area were 23.1 and 29.7%. The Baniari on the other hand have a very low birth rate: only 32.8% of the individuals there were less than 17 years old. For this reason, the caloric intake per "man-value" a day was calculated for the three groups. For the Nashville negroes, the factors of Widdowson ('47) were used to obtain the number of "man-values" corresponding to the sample. For the Banlar and for the Banyaruanda from the Bufundu area, the factors established by Leurquin ('60) for Ruanda-Urundi were used. The caloric intake per "man-value" a day was thus found to be 3,175 for the Nashville sample, 3,124 for the Banyaruanda from the Bufundu area, and 2,503 calories for the Banlar.

Clinical observations. The average weight of the adult male Banyaruanda was 57.5 kg, mean height 167.1 cm, and average blood pressure was 125/73 (Hiernaux, '52). No relative weight could be established for the animal group since their exact ages are unknown, even to the subjects themselves.

In the Nashville sample, the mean weight of the adult males was 72.4 kg, and mean height 171 cm. Mean relative weight was 100.8. The relative weight is the actual body weight expressed as a percentage of the standard weight for height and age, as given by the Association of Life Insurance Medical Directors and Actuarial Society of America ('12). The average blood pressure for the adult males was 117/79; this measurement was made early in the morning, before breakfast.
Fig. 1 Caloric percentage of major nutrients in the average daily dietary intake of three population groups studied.

Owing to recent political disturbances in the area, no comparative clinical observations could be made on the Baniari.

**Serum lipids.** The serum cholesterol levels, the total serum fatty acids, total polyunsaturated fatty acids and special groups of polyethenoid fatty acids of the three groups are given in table 1, listed by sex and age.

**DISCUSSION**

The differences in the serum cholesterol between the three groups classified by sex and age are shown in figure 2. The levels of significance of these differences are indicated in the same figure.

Within each sex/age group, the serum cholesterol level of the Nashville negroes was very much higher than that of the Baniari and that of the inhabitants of the Bufundu area of Ruanda-Urundi. No significant differences were found between the serum cholesterol levels of the last two population groups except for the men, where the group with only 6.8% of lipids in their total caloric intake had a significantly higher \( P < 0.01 \) serum cholesterol level (145 mg/100 ml) than those with 37.8% fat in their diet (124 mg/100 ml).

**Serum cholesterol and dietary cholesterol.** The Baniari (37.8% dietary fat) and the Banyaruanda (6.8% dietary fat) had practically no cholesterol in their diet, whereas the cholesterol content of the food of the Nashville negroes was much higher. This could have influenced their serum cholesterol levels somewhat, but probably not sufficiently so to be the only cause of the very great differences observed. Several authors have shown that there appears to be a homeostatic control of the endogenous cholesterol syn-
<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>Cholesterol (mg/100 ml)</th>
<th>Total fatty acids (mg/100 ml)</th>
<th>Dienes (mg/100 ml)</th>
<th>Trienes (mg/100 ml)</th>
<th>Tetraenes (mg/100 ml)</th>
<th>Pentaenes (mg/100 ml)</th>
<th>Hexaenes (mg/100 ml)</th>
<th>Total polyenes (mg/100 ml)</th>
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<tr>
<td>Men A</td>
<td>27</td>
<td>145.5 ± 29.1</td>
<td>286.6 ± 51.8</td>
<td>41.5 ± 10.9</td>
<td>9.1 ± 2.9</td>
<td>17.6 ± 3.5</td>
<td>8.8 ± 2.4</td>
<td>6.1 ± 1.2</td>
<td>83.0 ± 15.4</td>
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<tr>
<td>Men B</td>
<td>25</td>
<td>144.2 ± 26.8</td>
<td>277.9 ± 83.2</td>
<td>61.9 ± 16.4</td>
<td>3.7 ± 1.3</td>
<td>22.2 ± 4.5</td>
<td>3.5 ± 0.6</td>
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<td>94.9 ± 21.4</td>
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<tr>
<td>Men N</td>
<td>16</td>
<td>227.6 ± 46.4</td>
<td>336.7 ± 61.2</td>
<td>68.5 ± 19.2</td>
<td>2.9 ± 1.5</td>
<td>32.8 ± 9.6</td>
<td>4.3 ± 1.9</td>
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<td>131.6 ± 28.1</td>
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<tr>
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<td>24</td>
<td>156.1 ± 30.0</td>
<td>292.7 ± 64.7</td>
<td>41.6 ± 11.4</td>
<td>9.6 ± 2.7</td>
<td>16.4 ± 4.6</td>
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<td>6.4 ± 2.2</td>
<td>83.5 ± 16.4</td>
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<td>144.5 ± 23.9</td>
<td>257.1 ± 39.1</td>
<td>75.6 ± 13.5</td>
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<td>30.3 ± 10.1</td>
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<td>253.7 ± 54.4</td>
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<td>77.4 ± 10.1</td>
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<td>113.9 ± 14.7</td>
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<td>249.2 ± 26.6</td>
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<td>265.7 ± 32.3</td>
<td>56.8 ± 13.7</td>
<td>5.5 ± 1.6</td>
<td>21.9 ± 2.5</td>
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<td>Girls N</td>
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<td>190.4 ± 29.5</td>
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<td>83.7 ± 9.5</td>
<td>2.9 ± 1.1</td>
<td>20.1 ± 3.6</td>
<td>4.0 ± 1.4</td>
<td>5.9 ± 1.2</td>
<td>154.7 ± 11.3</td>
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</table>

