Cholesterol transfer between $\alpha$- and $\beta$-lipoprotein fractions in serum
Method of estimation and some factors influencing the cholesterol translocation

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Summary

An in vitro incubation procedure for the estimation of some cholesterol dynamics in serum was devised, taking advantage of a purified specific goat antiserum against human serum $\beta$-lipoprotein to achieve the fractionation of $\alpha$- and $\beta$-lipoprotein fractions. The new method was based on the two principles that the decrease of free cholesterol (FC) in the $\beta$-lipoprotein fraction during incubation should represent the amount of FC transferred from $\beta$- to $\alpha$-lipoproteins and that the increase in the amount of esterified cholesterol (EC) in the $\beta$-lipoprotein fraction during incubation should be equal to the amount of EC transferred from $\alpha$- to $\beta$-lipoproteins since the esterification of cholesterol occurs only in $\alpha$-lipoprotein particles. The amount of esterified cholesterol which was transferred from $\alpha$- to $\beta$-lipoproteins was calculated. The values of free cholesterol and esterified cholesterol in $\alpha$- and $\beta$-lipoprotein fractions before and after the incubation were estimated with various serum samples taken from young and old women. The results obtained in this study indicated that the cholesterol dynamics are influenced considerably by several factors, such as LCAT activity, and the levels of cholesterol, triglyceride and phospholipid in the serum. Differences in the cholesterol dynamics between the young and old women indicated the possibility that the new method is useful for investigating the pathogenesis of atherosclerosis.

Key words: cholesterol-translocation in serum, $\alpha$-lipoprotein, $\beta$-lipoprotein, LCAT, young and old

Introduction

The relationship of the serum cholesterol level toward atherosclerosis has been well documented and the so-called atherogenic index has been the subject of many discussions. As a means of studying the level of HDL-C or atherogenic index as well as the cholesterol dynamics in serum, the serum cholesterol binding reserve (or capacity), abbreviated as SCBR (or SCBC), has been widely adopted. However, we have encountered the problem that when we applied this SCBR-method to Japanese subjects, we could not obtain reliable values probably because the
serum cholesterol level in Japanese is relatively low. This prompted us to devise a new method to estimate the cholesterol dynamics which could be applied to the sera of normo- or hypo-cholesterol levels. Our new method, which actually deals with the cholesterol transfer between the \( \alpha \) and \( \beta \)-lipoprotein fractions and could be called briefly CTBL, is based on the following principles: (1) the decrease of free cholesterol in the \( \beta \)-lipoprotein fraction during incubation should represent the amount of FC translocated from \( \beta \)- to \( \alpha \)-lipoproteins, and the amount can be calculated as the difference of the amount of FC in \( \beta \)-lipoprotein fraction before and after the incubation, (2) the esterification of cholesterol occurs only in \( \alpha \)-lipoprotein particles and (3) fractionation of \( \alpha \) and \( \beta \)-lipoprotein fractions could be achieved by employing the purified specific goat antiserum against the human serum \( \beta \)-lipoprotein.

In the present paper we report on the practical procedures of our CTBL method and some characteristic findings on cholesterol dynamics in two groups of women, young and old, which were obtained by applying the CTBL method.

**Materials and Methods**

1. **Serum samples**
   The serum samples to be analyzed were taken from 50 young women from the Nursing School of Nippon Medical School (designated as “Young group” in the present study) and from more than 100 house-wives of 51 to 76 years of age in Bunkyo-ku, Tokyo (“Old group” in this study).

2. **Reagents**
   Reagents for the determination of serum lipid... Total cholesterol (TC), triglyceride (TG), free cholesterol (FC) and phospholipid (PL) in serum were determined by the enzymatic methods using commercially available kits from Nissho Chemical Company, Osaka. p-chloromercuriphenyl sulphonic acid (PCMPS) monosodium salt was purchased from Sigma, St. Louis, Mo.

