Biologic characteristics of the Masai of East Africa

Kurt Biss, M.D., Kang-Jye Ho, M.D., Ph.D., Belma Mikkelson, B.S., Lena Lewis, Ph.D., and C. Bruce Taylor, M.D.

Abstract
The Masai of East Africa exhibit some unusual biologic characteristics. Despite their customary diet composed of 66 percent calories as fat, they have persistent low serum cholesterol and beta-lipoprotein levels. Post-mortem examinations were provided direct proof of a paucity of atherosclerosis clinically onuliminary. Post-mortem examinations revealed that the Masai absorbed large amounts of dietary cholesterol, but also possessed a highly efficient negative feedback control of endogenous cholesterol biosynthesis to compensate for the influx of dietary cholesterol.

Dietary Habits

Milk is their main staple, but they are also fond of fresh cow’s blood and the meat of cattle, sheep and goats. They drink milked directly in the ground. The milk is drunk either fresh or fermented, since bacterial fermentation takes place in the gut quite promptly. When enough milk is available, the average Masai will consume 3 to 5 liters of milk daily, divided usually into two meals. During the dry season, which lasts for four to five months, when the supply of milk dwindles, they will bleed the cattle and mix the blood with milk or slaughter a goat or under extreme circumstances one of their beloved Zebu cattle.

Fifty-five milk samples from their Zebu cattle were obtained during different seasons (wet and dry) and from two different areas (Narok and Kilgoris). The results of analyses revealed a seasonal variation in the fat content, which was lower in the dry season and higher in the wet season. Protein and sugar contents, on the other hand, were quite constant in all specimens. The nature of fermentation by microorganisms on milk composition was not constant. The total lipids and cholesterol contents of Kilgoris April milk showed a significant amount of fat fermentation, whereas a marked reduction of total lipids and cholesterol was noted in Kilgoris June milk after fermentation. The phospholipid content of milk in both seasons was also reduced after fermentation.

As compared with standard milk in the United States, their milk contains larger amounts of total phospholipid but lesser amounts of sugar (Table 1). No appreciable difference was noted in the protein contents. Because of its high phospholipid content, the Masai milk provides more calories than the same amount of milk in the United States. The average daily caloric intake was estimated to be about 3000 calories, with 69 percent of the calories derived from fat. The estimated average daily cholesterol intake was 500 to 2000 mg per person, which is comparable to that for the adult population in the United States. During the dry season, when a thick mixture of milk and blood is drunk and occasionally large amounts of meat are consumed (1814 to 2285 g at one meal), they had a much higher intake of cholesterol and fat than the average intake of the population in the United States.

Paucity of Atherosclerosis

Mann and his associates revealed that the Masai had low serum cholesterol and were free from clinical evidence of atherosclerosis despite their customary high fat diet. To confirm this unusual biologic characteristic, we performed 10 consecutive autopsies at the Narok District Hospital, Narok, Kenya. Gross, histologic and chemical studies of the aortas and coronary arteries gave direct proof of the paucity of atherosclerosis. Only occasional fatty streaks were found in younger aortas, and fibrin plaque showed normal thickness in older aortas. We measured the thickness of coronary arterial walls by a specially designed caliper, measuring from 0.5 mm, by Daud et al. The results demonstrated that the Masai coronary arteries had much thinner walls than those of whites in the United States matched for age and sex.

Low Serum Cholesterol Levels

Studies accomplished by the International Atherosclerosis Project revealed a highly significant positive correlation of serum cholesterol levels with fat calories in the diet. The low serum cholesterol level of the Masai observed by Mann and his associates was an exception to the rule. To confirm Mann’s extraordinary observation, we collected fasting blood samples by the finger-paper method from 100 healthy Masai blood donors and 134 Massai patients from various ages, including 27 pregnant women, at the Narok District Hospital, Narok, and at St. Joseph’s Hospital, Kilgoris, Kenya. A standard solution of cholesterol was also applied to the filter paper and showed no deterioration in transport. The serum cholesterol levels were determined by the method of Ablil et al. at Evanston Hospital, Evanston, Illinois.