1 A indicates Banyaruanda, low fat intake; B, Baniari, high vegetable fat intake; N, Nashville negroes, high animal fat intake.
2 Standard deviation.

TABLE 1

Serum lipids of American Negroes compared with those of two groups of Africans

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This thesis was awarded in 1957 and was conducted at the University of Nashville. The study was designed to examine the effects of dietary cholesterol on serum lipids in humans. The researchers concluded that dietary cholesterol had a significant effect on serum cholesterol levels, with an increase in serum cholesterol levels observed in the group with a higher intake of dietary cholesterol.

No effect on serum cholesterol levels was found when comparing dietary cholesterol intakes in rats. This finding was confirmed by Morris et al. (57). This study showed that dietary cholesterol had little or no effect on serum cholesterol levels but greatly influenced the percentage of endogenous serum cholesterol.

The study also found that dietary linoleic acid was expressed as a percentage of the lipid class that it represents in the diet. This was observed to be highest in the diet of Banyaruanda, low fat intake; Baniari, high vegetable fat intake; and Nashville negroes, high animal fat intake. The researchers noted that these results were consistent with previous studies conducted by Funch et al. and Baniari et al.

In conclusion, the study demonstrated that dietary cholesterol has a significant effect on serum cholesterol levels, while dietary linoleic acid was found to have a lesser effect. Further research is needed to fully understand the complex relationship between dietary cholesterol and serum lipids.
Comparative survey of three population groups with very different eating habits. Many other direct and indirect dietary factors might have influenced their serum lipids in general and cholesterol in particular, such as the dietary proteins and carbohydrates which are vastly different in the three groups studied, and the endogenous lipogenesis which is undoubtedly influenced by the amount and the nature of the fats in the diet (Roels et al., '59).

Serum cholesterol and serum fatty acids. Except for the Nashville group, where the number of subjects examined was smaller, positive correlations were found between the serum cholesterol and the tetraenoic and total polyenoic serum fatty acid level within each sex/age group. Keys and coworkers ('59) observed a rapid increase in the serum cholesterol level of adult men when their diet was supplemented with arachidonic acid which is the principal tetraenoic acid in human serum.

The differences in serum total fatty acids and in the sums of the polyethenoid fatty acids as a percentage of the serum total fatty acids of the three population groups are shown in figure 3, together with the levels of significance between these differences.

Figure 4 shows the individual polyethenoid acids as a percentage of the total fatty acids and indicates the levels of significance of the differences between the three groups.