3. **Goat antiserum against human \( \beta \)-lipoprotein**
   This was obtained from Nissui Seiyaku Co., Tokyo, and was purified in the following way. To 100 ml of this sample was added an equal volume of saturated ammonium sulfate solution. The precipitate, collected by centrifugation in cold, was dissolved in distilled water and dialyzed against 0.1 mM phosphate buffer pH 6.3 overnight. The antiserum solution was applied to a DE 52 column (Whatman, 2.6 × 70 cm), which was equilibrated with 0.1 mM phosphate buffer pH 6.3, and eluted with the same buffer. First 50 ml of eluate was discarded and the next phase of the eluate, about 65 ml, was collected. The concentration of the purified antiserum solution was adjusted by dilution so that it can precipitate the \( \beta \)-lipoprotein in serum having 350 mg/dl of total cholesterol. When the concentration of the antiserum preparation was too low, the ammonium sulfate precipitation procedure was repeated. The purified antiserum solution was stored in a refrigerator at 4–6°C. To evaluate the antibody specificity, cross reactivity between the antiserum and \( \beta \)-lipoprotein or HDL-fraction was examined by the Ouchterony method or by the immunoelectrophoresis procedure. The concentration of cholesterol in this purified antiserum, measured by the enzymatic method, was nearly zero. Phospholipid and triglyceride were not detectable either.
General procedures for the estimation of FC transfer, EC transfer and esterification of FC to EC

A heparinized blood sample was centrifuged at 3,000 rpm for 5 min in cold and the plasma obtained was divided into two portions. One portion was stored immediately below −40°C. The other was placed in a glass-stoppered tube and incubated for 24 h at 37°C in a water bath. Total and free cholesterol in the plasma before and after incubation were measured by the enzymatic method using a portion of the plasmas. Using another portion of the incubated and unincubated plasma, the α-lipoprotein fraction was prepared separately from β-lipoprotein and then total and free cholesterol in the α-lipoprotein were also determined; separation of α-lipoprotein from β-lipoprotein was achieved in the following way by using purified goat antiserum against human β-lipoprotein. To 200 µl of the sample serum an equal volume of the antiserum was added and well mixed; after 2 min the precipitate that appeared was removed by a high speed centrifuge at 11,000 rpm for 2 min. A 100 µl portion of the clear supernatant was saved for FC-determination and the 50 µl was saved for cholesterol determination (abbreviated to LpC and LpFC). From the values thus obtained the amount of FC transferred from β-lipoprotein to α-lipoprotein and the amount of EC translocated from α-lipoprotein to β-lipoprotein were calculated respectively according to the methods presented schematically in Fig. 1.

Results

1. Comparison of the rates of cholesterol transfer between α- and β-lipoprotein fractions in the young and old groups

Remarkable differences were noticed between the young and old groups with respect to the

Fig. 1 Schematic presentation of the methods of calculation of lipid transfer between α-lipoprotein and β-lipoprotein

1. Amount of FC transferred from β- to α-lipoproteins (mg/dl): (βLpFC before incubation) minus (βLpFC after incubation)

2. Amount of EC esterified during incubation (mg/dl): ([FC transferred from β- to α-lipoproteins] plus (αLpFC before incubation)) minus (αLpFC after incubation)=([FC before incubation] minus (FC after incubation)

3. Amount of EC transferred from α- to β-lipoproteins (mg/dl): ([EC esterified during incubation] plus (αLpFC before incubation)) minus (αLpEC after incubation)

4. LCAT activity (%/h)

EC esterified during incubation x 1 x 100
(FC, transferred from β- to α-lipoproteins) plus (αLpFC before incubation)

(hours of incubation)
values of cholesterol transfer as estimated by our CTBL method and, as can be seen from Table 1, the difference in the values of EC transfer was most significant when compared under the conditions where the values of TC and TG were matched to be comparable between the young (n=33) and old (n=22) groups. On the other hand, the difference in FC (β to α) between the two groups was relatively small. Consequently, the value of ΔαLpC, which represents the difference of FC (β to α) and EC (α to β), was also considerably different between the two groups. Also the value of ΔαLpEC, which represents the difference of the amount of EC esterified during the incubation and the amount of EC transferred from α- to β-lipoproteins during the incubation, was considerably different between the young and old groups. The significance of these findings will be discussed later.

