Lindgren and co-workers (1) have described the properties and methodology for the ultracentrifugal study of the entire spectrum of lipoproteins present in human serum. The three most dense lipoproteins described by Lindgren et al. represent the subject of present considerations. These lipoproteins have been identified by their estimated hydrated densities as a lipoprotein of density 1.05 gm/ml, a lipoprotein of density 1.075 gm/ml, and a lipoprotein of 1.145 gm/ml. At present the precise interrelationship of the chemically isolated alpha lipoproteins described by Oncley et al. (2) to these three ultracentrifugally defined high density lipoproteins is not completely clear. Since the exact relationship to chemically isolated alpha lipoproteins remains indeterminate we have avoided the designation of the three high density lipoproteins as alpha lipoproteins and instead have utilized the following nomenclature: the lipoprotein of hydrated density 1.05 gm/ml = HDL1; the lipoprotein of hydrated density 1.075 gm/ml = HDL2; the lipoprotein of hydrated density 1.145 gm/ml = HDL3.

The role of the low density lipoproteins (of hydrated density less than 1.04 gm/ml) in the systematics of serum lipid transport have been described (3–5). Ultimate further understanding of lipid transport requires similar knowledge of the population distributions of the three high density lipoproteins. The high density lipoprotein determinations described below were made on 566 randomly chosen clinically healthy adults of both sexes, between the ages of 18 and 69 years of age.

MATERIALS AND METHODS

The procedure of Lindgren et al. involving preparative and analytic ultracentrifugation is in a sodium chloride-D2O-H2O system of solvent density equal to 1.24 gm/ml. All three high density lipoproteins were observable in the analytical ultracentrifugation at a density of 1.24 gm/ml. This analytical method allowed the quantitative determination of the sum of the concentrations of the HDL1 plus HDL2, but was unsatisfactory for the complete resolution of the HDL1 from HDL2 so that their individual concentrations could be measured. For this reason an additional ultracentrifugal procedure at a density of 1.125 gm/ml was designed for the separate measure of the concentration of the HDL2. The detailed description of the preparative and analytical ultracentrifugal procedures including film analysis employed for the determination of the three high density lipoproteins is described by deLalla and Gofman (6).

The clinically healthy subjects for this study were obtained from two sources. Males and females below the age of 30 years were volunteers from the student population of the University of California at Berkeley. All other subjects, above the age of 30 years, were randomly selected from the population under study by the Heart Epidemiology Study Group at Framingham, Massachusetts. All subjects had had a recent complete medical examination. The subjects included in the present study as clinically healthy individuals represented those meeting certain requirements in their medical examination. All individuals included in the study had blood pressures, at examination, below 140 mm Hg systolic and 90 mm Hg diastolic. The following illnesses recorded in the medical history or discovered during examination were sufficient to exclude individuals from this study; diabetes, nephritis, rheumatic fever, rheumatoid arthritis, syphilis, tuberculosis, peptic ulcer, asthma, hepatitis, cardio-

Received for publication May 3, 1954.
vascular disease, cancer, epilepsy, hyper- or hypothyroidism, adrenal disease, Raynaud’s disease and pernicious anemia.

A laboratory finding of an abnormal electrocardiogram, abnormal chest roentgenogram, anemia, albuminuria or glycosuria was considered sufficient to exclude a subject from this study. It is evident that if any of these abnormalities or disease states were not overt to the examiner, the subject would be included in the study. Thus the subjects included are to be regarded as clinically healthy within the limits of the criteria described. The total number of subjects who qualified with these criteria were 566 in all, 229 of whom were males, and 337, females. The analysis of all three high density lipoproteins was made for all these subjects; segregating the population studied by sex and into the following age groups, 18-19, 20-29, 30-39, 40-49, 50-59 and 60-69 years.

RESULTS

HDL₁. The mean serum concentrations of HDL₁, the standard deviations of the distribution and the standard errors of the means are presented in table 1A.

HDL₂. The mean serum concentrations of the HDL₂, the standard deviations of the distribution and the standard errors of the means are presented in table 1B.

HDL₃. The mean serum concentrations of the HDL₃, the standard deviations of the distribution, and the standard errors of the means are presented in table 1C.