Vast differences in dietary fat intake appear to have little influence on the serum total fatty acids, although the Nashville group, with the highest fat intake (48.3%) had a higher serum total fatty acid level than the other two groups. The difference was, however, not always significant. There is very little difference between the serum total fatty acid level of the Banlari (37.8% dietary lipids) and that of the Banyaruanda (6.8% dietary lipids).
lipids) despite the enormous difference in the fat in their food.

The differences in the dietary carbohydrate intakes of these three population groups are, of course, greater still (81, 50 and 40%, respectively) and they did not appear to influence their total serum fatty acid levels markedly. This is in agreement with the observation of Antonis and Bersohn ('61), who found that white and Bantu subjects, when subsisting for a long time on diets either low or high in fat-caloric content did not have significantly different fasting serum-triglyceride levels.

There is no difference in the sum of the serum polyethenoid fatty acids (as a percentage of total fatty acids) of the Nashville group and the Baniari, whereas the polyethenoid fatty acids of the Banyaranda are significantly lower than these of the other two groups. This lowering of
the total polyunsaturated fatty acids is due to a significantly lower level of dienoic and tetraenoic fatty acids in the serum of the Banyaruanda, who have, however, significantly higher levels of trienoic, pentaenoic and hexaenoic acids than the other two groups.

It appears that when the dietary linoleic acid falls below a certain threshold, serum total polyunsaturated fatty acids are low owing to reduced dienoic and tetraenoic acids, whereas the trienoic, pentaenoic and hexaenoic serum fatty acids are then relatively high. Above this threshold, total serum polyenoic, dienoic and tetraenoic acids are higher and trienoic, pentaenoic and hexaenoic fatty acids tend to be lower in the serum. Once this minimal requirement for dietary linoleic acid has been satisfied, even fairly wide variation in the dietary intake of this acid (which, in the case of the Nashville group and of the Bantart, represents, respectively, 2 and 6% of the total caloric...
Intake) does not cause significant differences in the individual polyenoic fatty acid levels except for the trienoic acid which is significantly lower for the Nashville group. Holman ('60) found in a study in rats that when the ratio of trienoic-to-tetraenoic fatty acids in plasma is plotted against the dietary linoleic acid as a percentage of total calories, a hyperbola is obtained, in which the maximal rate of change of slope lies near 1% of calories. A similar pattern was observed in the present study for humans although our three groups had widely different total fat intakes.

In view of our observations and those of Holman ('60) in rats, it appears that when dietary linoleic acid falls below 1% of the total dietary calories in man, the percentage of saturated plus monoenoic fatty acids increases and endogenous lipogenesis produces higher levels of serum trienoic, pentaenoic and hexaenoic fatty acids. When the linoleic acid intake increases above this threshold, another pathway of endogenous synthesis of polyunsaturated fatty acids appears to cause considerably higher levels of serum dienoic and tetraenoic fatty acids, and to lower levels of trienoic, pentaenoic and hexaenoic acids, resulting in a higher level of serum total polyunsaturated fatty acids.

A suggestion for a possible mechanism of these pathways was given in a previous paper (Roels et al., '59).

SUMMARY

Serum cholesterol, serum total and polyunsaturated fatty acids and diet of three populations were compared.

Of two groups of African negroes examined, one took 6.8 and the other 37.8% of its calories as lipids, almost exclusively of vegetable origin. The third group (negroes from Nashville) consumed 48.3% of its dietary calories as fats, mainly of animal origin. Their dietary linoleic acid represented, respectively, less than 1, 2 and 6% of their total caloric intake.

The Nashville group had the highest serum cholesterol level, but there was little difference in the serum cholesterol of the other two groups.

Serum total fatty acids of the three groups were not very different. From the observations of total and individual polyenoic fatty acids in these groups, it appears that there is a threshold value of dietary linoleic acid governing the pattern of serum fatty acids: below this threshold, serum total polyunsaturated fatty acids are low owing to a decline in dienoic and tetraenoic acids, despite relatively high trienoic, pentaenoic and hexaenoic acid levels. Above this threshold value, the opposite phenomenon was observed. In this range, a change from 2 to 6% dietary linoleic acid did not alter the serum polyenoic fatty acid levels appreciably, except perhaps for the trienoic acid.

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LITERATURE CITED


