Relationship between Total Cholesterol and Cholesteryl Esters with Age in Human Blood Plasma

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Evidence continues to accumulate in support of the concept that the serum cholesterol level may be influenced by the dietary intake of cholesterol and the type and amount of fat in the diet; other factors such as the dietary intake of simple sugars, age, exercise, weight, and metabolic abnormalities such as diabetes mellitus may also be influential. It is also generally agreed that elevations of serum cholesterol from whatever cause may be one of the key factors associated with the pathogenesis of coronary artery atherosclerosis. These facts provide the basis for relating nutrition to the etiology of atherosclerosis.

Although cholesterol circulates in the blood primarily in the esterified form, relatively few studies have been reported on large numbers of subjects to explore the significance of variations in cholesterol esters in the atherosclerotic process or indeed to determine how the cholesterol ester fractions fluctuate with age.

The relative proportion of free to esterified cholesterol in the plasma is generally considered to be relatively constant. As early as 1936, Sperry stated (1, 2) that the proportion of free to esterified cholesterol remains quite stable despite large differences in the total amount of cholesterol present. Similar observations have been made by Boyd (3) and Foldes and Murphy (4). However, very recently Leonard et al. (5) studied Africans and Asians in East Africa, and reported that “as the total cholesterol level increased, the increase in the free cholesterol fraction was proportionally greater than the increase in the esterified fraction.” Most of the available information on cholesterol esters deals with this fraction as a single entity, but there are reasons to believe that the distribution of cholesterol esters as individual components may be important in determining the physiological influence of the cholesterol fractions of the plasma. Difficulties in methodology have limited the development of information and understanding of the physiological significance of the esterified fractions of cholesterol.

In 1965 (6) we published evidence confirming the existence of “types” of cholesterol ester fatty acid (CEFA) patterns: an “adult” type in which cholesteryl linoleate predominates over cholesteryl oleate, and an “infant” type in which the reverse is observed, i.e., a predominance of cholesteryl oleate over cholesteryl linoleate. A nutrition survey among children and a study of coronary proneness in working adults in Iowa provided an abundance of plasma samples with which to evaluate the cholesterol and CEFA pattern in young...
and adult people and to extend the knowledge concerning the changing cholesterol ester pattern with age.

The present study, therefore, deals with the changes which occur with sex and age in the plasma of total and esterified cholesterol in a large group of individuals ranging from 12 to 70 years of age.

**METHODS**

The younger subjects of the group were students participating in the Iowa nutrition survey. The statistical planning of this school survey has been previously discussed (7). Similar planning was involved in the organization of the industrial survey which provided the plasma samples of the older individuals studied.

Blood was obtained in the field as a casual specimen from the antecubital vein 1–3 hr after meals, the intervening period being devoted to routine school or factory work. Serum was separated promptly, frozen for preservation, and subsequently prepared for lipid analysis, as described elsewhere (8).

Total cholesterol was determined using the Technicon automated method which is a modification of the one by Zlatkis, Zak and Boyle (9). This method is based on the reaction of concentrated sulfuric acid and ferric chloride in acetic acid upon steroids having the 5-N, 3-β-ol grouping. It has been claimed, however, that this method tends to give lower values for total cholesterol (10, 11) because of the incomplete and variable color development of cholesterol esters as compared to free cholesterol (12).

Plasma CEFA were determined by a glass paper chromatographic method based upon the one described by Muldrey et al. (13). This consists of chromatography of the serum or plasma extract on silica-gel impregnated glass paper and densitometry of the charred spots which are produced with sulfuric acid and heat. Four well-separated and defined spots are obtained by this method corresponding to four different classes of cholesteryl esters as indicated in Fig. 1.

**RESULTS**

Information relative to the age and sex distribution of the subjects comprising our sample is given in Fig. 2. The larger number of young people reflects the very extensive nutrition survey carried out over a 3-year period in the school population of Iowa, and the preponderance of men versus women in the more advanced ages relates to the characteristics of the labor force of the factories studied in the coronary proneness survey.

**Total cholesterol.** Figure 3 represents the distribution of values for total serum cholesterol. It shows an almost normal (Gaussian) distribution of the values, although slightly skewed. This skewness is not significant and probably results from the presence of older people in the group.