2. Relation of CTBL to the level of serum TC

The 61 old women (58 to 72 years of age with a mean age of 61.4 years) were divided into three groups according to the TC level in serum and the data obtained in individual groups were classified as in Table 2. The amount of FC transferred from β- to α-lipoproteins during incubation was larger in the groups showing higher TC levels. However, the efficiency of FC transfer, defined as the ratio of the amount of FC transferred against the amount of FC which was present in the β-lipoprotein before incubation was lower in the groups with higher TC levels,

<table>
<thead>
<tr>
<th>Groups</th>
<th>TC (mg/dl)</th>
<th>FC (β to α) (mg/dl)</th>
<th>EC (α to β) (mg/dl)</th>
<th>ΔTC in α-lipoprotein (ΔαLpC) (mg/dl)</th>
<th>TG (mg/dl)</th>
<th>EC esterified (mg/dl)</th>
<th>FC in β-lipoprotein before incubation (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young (n=33)</td>
<td>173.5 ± 34.6</td>
<td>16.2 ± 3.8</td>
<td>11.0 ± 4.7</td>
<td>5.3 ± 2.9</td>
<td>77.8 ± 31.6</td>
<td>20.9 ± 4.0</td>
<td>32.1 ± 8.5</td>
</tr>
<tr>
<td>Old (n=73)</td>
<td>212.1</td>
<td>p&lt;0.02</td>
<td>p&lt;0.02</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC matched to that of young (n=22)</td>
<td>173.9 ± 9.8</td>
<td>17.0 ± 3.3</td>
<td>13.8 ± 4.1</td>
<td>3.2 ± 2.9</td>
<td>77.9 ± 30.2</td>
<td>20.9 ± 2.8</td>
<td>33.6 ± 4.2</td>
</tr>
</tbody>
</table>

1) Change in cholesterol content in α-lipoprotein (ΔαLpC) was calculated as the difference of FC (β to α) minus EC (α to β).
2) Change in EC in α-lipoprotein was calculated as the difference of the amount of EC esterified during incubation minus EC (α to β).

<table>
<thead>
<tr>
<th>TC group (mg/dl)</th>
<th>FC (β to α) (mg/dl)</th>
<th>βLpPC before incubation (mg/dl)</th>
<th>Ratio of (a)/(b)</th>
<th>LCAT activity (%/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Below 189 (n=22)</td>
<td>17.0±3.3</td>
<td>33.6±4.2</td>
<td>0.51</td>
<td>2.90±0.23</td>
</tr>
<tr>
<td>(173.9±9.8)*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>190~219 (n=22)</td>
<td>17.3±4.6</td>
<td>38.2±7.2</td>
<td>0.45</td>
<td>2.80±0.38</td>
</tr>
<tr>
<td>(202.5±8.3)*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>220~249 (n=2)</td>
<td>19.4±3.6</td>
<td>46.3±5.4</td>
<td>0.42</td>
<td>2.86±0.34</td>
</tr>
<tr>
<td>(234.6±10.6)*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Mean±S.D.
Table 3 Relationship of LCAT activity and CTBL