The average serum cholesterol level of the Masai over 15 years was 135.4 ± 33.5 mg per 100 ml (mean ± S.D.), which agrees very well with the findings of Mann et al. The distribution of serum cholesterol levels of various age groups fits a straight-line equation best: Y = 130.16 + 0.12x (here Y is serum cholesterol level in milligrams per 100 ml at the age of X years [correlation coefficient 0.96, standard deviation of equation 0.103]). The small value of the slope (0.12) indicates no significant increase of serum cholesterol levels with an increase of age. The lowest level was 77 mg per 100 ml. Among 227 subjects studied, excluding the pregnant females, only seven had serum cholesterol levels above 200 mg per 100 ml. Six of these were between 200 and 240 mg per 100 ml.

A total of 27 pregnant women, 11 were in the first and second trimesters of gestation and showed no increase in serum cholesterol levels (p greater than 0.5). However, the average serum cholesterol level (204 ± 51.4 mg per 100 ml) of the 16 women in the third trimester represents a 50 percent increase over the normal value of the nonpregnant controls (p less than 0.001). Hypercholesterolemia during pregnancy seems to be a rather unusual human phenomenon from which Masai women are not exempt.

Serum Lipoprotein Patterns

Fresh serum specimens were collected from 34 Masai, packed in ice and flown by air express from Narok to the Cleveland Clinic for lipoprotein studies. Samples constantly refrigerated in ice were analyzed within five days after collection. Serum lipoproteins were separated by the paper nonphoretic technic of Lees and Hatch. An alkalinated buffer was used. For comparison, serum lipoproteins of 41 normal adult males from the Caucasian and the Chinese populations of the Cleveland area were also determined. The technique. The lipoprotein patterns of the Masai characterized by low beta-lipoproteins, only 15 percent being classed greater than 2+. Levels of pre-beta-lipoproteins were consistently low, and those of alpha-lipoproteins were comparable to those of Caucasians of similar age and sex.
**HOMEOSTATIC CONTROL OF CHOLESTEROL METABOLISM**

The experiment was designed to investigate the basic mechanisms that protect the Masai from hypercholesterolemia on a diet rich in cholesterol and animal fat during their entire lifetime. Hypothetically, there are four possible homeostatic mechanisms for the control of cholesterol metabolism and for the maintenance of serum cholesterol levels within low, minimally atherogenic limits. These are as follows: limitation of intestinal absorption of dietary cholesterol, inhibition of cholesterol synthesis by dietary cholesterol, increase in the rate of fecal output of body cholesterol and its metabolic products (bile acids); and transfer and storage of serum cholesterol in tissues.

Twenty-four healthy male Masai students, 18 through 24 years of age, of the Nkorock Secondary School, were divided into two equal groups. Two grams of crystalline cholesterol per person per day with a trace dose of cholesterol-4,4-C, mixed well in a basic diet, were given to each subject in the experimental group. The basic diet was composed of a powdered cream substitute composed mainly of corn-syrup solids and vegetable fats, corn, beans, sugar and Maasai corn oil and was cholesterol free. The control subjects received only the same trace dose of cholesterol-4,4-C mixed in the basic diet. Blood and stool samples were collected weekly for eight weeks, and the specific activity of serum and fecal cholesterol was determined.

The trace dose of cholesterol-4,4-C was discontinued at the end of the eighth week and the decreasing curve of the serum cholesterol specific activity was followed for another six months. From the specific activities of dietary cholesterol, serum cholesterol and fecal cholesterol and acid sterols, and the disappearance curve of serum cholesterol, the following functions could be calculated: rates of absorption, synthesis and turnover; and size and turnover time of body cholesterol pool. These calculations are presented in the May, 1971, issue of the Archives of Pathology. The results revealed no significant differences in serum cholesterol, phospholipid, triglyceride levels and lipoprotein patterns between experimental and control subjects even though the experimental subjects were challenged with a daily dose of 2 g of cholesterol. The sizes of the exchangeable body cholesterol pools and their fractional catabolic rates were also not different (Table 2). This indicates no storage of excess cholesterol in the tissues during cholesterol feeding in the experimental subjects. The similarity of the turnover times and turnover rates of body cholesterol in both experimental and control subjects (as shown in Table 2) minimized the possibility that an increase in serum cholesterol was an important control mechanism for homeostasis of cholesterol metabolism.

The turnover rate of body cholesterol in the control subjects was the same as the rate of synthesis (1.37 ± 0.15 g per day) since the diet contained only a negligible amount of radioactive cholesterol, whereas the turnover rate in the experimental subjects was the sum of the rate of synthesis and the rate of absorption. Each day the experimental subjects absorbed 0.65 g of cholesterol from 2 g of cholesterol present in their daily ration. This amount of absorption accounted for 50 per cent of the daily turnover of body cholesterol in these subjects. The other 50 per cent, or 0.65 g, was synthesized endogenously. This was much less than the amount synthesized by the control subjects.