For comparing the relationship between age and serum cholesterol concentration of males and females, measurements were
made on 2,691 individuals (1,653 males and 1,038 females). The data are summarized in Table 1 and Fig. 4. It is noted that before the age of 20, there is only a slight difference between the serum cholesterol values of girls and boys, with slightly higher values for the girls. From age 20 to ages 45–50, in both men and women, the plasma cholesterol continues its upward trend with age, with higher values for men than the women. From then, the level of plasma cholesterol in women tends to overtake and surpass that of men of comparable age, probably reflecting the hypercholesterolemic effect of the menopause.

In order to evaluate the identity of the two sexes in regard to cholesterol, regression lines of the relationships between serum cholesterol and age were calculated for both sexes. From these data summarized in Table II, it can be concluded that there is a significant identity between the two sexes in regard to the relationship of plasma cholesterol values and age (the differences between the slopes in both sexes are not statistically significant); however, in both sexes the average annual change of total serum cholesterol in mg/100 cc is statistically significantly different from zero slope. The identity of the two sexes is even more marked if we calculate the cholesterol values for both sexes for

![Graph](https://academic.oup.com/ajcn/article-abstract/20/8/808/4787819/fig3)

**Fig. 3. Distribution of the total plasma cholesterol values of the sample.**

### Table 1

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* Milligrams/100 ml.
identical mean ages using the respective regressive equations. For example, at age 22, the mean cholesterol value for men is 165.7 mg/100 ml versus 166.6 for women of the same age; at age 32 (mean age of the men in our group) men have a cholesterol of 179.3 mg/100 ml and women, 181.3; at age 28 (mean age for our female group) men have 173.8 mg/100 ml and women, 175.8 mg/100 ml.

Since there is no significant difference in the plasma total cholesterol between normal males and females, we calculated the regression line for both sexes combined:

\[ y = 134.8 + 1.49x \]

which represents the change in plasma cholesterol with age in a total of 2,691 individuals ranging in age from 12 to 74 years.

Cholesteryl esters. In Figs. 5 and 6, the changing trend with age of the total esterified cholesterol and of each individual ester is presented for both males and females. It can be observed that there is a general tendency toward increased values with age, at least until 45-50 years, after which a plateau seems to be reached. The regression equations (Table II) show a positive correlation with age for each of the cholesteryl esters in both sexes. This correlation is statistically significant for each of the esters except for the S group of esters (cholesterol esterified with saturated fatty acids).

What is the contribution of each ester to the yearly increase in the level of esterified cholesterol? In Table I are the values for each of the cholesteryl esters ex-

![Graph showing total plasma cholesterol levels by age in males and females.](https://academic.oup.com/ajcn/article-abstract/20/8/808/4787819/66x36to546x756)

**Fig. 4.** Total plasma cholesterol levels by age in males and females.

### Table II

<table>
<thead>
<tr>
<th>Item</th>
<th>No.</th>
<th>A0</th>
<th>A1</th>
<th>SSE</th>
<th>Mean Age</th>
<th>r²</th>
<th>p</th>
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<td>133.72</td>
<td>1.362</td>
<td>1,154.20</td>
<td>32.50</td>
<td>0.505</td>
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<td>S</td>
<td>972</td>
<td>24.52</td>
<td>0.056</td>
<td>210.02</td>
<td>31.28</td>
<td>0.056</td>
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<td>O</td>
<td>976</td>
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<td>187.67</td>
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<td>L</td>
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<td>88.04</td>
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<td><strong>Females</strong></td>
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<td>Total Cholesterol</td>
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<td>1,063.94</td>
<td>28.42</td>
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<td>0.059</td>
<td>190.81</td>
<td>27.93</td>
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<td>30.01</td>
<td>0.282</td>
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<tr>
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<td>89.81</td>
<td>27.96</td>
<td>0.347</td>
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</table>

\[ y = A_0 + A_1x. \]

*a SSE = residual sum of squares.

*b r = correlation with age (linear correlation coefficient).

pressed as percent of the total esterified fraction. Although there seems to be a tendency for a decrease in the percent of cholesteryl linoleate as age increases, this tendency does not seem to be significant.

In Fig. 7 are represented the regression lines for total cholesterol and its esters. This figure shows an annual change (slope) of total cholesterol larger than the annual change of total ester, which implied that the nonesterified fraction of cholesterol increases with age. However, the differences in the slopes are not statistically significant. In order to evaluate further this possibility, in Fig. 8 we have plotted the percent of cholesterol which is esterified versus age, and it was found that the percentage contribution of age factor is 1.8 which is not significant ($P > 0.05$).