<table>
<thead>
<tr>
<th>Group classified by LCAT activity ( %/d )</th>
<th>TC, average (mg/dl)</th>
<th>αLpEC before incubation (mg/dl)</th>
<th>EC esterified during incubation (mg/dl)</th>
<th>EC(α to β) during incubation (mg/dl)</th>
<th>βLpEC before incubation (mg/dl)</th>
<th>Efficiency of transfer of EC (c)</th>
<th>FC (d)</th>
<th>Efficiency of transfer of FC (c) + (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A) Group TC below 220 mg/dl</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.1~2.4(n=3)</td>
<td>198.9±7.4</td>
<td>71.6±13.3</td>
<td>19.5±1.8</td>
<td>5.1±3.3</td>
<td>22.0±4.1</td>
<td>12.3±0.9</td>
<td>29.0±4.4</td>
<td>0.04</td>
</tr>
<tr>
<td>2.5, 2.6(n=9)</td>
<td>192.2±20.7</td>
<td>55.3±8.9</td>
<td>19.2±2.1</td>
<td>9.4±1.5</td>
<td>17.6±3.0</td>
<td>13.8±1.3</td>
<td>33.4±5.4</td>
<td>0.13</td>
</tr>
<tr>
<td>2.7, 2.8(n=8)</td>
<td>181.5±12.9</td>
<td>43.3±5.1</td>
<td>19.9±1.9</td>
<td>11.6±1.3</td>
<td>13.2±1.1</td>
<td>16.8±2.3</td>
<td>35.0±2.8</td>
<td>0.18</td>
</tr>
<tr>
<td>2.9, 3.0(n=9)</td>
<td>178.1±9.5</td>
<td>43.9±5.8</td>
<td>21.1±1.5</td>
<td>14.2±2.3</td>
<td>12.7±1.4</td>
<td>16.9±2.0</td>
<td>33.6±3.7</td>
<td>0.22</td>
</tr>
<tr>
<td>3.1~3.5(n=11)</td>
<td>190.9±15.9</td>
<td>36.1±8.0</td>
<td>25.2±2.1</td>
<td>19.0±5.1</td>
<td>10.8±1.8</td>
<td>21.6±2.6</td>
<td>41.5±6.0</td>
<td>0.31</td>
</tr>
<tr>
<td>B) Group TC over 220 mg/dl</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.1~2.4(n=4)</td>
<td>242.1±24.0</td>
<td>71.6±4.9</td>
<td>18.8±2.3</td>
<td>8.1±1.2</td>
<td>21.2±1.2</td>
<td>13.1±3.3</td>
<td>40.6±5.7</td>
<td>0.09</td>
</tr>
<tr>
<td>2.5, 2.6(n=4)</td>
<td>253.0±18.0</td>
<td>68.2±3.8</td>
<td>22.9±2.4</td>
<td>10.9±2.4</td>
<td>20.8±2.8</td>
<td>16.7±0.9</td>
<td>43.7±3.3</td>
<td>0.12</td>
</tr>
<tr>
<td>2.7, 2.8(n=7)</td>
<td>241.3±14.2</td>
<td>49.6±9.5</td>
<td>23.5±2.2</td>
<td>14.4±2.3</td>
<td>15.2±2.5</td>
<td>20.5±1.4</td>
<td>49.6±4.1</td>
<td>0.21</td>
</tr>
<tr>
<td>2.9, 3.0(n=12)</td>
<td>245.3±12.6</td>
<td>45.7±7.4</td>
<td>22.9±2.2</td>
<td>16.9±2.3</td>
<td>12.9±1.9</td>
<td>19.3±2.8</td>
<td>49.1±5.0</td>
<td>0.24</td>
</tr>
<tr>
<td>3.1~3.5(n=6)</td>
<td>245.4±19.8</td>
<td>39.4±6.0</td>
<td>27.7±1.3</td>
<td>23.5±3.6</td>
<td>11.3±2.0</td>
<td>24.1±1.3</td>
<td>54.2±5.2</td>
<td>0.36</td>
</tr>
</tbody>
</table>

because of larger values of FC amount in the β-lipoprotein before incubation in the groups of high TC level.

3. Relation of CTBL of LCAT activity

In contrast to the cases of FC transfer from β- to α-lipoprotein fractions, the rate of EC transfer from α- to β-lipoproteins appeared to be highly dependent upon the activity of LCAT as can be seen from Table 3. In Table 3, the data were classified into two groups, A and B, according to the TC levels below 220 mg/dl or over 221 mg/dl, and the data in each group were further classified according to the activities of LCAT. In both TC groups, the amount of EC esterified during incubation, as well as the amount of EC transferred from α- to β-lipoprotein fractions, was larger when the LCAT activity was higher, suggesting that the content of EC in the β-lipoprotein may be significantly influenced by the action of LCAT. It should be noted, however, that the content of EC in the α-lipoprotein fraction before incubation was considerably smaller in the group with higher LCAT activity in both the high TC group and the low TC group. Therefore, if one compares the efficiency of EC transfer from α- to β-lipoproteins, which could be formulated as the ratio of the amount of EC (α to β) against the sum of the amounts of αLpEC before incubation and EC esterified during incubation, the efficiency of the EC transfer appears to be much higher in the groups with higher LCAT activity. In other words, LCAT may play an important role in the transfer of EC from α- to β-lipoprotein fractions.

It seems paradoxical that the amounts of FC in the β-lipoprotein fraction before incubation were much higher in the high LCAT group than in the low LCAT group. At present we should like to reserve our interpretation of this finding.

It seems paradoxical that the amounts of FC in the β-lipoprotein fraction before incubation were much higher in the high LCAT group than in the low LCAT group. At present we should like to reserve our interpretation of this finding.

In order to obtain further evidence for the correlation of EC transfer to the LCAT activity, altogether 31 individual persons were subjected to the examination twice at an interval of two weeks, and the data obtained are summarized in Fig. 2. It is apparent that the extent of the EC transfer increases with the increased LCAT activity, although there were some which did not appear to agree with this conclusion.
Fig. 2 Correlation between LCAT activity and EC transfer from α- to β-lipoprotein fractions

One straight line represents the relation of change of EC (α to β) to LCAT activity at 2 week intervals in the same person.

