Hyper-Low Density Lipoproteinemia in United States Air Force Recruits

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SUMMARY
Six hundred and three selected, lean, healthy United States Air Force recruits, ranging from age 18 to 22, were screened for hyperlipoproteinemia shortly after military induction. There were 307 men and 296 women. Mean plasma lipid levels were lower than generally observed in the population at large: cholesterol = 139 ± 39 (SD) mg%; low density lipoprotein (LDL) cholesterol = 86 ± 40 mg%; high density lipoprotein (HDL) cholesterol = 59 ± 23 mg%; total plasma triglyceride (TG) = 40 ± 17 mg%. The low total plasma cholesterol was largely attributed to the LDL cholesterol fraction. White women had lower total and LDL cholesterol levels (P < 0.01) than men, but no other significant sex or race differences were observed. Twenty-six individuals (4.3%) had values for either one or both total plasma cholesterol and LDL cholesterol concentrations above the limits arbitrarily established for this group and are regarded as having hyper-low density lipoproteinemia. More individuals were identified as abnormal by a determination of total plasma and LDL cholesterol levels than by measuring total plasma cholesterol alone. No other lipoprotein abnormalities were observed. The lipid concentrations observed in these lean, healthy, young subjects are probably a reflection of ideal values around age 20.

Additional Indexing Words:
Cholesterol levels Coronary artery disease

Although the occurrence of asymptomatic hyperlipoproteinemia in the population at large is well documented, its frequency remains largely speculative.1 The incidence of hyper-low density lipoproteinemia is of particular importance because of its close association with development of coronary artery disease.2 It is not certain that therapy for lipid disorders will reduce the incidence of coronary atherosclerosis, but information now accumulating suggests that this is the case.3, 4 Since young persons dying of trauma frequently have evidence of early coronary artery atherosclerosis,5 the recent trend to diagnose hyper-low density lipoproteinemia in the young seems logical.

A variety of biologic variables may influence lipoprotein levels. Therefore, it is necessary to adjust the ranges considered “normal” to account for the influence of such variables as the age of the patient. Although a large number of infants6 and older adults7 have been screened to assess the probable limits of “normal” for plasma lipoproteins and the frequency of the various forms of hyperlipoproteinemia, the limits of lipoprotein levels which are clearly normal, and the frequency of asymptomatic hyperlipoproteinemia in the asymptomatic young adult, is largely undetermined.

Accordingly, a group of young, healthy men and women were studied shortly after their induction into the Air Force. An attempt was made to determine the frequency of lipid abnormalities in general and the incidence of hyper-low density lipoproteinemia in particular. The advantages of studying such a group are their young age and the opportunity to study them under controlled conditions.

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The views expressed herein are those of the authors and do not necessarily reflect the views of the United States Air Force, or the Department of Defense.

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Methods

Six hundred and three healthy United States Air Force recruits ranging from age 18 to 22 years, with a mean age of 19.6 years, were studied. Groups of recruits were sequentially studied as soon as training flights were assembled. Participation from each group was almost total, and only about 30 recruits refused to have blood drawn. The study group was thus felt to be a representative sample of the young volunteers entering the United States Air Force. The group consisted of 307 (50.9%) men and 286 (49.1%) women of whom 493 (82%) were white, 99 (16%) black, and 11 were of other races. The subjects were from all areas of the United States and had widely divergent genetic and socioeconomic backgrounds.

Prior to study, each subject had a physical examination, chest X-ray, hemoglobin and urinalysis, and all were normal. One of us (D.B.K.) took a history, and specifically excluded subjects who were taking medication and those with illnesses which might secondarily alter lipid values. He also evaluated the family history of coronary artery disease. Thus, any volunteer with significant chronic or acute illness, a recent weight loss of more than five pounds, or who was taking medicine (such as birth control pills) known to alter plasma lipid levels was excluded from the study. Only 20 subjects were eliminated for these reasons. A family history of coronary artery disease before the age of 60 in siblings, parents, or grandparents was considered as positive and arbitrarily scored with one point for each affected sibling or parent and one half point for each grandparent. It was sometimes difficult to determine with certainty the presence of coronary artery disease within the family, but generally a "heart attack" or "coronary" were the only events included as a positive response.

After volunteering for study, recruits were counseled both in groups and individually, and fully informed written consent was obtained from each. On the sixth day after arrival at Lackland Air Force Base and before the physical fitness program or routine immunizations were started, blood was drawn for study. Each subject for five days prior to the study had eaten regularly in the dining halls a diet which generally provided 43% of the caloric intake as carbohydrate and 42% as fat (75% saturated and 25% unsaturated).

After an overnight supervised fast of 12 to 14 hours, 20 ml of blood from each subject was collected in EDTA tubes. The blood was immediately placed in ice and within an hour centrifuged at 3°C Centigrade. The plasma was then refrigerated at the same temperature. Plasma was observed for chylomicrons or turbidity after overnight refrigeration.

Except for the ultracentrifugation studies, all plasma lipid determinations were done in the Endocrinology Research Laboratory, Wilford Hall USAF Medical Center. Preparative ultracentrifugation was done by D.J.K. at the University of Texas Medical School in San Antonio.