Intercorrelations of the High Density Lipoproteins. In table 2 are given the calculated Pearson product-moment correlation coefficients between the HDL₁ vs HDL₂, HDL₁ vs HDL₃ and HDL₂ vs HDL₃, respectively, for both sexes and for the several age categories.

DISCUSSION

HDL₁ (see table 1A). Inspection of the mean HDL₁ levels in either sex reveals no large trends of lipoprotein concentrations with age. Application of the t test demonstrates that the recorded differences in mean levels with age fail to show significance at the 1% level. However the t test does reveal that, for the age group under 40 years the mean level of HDL₁ appears to be significantly lower in the female than in the male (1% level of significance). It is not possible from these data to demonstrate any significant sex difference in HDL₁ level beyond the age of 40 years.

HDL₂ (See table 1B). One feature is outstanding in the results of the measurement of the HDL₂, namely the large difference between the male and female sex for the age range from 20 years to 60 years. For each decade in this range the mean HDL₂ level is much higher in the female than in the male. For the 18-19-year age group the same trend appears to be maintained, although the difference can be proven significant only between the 1 and 5% level from these data. Above the age of 60 years no significant male-female difference can be demonstrated within the limited data now available. In the male sex there is a fall in HDL₂ concentration in the 20-29-year age group as compared with the 18-19-year age group that is significant at the 1% level. In the range from 20 years to 60 years there is no further significant change in HDL₂ concentration in males with age. There is an apparent rise in HDL₂ concentration in the 60-69-year-old male compared with 50-59-year age group, significant between the 1 and 5% level. It is evident that a larger scres of males beyond 60 years of age is required to determine more

<table>
<thead>
<tr>
<th>Age group</th>
<th>No. of subj.</th>
<th>Mean HDL</th>
<th>S.D. of distr.</th>
<th>S.E. of mean</th>
<th>No. of subj.</th>
<th>Mean HDL</th>
<th>S.D. of distr.</th>
<th>S.E. of mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18-19</td>
<td>15</td>
<td>21.8</td>
<td>3.0</td>
<td>1.0</td>
<td>22</td>
<td>17.6</td>
<td>6.2</td>
<td>1.4</td>
</tr>
<tr>
<td>20-29</td>
<td>28</td>
<td>21.0</td>
<td>7.7</td>
<td>1.5</td>
<td>28</td>
<td>15.8</td>
<td>5.8</td>
<td>1.1</td>
</tr>
<tr>
<td>30-39</td>
<td>82</td>
<td>17.2</td>
<td>7.9</td>
<td>0.9</td>
<td>93</td>
<td>14.3</td>
<td>5.5</td>
<td>0.0</td>
</tr>
<tr>
<td>40-49</td>
<td>54</td>
<td>18.4</td>
<td>10.5</td>
<td>1.4</td>
<td>104</td>
<td>15.9</td>
<td>6.1</td>
<td>0.0</td>
</tr>
<tr>
<td>50-59</td>
<td>39</td>
<td>18.5</td>
<td>8.3</td>
<td>1.4</td>
<td>44</td>
<td>10.3</td>
<td>5.9</td>
<td>0.9</td>
</tr>
<tr>
<td>60-69</td>
<td>11</td>
<td>23.5</td>
<td>7.8</td>
<td>2.5</td>
<td>7</td>
<td>10.6</td>
<td>12.7</td>
<td>5.2</td>
</tr>
<tr>
<td>Females</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18-19</td>
<td>15</td>
<td>21.8</td>
<td>3.0</td>
<td>1.0</td>
<td>22</td>
<td>17.6</td>
<td>6.2</td>
<td>1.4</td>
</tr>
<tr>
<td>20-29</td>
<td>28</td>
<td>21.0</td>
<td>7.7</td>
<td>1.5</td>
<td>28</td>
<td>15.8</td>
<td>5.8</td>
<td>1.1</td>
</tr>
<tr>
<td>30-39</td>
<td>82</td>
<td>17.2</td>
<td>7.9</td>
<td>0.9</td>
<td>93</td>
<td>14.3</td>
<td>5.5</td>
<td>0.0</td>
</tr>
<tr>
<td>40-49</td>
<td>54</td>
<td>18.4</td>
<td>10.5</td>
<td>1.4</td>
<td>104</td>
<td>15.9</td>
<td>6.1</td>
<td>0.0</td>
</tr>
<tr>
<td>50-59</td>
<td>39</td>
<td>18.5</td>
<td>8.3</td>
<td>1.4</td>
<td>44</td>
<td>10.3</td>
<td>5.9</td>
<td>0.9</td>
</tr>
<tr>
<td>60-69</td>
<td>11</td>
<td>23.5</td>
<td>7.8</td>
<td>2.5</td>
<td>7</td>
<td>10.6</td>
<td>12.7</td>
<td>5.2</td>
</tr>
</tbody>
</table>