Table 4 Effect of inhibition of LCAT by PCMPS** upon CTBL*

<table>
<thead>
<tr>
<th>Group</th>
<th>FC(β to α) (mg/dl)</th>
<th>EC(α to β) (mg/dl)</th>
<th>αLpEC before incubation (mg/dl)</th>
<th>EC esterified during incubation (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young (n=33)</td>
<td>1.02±1.24</td>
<td>4.88±2.35</td>
<td>44.0±8.20</td>
<td>0.69±1.10</td>
</tr>
<tr>
<td>Old (TC matched)</td>
<td>0.97±1.04</td>
<td>6.71±2.18</td>
<td>42.2±7.49</td>
<td>0.86±0.65</td>
</tr>
</tbody>
</table>

*For the control values without PCMPS, see Table 1

**PCMPS=p-chloromercuriphenyl sulfonic acid

The LCAT activity in serum can be reduced to about 1/30 of the original activity by the addition 10⁻³⁰ M p-chloromercuriphenyl sulfonic acid in the assay mixture (data not shown). Under such conditions the FC transfer from β- to α-lipoprotein fractions was greatly reduced, whereas there was still a considerable transfer of EC from α- to β-lipoproteins (Table 4). These observations suggest that the esterification of FC by LCAT in the α-lipoprotein fraction is an important factor in the transfer of FC from β- to α-lipoprotein fractions.

4. Relation of CTBL to serum TC level

The data from those subjects showing comparable levels of serum TC were classified into 5 groups according to their serum TG levels and various parameters in individual groups were compared (Table 5). The LCAT activity was found to become higher as the TG level increased. Also the amount of FC transferred from β- to α-lipoproteins as well as the amount of EC transferred from α- to β-lipoproteins, increased considerably with the increased TG level in the serum. The esterification of FC was also more active in the groups with higher TG levels, although the degree of increase was relatively small. An especially notable finding was that in the
5. Relation of CTBL to the serum phospholipid level

Serum lipoproteins also contain a large quantity of phospholipid which is highly polar and hence, it is interesting to examine the influence of phospholipid upon the value of CTBL. As shown in Table 6, the subjects were classified into three groups according to the serum TC level. The first group consisted of 24 subjects showing TC levels below 189 mg/dl, the second group consisted of 17 subjects having a TC value of 190 to 219 mg/dl, and the third group consisted of 27 subjects with a TC value of 220 to 249 mg/dl. Each group was further divided into two subgroups according to whether the content of phospholipid was smaller or larger than the TC content in the sample specimen. In all TC groups, the value of ΔαLpC was larger in the PL>TC group than in the PL<TC group and the difference in ΔαLpC between the subgroups was significant in two low TC groups, whereas it was insignificant in the highest TC group. On the other hand, the differences in LCAT activity and in TG level between the two subgroups in each TC group were not significant.

In the cases of high serum TC but with relatively small serum content of polar lipid (=phospholipid), a larger amount of β-lipoprotein should be required to maintain the stability of lipoprotein molecule in the aqueous phase. The observation that the value of ΔαLpC was smaller when the ratio of PL/TC ratio was relatively low, may be accounted for by assuming that the
serum containing relatively low amounts of phospholipid contained relatively large amounts of \( \beta \)-lipoprotein which is capable of binding non-polar lipid.

**Discussion**

It is well known that the increase of lipids in serum facilitates the progress of atherosclerosis\(^1\)\(^\text{-}^3\). However, the significance of the translocation of cholesterol between various body compartments in atherosclerosis has not been well understood, except for the translocation of FC from the endothel cell surface of arteries to HDL-particles\(^6\)\(^,\)\(^13\). One of the reasons for this may be that the origin of FC in HDL could multiple; it may come from not only endothel cells but also many other cells and, in addition, \( \beta \)-lipoproteins could also be the source of FC either directly or through the TC rich lipoprotein HDL-cycle\(^14\)\(^-\)\(^\text{16}\). Another reason may be that the role of LCAT in cholesterol dynamics in serum is quite complicated. LCAT stimulates the extraction of FC from cell surfaces by way of decreasing the FC content in HDL-particles, but the increased EC in HDL resulting from the action of LCAT, in turn, would bring about an increased translocation of EC from \( \alpha \)-to \( \beta \)-lipoprotein fractions, and the elevated LDL-EC would again give rise to an increased cholesterol content in the cells such as the endothel cells of arteries. At present, a high HDL-C value is generally accepted as an antirisk factor against atherosclerosis. The HDL-C level may vary according to changes in various factors, such as the rate of HDL-apoprotein synthesis, FC input into HDL-particles, degradation of HDL (possibly HDL 2), EC transfer from \( \alpha \)-to \( \beta \)-lipoprotein fractions, and so on. Therefore, it is essential to have information on those factors to evaluate correctly the role of HDL as the antirisk factor. Our present work concerns these points. We took advantage of purified goat antiserum against human \( \beta \)-lipoprotein which enabled us to obtain human \( \alpha \)-lipoprotein without any interference from the subsequent determination of \( \alpha \)\( \text{LpC} \) and \( \alpha \)\( \text{LpFC} \).