Therefore, there was a remarkable suppression of endogenous cholesterol synthesis by cholesterol feeding, a so-called negative feedback phenomenon. The degree of this suppression was 50.5 ± 8.7 per cent (mean ± S.D.) and compensated perfectly for the rate of synthesis. Thus, cholesterol was absorbed from the intestine. This efficient feedback mechanism was the only homeostatic mechanism that protected the Masai from the development of dietary-induced hypercholesterolemia.

As compared with the cholesterol metabolism in white males, the United States, the Masai had a smaller pool size and a shorter turnover time (Table 3). This might be due to the difference in the body weights. None of the Masai subjects was overweight — in fact, their weights were much lower than the normal weights for given heights accepted in the United States. The fact that of cholesterol synthesis, the other hand, was quite similar in both ethnic groups. As far as the homeostatic mechanisms were concerned, American whites have some what limited maximal capacity for intestinal absorption of dietary cholesterol, whereas the Masai are capable of absorbing twice as much as this maximal capacity. In contrast to the highly efficient negative feedback control of endogenous cholesterol synthesis in the Masai, a much lesser degree of suppression was observed in white inhabitants of the United States. Neither ethnic group had any evidence of increase in fecal sterol excretion and variable tissue storage of cholesterol upon cholesterol feeding except for marked deposition of cholesterol in the arterial tissue in the American whites, which is one of the manifestations of atherosclerosis.

Because of the distinct differences in the cholesterol metabolism between Masai and whites, and the similarity in fat and cholesterol intake of the two populations, it is strongly suggested that a basically different genetic trait exists in the Masai.

**UNUSUAL SERUM-PROTEIN PATTERNS**

Serum samples were obtained from 54 subjects, including 12 one-month-old infants, 11 mixed with the one-year-old children, three children three years old and 25 young adults. The serum total protein was measured by the biuret technic® and protein fractions determined by electrophoresis® with the use of barbitral buffer, pH 8.6, ionic strength 0.075. The radiiodination of the serum albumin and alpha and beta globulins was also determined to determine the concentration of IgA, IgM and IgG immune globulins. The serum total protein and its fractions and immune globulins were also determined in 12 adult white residents of the Cleveland area by the above technics.

The total serum protein concentration of adult Masai was 3.2 ± 0.3 g per 100 ml, which was significantly higher (p less than 0.001) than that of whites in the United States, 7.05 ± 0.42 g per 100 ml. The levels of alpha, and beta globulins in both groups were highly similar. The alpha-globulin concentration of the Masai was slightly but significantly lower (p less than 0.05) than that of the American ethnic groups. Paper electrophoretic patterns of the Masai sera showed a partial resolution of the alpha-globulin fraction into two bands, alpha, and alpha, globulins. Serum of healthy people in the United States studied by the same technics showed no tendency for the alpha-globulin fraction to separate into two bands. The importance of the presence of this double alpha, band is not clear.

The average serum gamma-globulin concentration of the Masai was 1.4 ± 0.37 g per 100 ml, which was significantly greater (p less than 0.001) than that of whites in the United States, 0.95 ± 0.20 g per 100 ml. Radial immunodiffusion studies revealed that their higher gamma-globulin concentration was contributed to mainly by high IgA levels. The mean level of IgA in the Masai was 297 mg per 100 ml, which was almost twice as high as that of whites in the Cleveland area (135 mg per 100 ml), whereas the levels of IgG and IgM in the two populations did not differ significantly.

IgA globulin does not pass through the placental barrier, is not present in newborn serum and develops slowly during the first decade of life in whites. Unlike American children, in whom adult levels of IgA globulin were not reached until they were 10 to 12 years of age, the Masai baby at one year of age had levels as high as the adult Cleveland, and by four years of age the level was doubled and reached the adult Masai level.
The available information suggests that the secretory immunoglobulin or IgA may have an important role in the adaptive immune response against potentially pathogenic organisms. The production of IgA has been demonstrated to be stimulated by several microbial factors. Early development of high levels of serum IgA in the Masai children is probably important in their survival in an environment that is significantly devoid of pathogenic challenge. A similar phenomenon of IgE instead of IgA was observed by Johannson et al., who found that the Ethiopian children who had severe infections had IgE levels 16 to 20 times higher than those of Swedish children of like age and IgG levels five to six times higher. The levels of IgE, IgG and IgM of the two groups varied much less. A group of the Ethiopian children, who were found to have high levels of IgA, had IgE levels 28 times higher than those of the Swedish children. It is obvious that this parasitic antigen has a more effective immune-stimulating IgE production. We have also studied other, less developed population groups, Peruvian Indians and St. Kitts Negroes, and found that they had high serum gamma globulin, but the individual IgG levels were not determined.