Total subjects 564.

In the two groups designated one case had analyses for the HDL₄ and HDL₅ but did not have the HDL₃ analysis because of insufficient quantity of serum available.
conclusively whether this rise is real. In the female sex no significant difference in mean HDL₂ concentration can be demonstrated for 18–19 year-olds as compared with the 20–29-year-old females. However, a decrease, significant at the 1% level, in mean HDL₂ concentration is observed in the 30–39-year-old females as compared with the 20–29-year-old females. It must be noted, however, that the age of 30 years represents the dividing line between the younger age group of females collected in Berkeley, California and the older age groups collected in Framingham, Massachusetts. Therefore, we cannot rule out the possibility that the observed significant difference in HDL₂ level in the 20–29-year-old females as compared with the 30–39 year-old females is due to a factor in some way geographically determined. On the other hand the observed difference may very well reflect a metabolic alteration with age in the female, independent of geographical considerations. In any event the observed differences between 18–19-year-old males as compared with 20–29-year-old males cannot be geographically determined, since both groups of subjects were of the Berkeley sampling. Beyond the age of 30 years in the female no significant alterations in HDL₂ concentrations with age are demonstrable.

If the assumption is made that the possible geographic factor alluded to above is unimportant, then it appears that, within the age spans studied, the female shows a drop in mean HDL₂ level from the peak value apparently ten years later than the male.

HDL₃ (see table 1C). In the 18–19-year age group no significant difference between males and females in the mean HDL₃ level can be demonstrated. However, for the age span 20–60 years, in each decade it can be demonstrated that the mean HDL₃ level in females is higher, at the 1% level of significance, than in the corresponding males.

In the age span from 18–29 years, for either sex alone, no significant change in mean HDL₃ level as a function of age can be demonstrated. However for both sexes there appears to be a drop in mean HDL₃ level in the 30–39 year age group as compared with the 20 to 29 year age group, the significance being at the 1% level in the female and between the 1 and 2% level in the male. Again, as was mentioned for the HDL₂ data, the factor of possible geographic influence cannot be overlooked for differences which become manifest at the dividing line of 30 years of age.

In the males no significant age trends can be demonstrated beyond 30 years of age. In the female, however, there appears to be some increase in mean HDL₃ level in the 50–59 year age group as compared with the 30–39 year age group. However, this apparent increase must be regarded only as borderline, since it is at the 5% level of significance.

It is of interest that while the female in general has been shown above to be higher than the corresponding male in both the HDL₁ and HDL₃ levels, the magnitude of the sex difference is approximately 2–2.5 times as great in the HDL₁ levels as in the HDL₃ levels, when considered on a standard score basis (7).

Some data exist in the literature on the subject of so-called ‘alpha’ lipoprotein as measured by chemical fraction (8) and ultracentrifugally by Lewis and Page (9). The work of Russ, Eder and Barr provide data for the cholesterol recovered in fraction A of the Cohn fractionation method 10 (10). These authors state that the ‘α lipoprotein’ is to be found in fraction A. However, they make no comment concerning the possible presence of several components in this fraction nor do they give the percentage that the cholesterol represents of the total lipoprotein. As a result it is impossible to compare their findings of the quantity of cholesterol, in whatever form it is present in fraction A, with our results on the individual HDL₁, HDL₂, and HDL₃. Further their limited data on 20 subjects, over the age span 18–35 years for females and 24 subjects over the 18–35 year span for males would have completely obscured the changes observed in the present work within this age span, even if they were measuring the HDL₂ and HDL₃ components combined. Lewis and Page have utilized the method of Lundgren et al. (1) for ultracentrifugal study of the lipoproteins in a medium of density 1.21, except for the minor modification of substituting KBr for NaCl and D₂O in achieving the density 1.21 gm/ml they