To isolate the \( \alpha \)-lipoprotein fraction in its native form, it is important to carry out the fractionation as quickly as possible. HDL-subfractions are in dynamic equilibrium with one another and also with \( \beta \)-lipoprotein particles\(^17\)\(^,\)\(^18\), and therefore if the fractionation took a long time, for instance, as in the case of ultracentrifugation, the risk would arise that the individual lipoprotein components might dissociate and exchange with the components of other lipoprotein particles. Thus the methods of analysis requiring a long time are inadequate for the purpose of work as intended in the present study. In this sense, our CTBL method using the antiserum has a great advantage in that it would minimize the apoprotein dissociation during the course of analysis.

The CTBL method is based principally on the well established fact that LCAT acts only in the \( \alpha \)-lipoprotein particles; the binding of LCAT and Apo A-I has been reported by Fielding\(^18\). Thus, the amount of FC transferred from \( \beta \)-to \( \alpha \)-lipoproteins during incubation should be equal to the amount of FC which was found to be decreased in \( \beta \)-lipoprotein fraction after incubation. On the other hand, the increase of EC in \( \beta \)-lipoprotein fraction after incubation should represent the amount of EC transferred from \( \alpha \)-lipoprotein during incubation, because esterification of cholesterol does not occur at all in the \( \beta \)-lipoprotein fraction.

The activity of LCAT determined in this study should be closer to the real value than that obtained by the routinely used previous method\(^19\)\(^,\)\(^20\), which simply measures the decrease of FC in
the whole serum or plasma after incubation and does not take into consideration the FC translocation from β- to α-lipoprotein and the site of FC esterification. However, our method still has an insufficiency in that it neglects the amount of EC translocated from α- to β-lipoproteins in the calculation; removal of EC from α-lipoprotein may increase the apparent activity of LCAT by releasing the product inhibition\(^{21}\). Further improvement of the assay method is desirable in view of the fact that the LCAT activity has been thought to be a very important factor in the control of atherosclerosis.

The serum samples showing very high TG levels in Table 5 were perhaps rich in VLDL (the serum samples were taken at least 6 h after meals, and if so, the observed high degree of esterification and an extensive FC transfer from β- to α-lipoproteins are quite understandable since the FC in VLDL is supposed to be also available for LCAT in the α-lipoprotein particles. LDL contains a large amount of FC, but so far it is unclear whether the LDL concentration influences in any way the rate of EC formation in serum\(^{22}\).

It was seen in Table 3 that, between the high and low LCAT groups, the differences in EC (α to β) were more distinct than the differences in FC (β to α) and the differences in the amount of EC formed during incubation; namely, LCAT activity did not appear to exert the same effect upon the values of FC (β to α) and EC (α to β).

On the other hand, it should be noted that, if the esterified cholesterol remains persistently in α-lipoprotein particles, it might act to reduce the LCAT activity as a result of product inhibition and therefore, the rate of EC transfer from α- to β-lipoproteins would have a significant influence on the assay of LCAT activity by the routinely used method. Perhaps the LCAT activity as measured in this study may have also been influenced by the circumstances described above. The rate of EC transfer may also vary according to the fluctuation of the amount of apoprotein D\(^{18,23}\) or an exchange protein\(^{24}\), transfer protein\(^{25-27}\) and of some unknown factors in the serum.

The present finding that the values of CTBL in the young and old groups were significantly different indicates that the mechanism of the translocation of cholesterol between α- and β-lipoprotein fractions involves an age dependent event. Since the relation of atherosclerosis and age is a well-known and established fact, the CTBL method should be useful for investigation about the development of atherosclerosis. The significance of CTBL in cardiovascular diseases awaits further investigation.

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References


4) The Lipid Research Clinics Program: The lipid research clinics coronary primary prevention trial results. 2. The


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