Also the relationship between the absolute amounts of total-to-free cholesterol was calculated using the regression equations of Fig. 7. From these data the regression equation appears to be (Fig. 9):

Free cholesterol = 0.28 total cholesterol - 16.8

This equation could allow us to calculate the free cholesterol knowing the total cholesterol value, but Keys (14) has pointed...
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out the inadequacy of this type of calculation.

The Pearson's product moment correlations between the variables included in this study show a positive correlation ($r = 0.62$) between total cholesterol and esterified cholesterol; also, a positive correlation between cholesteryl linoleate and esterified cholesterol ($r = -0.75$) and a very high negative correlation between cholesteryl esters of saturated fatty acids and cholesteryl linoleate ($-0.97$).

**DISCUSSION**

Numerous reports have been made of the total serum cholesterol levels in man (15–19). These reports and the present findings agree that serum cholesterol increases with advancing age. The values reported here are somewhat lower for the same age groups than some of those reported in the literature for United States communities (17, 18). This may relate to differences in the methodology employed or to differences in the composition of the sample, or both. For example, Keys et al. (17) studied a geographically similar population as the one reported here, but theirs was an upper metropolitan population engaged mainly in business, professional, and scholastic pursuits, whereas ours was mainly a rural sample composed of vigorous school youngsters and active factory workers.

Regardless of the absolute values found, the mean annual increase of the total serum cholesterol value for men in the present study (3.2 mg/100 ml) is similar to the one reported by Adlersberg (18), 3.6 and 3.3 mg/100 ml in Keys' study.

Our study also shows (Fig. 4) the tendency to lower serum cholesterol values in older ages; as Keys has pointed out (17), this may very well reflect the result of a process of selection by which persons having lower cholesterol values tend to survive longer. The apparent sex difference (Fig. 4) in the plasma cholesterol values before age 19 and after ages 45–50 is not significant; these findings agree with the reports of Keys (17) and Adlersberg (18); and this individual sex difference, although not significant, could reflect the effect of menstruation and pregnancy during the reproductive years.

Sperry (1) probably was the first to show changes in the esterified fraction of cholesterol with age by comparing the values found in infants and adults; however, he established late (2) the relatively constant ratio of free and total cholesterol in healthy adults. He concluded that the regulatory mechanism which keeps the ratio constant with narrow limits in the adult is not fully established in the neonatal period. We also have shown the difference in the CEFA pattern of newborn and adult animals (6).

More recently Leonard et al. (5) reported that the relationship between the free and the total cholesterol levels was unaffected by age. In the present study (Figs. 7 and 8) we also found that with advancing age there is a trend toward a proportional increase in the nonesterified fraction of cholesterol, but this is not statistically significant. Our results are also in agreement with those of Adlersberg et al. (18) who found no significant differences in the serum cholesterol levels between sexes with change in age.

When the different fatty acids of the esterified fraction are studied, it is interesting to note that the one which correlates best with age is the cholesteryl linoleate.
(Table 11). Also cholesteryl linoleate is the ester which best correlates with the total esterified cholesterol fraction. If this means that changes in the esterified cholesterol fraction result mainly from changes in the cholesteryl-linoleate fraction, then this may be of clinical significance. We know that changes in the components of the cholesteryl ester fraction can be introduced by manipulations in the diet (20); further, it has been shown (21) that in patients with atherosclerosis there is a greater increase in the proportions of saturated and monounsaturated serum cholesterol esters and a greater decrease in the proportions of linoleate and arachidonate cholesteryl esters. In the present study, the tendency to decrease the proportion of cholesteryl linoleate with age was observed, but the change was not significant.

It is worth noting that in the present study there was a negative correlation between the values of cholesteryl linoleate and saturated cholesteryl esters. Undoubtedly, we have to learn more about the possible significance of these relationships and changes with age of the esterified fraction of cholesterol and its possible relationship with atherosclerosis.

**SUMMARY**

The concentration of total cholesterol and different cholesteryl esters has been determined in a group of 2,691 individuals.

There was an increase in the total serum cholesterol content and in the esterified fraction with advancing age.

The annual change (in milligrams/100 ml) of total cholesterol, was found to be larger than the annual change of total ester.

No significant differences were found between sexes in regard to the changes with age of the esterified fraction of cholesterol. Changes in the esterified fraction of cholesterol occurred with age in both sexes.

A highly positive correlation was observed between total and esterified cholesterol and between cholesteryl linoleate and total esterified cholesterol. A high negative correlation was observed between cholesteryl esters of saturated fatty acids and cholesteryl linoleate.

We wish to express our appreciation to Dr. P. Leaverton and Mr. V. Uthoff for their statistical help.

**REFERENCES**

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