3 Comparisons on a standard score basis refer to differences in mean as related to the standard deviation of the distribution.
used. As was pointed out earlier in this discussion (see MATERIALS and METHODS section) this type of run is unsatisfactory for the resolution of the HDL2 and HDL3. It appears further that Lewis and Page failed to utilize the length of time of centrifugation described in the Lindgren et al. paper and that at least for the HDL3, which is the most abundant high density lipoprotein, their analyses could not possibly have been quantitative under the conditions they describe. Direct evidence that their results may not be quantitative may be found by comparing the analyses reported by Lewis and Page for what they call α1- lipoprotein. If the 1.21 gm/ml run were used correctly according to the Lindgren et al. procedure they should have been able to measure the sum of HDL2 and HDL3 combined. From their data for so-called S = 4 lipoprotein (their ‘α1-lipoprotein’) for males 18–34 years; α1-lipoprotein = 153.7 mg%. From table 1B and table 1C, by combining our means for this age range for males, the combined HDL2 and HDL3 value would be 260 mg%. Thus the Lewis and Page value is about 40% low. Similarly for females our combined HDL2 and HDL3 would be 315 mg%. The Lewis and Page value for ‘α1-lipoprotein’ is 183 mg%, which is again approximately 40% low. It appears likely that the incomplete separation due to inadequate centrifugation is the basis for their seriously discrepant results. This renders any comparison of the results herein reported with those of Lewis and Page essentially without value. The values reported by Lewis and Page for ‘α2-lipoprotein’ are of the order of magnitude of those found by us for the HDL1. However their values are approximately 1/3 of our values for HDL1.

**Intercorrelations of the High Density Lipoproteins.** It is of interest biologically to know to what extent the factors involved in the control of the serum levels of the three high density lipoproteins are alike and to what extent they differ. One approach to an evaluation of this is the use of the Pearson product-moment correlation between the levels of any pair of lipoproteins. Such correlations are evaluated in table 2. It is evident from this table that no strong intercorrelations exist between the HDL1 and either the HDL2 or HDL3. For most of the age decades involved and for both sexes it is impossible even to demonstrate any correlation of significance. However, in 3 of the 12 groups there does appear to be a significant negative correlation between the HDL1 and HDL2. It appears therefore that even though there may exist a negative correlation between these two lipoproteins, it is probably of very low order. For most of the groups no significant correlation can be demonstrated between the HDL1 and HDL3 lipoproteins, although in three groups a significant positive correlation is noted. Again, it appears, therefore, that if the HDL1 and HDL3 are positively correlated, the interrelationship is of a low order.

For 8 of the 12 groups considered no significant correlation is demonstrable between the levels of HDL2 and HDL3. In three groups a low positive correlation of borderline significance was found and one group a low positive correlation, significant at the 1%
level, was demonstrated. Therefore, an overall relationship of HDLα and HDLβ levels, if positive, is of low order.

Consideration of all these intercorrelation studies indicates that the metabolic factors involved in the regulation of the serum level of any one of the high density lipoproteins are largely independent of those involved in the regulation of the level of either of the other high density lipoproteins.

SUMMARY

Measurements of three high density lipoproteins in the serum of 566 clinically healthy adults have been made. Analysis of the age and sex trends in mean high density lipoprotein levels for the age span 18–69 years has been made. Possible interrelationships in the serum levels of the three high density lipoproteins were evaluated.

The authors are indebted to Dr. Thomas R. Dawber and Mr. Felix Moore of the Heart Epidemiology Group at Framingham, Massachusetts (National Heart Institute) for their generous cooperation in provision of the blood samples and clinical data for the largest segment of the population considered in this report.

REFERENCES