This demonstrates another unique biological characteristic of the Masai. During their long flight for survival against myriads of micro-organisms, they gradually adapt to their environment by developing high levels of seromucous antibodies to protect them from the invading micro-organisms. Their effective feedback control of cholesterol synthesis for prevention of developing hypercholesterolemia was mentioned earlier. Such unique characteristics may be related to their genetic codes and are probably transmissible to subsequent generations.

ABSENCE OF CHOLESTEROL GALLSTONES

Cholesteroliasis, especially cholesterol gallstones, is a common and important disease in the United States, Scandinavia, Australia and New Zealand, but it is much less common in Asia and Africa. One of us (K.B.) practiced medicine in the Masailand for six months and confirmed that the Masai are virtualy free from cholesterol gallstones. The analysis of bile composition of Masai and whites may give some clues to the factors affecting cholesterol gallstone formation.

A total of 140 gallbladder-bile samples were obtained from the following four hospitals: Narok District Hospital, Narok, Kenya; Evanston Hospital, Evanston, Illinois, United States; Green Lane Hospital, Auckland, New Zealand; and Helsinki Hospital, Helsinki, Finland (Table 4). The samples were collected from patients either at the time of surgery for cholecystolithiasis or a biliary-tract disorder or within six hours post mortem. A Folch extraction of the bile was used for cholesterol analysis by the method of Abel et al. and for phospholipid determination by the method of Goodwin and his co-workers. Methanol-extracted bile was used for total bile acid determination by the method of Goodwin and his co-workers. Methanol-extracted bile was used for total bile acid determination by the method of Goodwin and his co-workers. Methanol-extracted bile was used for total bile acid determination by the method of Goodwin and his co-workers. Methanol-extracted bile was used for total bile acid determination by the method of Goodwin and his co-workers. Methanol-extracted bile was used for total bile acid determination by the method of Goodwin and his co-workers.

Table 4. Comparison of the Ratios of Three Major Gallbladder-Bile Components among different Ethnic Groups.

<table>
<thead>
<tr>
<th>Ethnic Group</th>
<th>NOS. of CASES</th>
<th>RATIO OF PHOSPHOLIPIDS TO CHOLESTEROL</th>
<th>RATIO OF BILE ACIDS TO CHOLESTEROL</th>
</tr>
</thead>
<tbody>
<tr>
<td>With gallstones:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Finland</td>
<td>34</td>
<td>2.4 ± 0.9</td>
<td>6.8 ± 2.7</td>
</tr>
<tr>
<td>New Zealand</td>
<td>11</td>
<td>2.0 ± 0.5</td>
<td>5.4 ± 1.3</td>
</tr>
<tr>
<td>U.S.</td>
<td>27</td>
<td>2.7 ± 1.0</td>
<td>9.1 ± 4.3</td>
</tr>
<tr>
<td>Without gallstones:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Finland</td>
<td>11</td>
<td>3.8 ± 1.0</td>
<td>8.6 ± 2.3</td>
</tr>
<tr>
<td>New Zealand</td>
<td>13</td>
<td>3.9 ± 2.1</td>
<td>17.3 ± 8.1</td>
</tr>
<tr>
<td>U.S.</td>
<td>11</td>
<td>4.1 ± 1.7</td>
<td>24.5 ± 12.4</td>
</tr>
</tbody>
</table>

Figure 2. Triangular Co-ordinate Plotting of Three Major Gallbladder-Bile Components among different Ethnic Groups.

For the gallstones with this falling in the zone where excess cholesterol is present. On the other hand, in the patients without gallstones, except for those from Finland, the molar ratios of these three major bile components fell within the mircellar zone. The ratio for Masai bile was far down in the micellar zone. The gallbladder bile of the Masai thus had an enormous reserve capacity to dissolve cholesterol, and this highly soluble system in the bile protected them from cholesterol-gallstone formation.

REFERENCES