Plasma samples were extracted within 24 hours by a minor modification8 of the method of Kessler and Lederer.9 Cholesterol levels were determined in duplicate with the Technicon autoanalyzer.10 Two standard reagents, Monitor (Dade Laboratories) and Versatol (Warner-Chilcott Laboratories) were used simultaneously in every run. In addition, plasma samples of known cholesterol content (100–300 mg%) were measured in duplicate during each daily assay. The coefficient of variation was 3.0% between assays and the difference between duplicate samples in the same assay was usually less than 5%. When the difference between samples exceeded 5%, plasma was reextracted and cholesterol remeasured in duplicate. In addition, results for both cholesterol and triglyceride were randomly compared on duplicate samples in two laboratories in the area: The University of Texas Medical School in San Antonio and the USAF School of Aerospace Medicine, Brooks Air Force Base, Texas. Quality control is monitored in both laboratories by periodic surveys performed by the American Society of Clinical Pathology. The results agreed within 10% of those obtained in our laboratory using similar methods.

Plasma triglyceride was determined fluorometrically and in duplicate on each sample by a minor modification11 of the method of Kessler and Lederer.8 The standard reagent, tripropionin (mol wt, 260.29), was obtained from the Eastman Organic Chemical Company. Millimolar quantities of this standard were used in the triglyceride analysis, and the results converted and expressed as milligrams of triolein. For example, 14.86 mg of the tripropionin standard is equivalent to 50 mg of triolein. The coefficient of variation between assays was less than 3% and duplicate samples varied less than 5%. Those exceeding this value were reextracted and redetermined in duplicate.

On 258 arbitrarily selected specimens, preparative ultracentrifugation (density (d) = 1.006) was performed with a Spinco ultracentrifuge (model L) at 105,000 g for 17–22 hours at 12°C Centigrade. Cholesterol and triglyceride content of the infranate (d > 1.006) and supranate (d < 1.006) fractions were determined.

The cholesterol content of the high density lipoprotein (HDL) fraction was also determined by the heparin-manganese precipitation method of Berstein and Samaillé11 on the 421 samples which offered sufficient plasma for study. The low density lipoprotein (LDL) cholesterol content was calculated by the formula: LDL, cholesterol = total cholesterol–(TG/5 + HDL cholesterol). This was considered a valid estimate of LDL cholesterol since no specimen had a triglyceride value in excess of 150 mg%. In a smaller number of samples (183) sufficient plasma was available for both ultracentrifugation and HDL cholesterol determinations. In this group LDL cholesterol was also calculated directly from the formula: LDL = infranate cholesterol – HDL cholesterol.

Lipoprotein electrophoresis was done on paper media in duplicate on all specimens on the same day they were obtained according to the method of Lees and Hatch.12 Repeat lipoprotein electrophoresis was done on some specimens after ultracentrifugation. Two of us (D.B.K. and E.L.M.) independently reviewed the lipoprotein patterns without knowledge of other data on
individual patients and arbitrarily graded the density of the beta and pre-beta bands as zero, 1+, 2+, or 3+. On almost all specimens, the intensity of these zones was judged to be within 1+ of its duplicate and both observers almost always agreed within 1+ of the other.

Statistical analysis was done by computer utilizing student's t-test, analysis of variance, Kolmogorov-Smirnov (K-S) statistics to determine normality of distribution, and standard methods for log transformation to determine fiducial limits.13

Results

The subjects as a group were lean and had a mean ponderal index of thirteen. Only 20 subjects had indices below twelve. This range is generally considered to define obesity; however, of these, some were muscular, nonobese men.

All but five of the 603 plasma specimens were clear after refrigeration. Three displayed turbidity and two had demonstrable chylomicronemia. Each of these five individuals consented to a repeat 12 hour fast and in every instance the second plasma specimen was clear. The five had apparently not fasted before the original sampling since no instance could other extenuating circumstances account for the initial hypertriglyceridemia.

Mean plasma lipid concentrations for the entire group are summarized in table 1. Total plasma cholesterol ranged from 40 to 329 mg per 100 ml with a mean of 139 ± 39 (sd).* The cholesterol content of the HDL fraction was determined by the heparin-manganese method averaged 59 ± 23 mg per 100 ml. Mean LDL cholesterol in 415 subjects was 79 ± 43 mg per 100 ml with a range of 10 to 233 when calculated by subtracting HDL cholesterol and TG/5 from the total plasma cholesterol. Adequate plasma was available for simultaneous ultracentrifugation and HDL cholesterol analysis in 183 specimens. In these subjects mean LDL cholesterol calculated from the bottom fraction cholesterol − HDL was 86 ± 40 mg per 100 ml. There was a good correlation (r = 0.88) between LDL cholesterol and total cholesterol values. The mean plasma triglyceride (TG) level for the entire group was 40 ± 17 mg per 100 ml. The mean TG concentration of the very low density lipoprotein (VLDL) fraction obtained after ultracentrifugation (d < 1.006) was 15 ± 1 mg per 100 ml. The TG values of the VLDL fraction correlated well (r = 0.73) with total plasma triglyceride levels.

Table 1

Comparison of Plasma Lipid Levels in Men and Women (mg per 100 ml)

<table>
<thead>
<tr>
<th>Lipid</th>
<th>All subjects</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td>139 ± 39†</td>
<td>144 ± 40</td>
<td>135 ± 36</td>
</tr>
<tr>
<td>Total</td>
<td>(603)</td>
<td>(307)</td>
<td>(296)</td>
</tr>
<tr>
<td>LDL</td>
<td>79 ± 43</td>
<td>84 ± 48</td>
<td>73 ± 36</td>
</tr>
<tr>
<td></td>
<td>(415)</td>
<td>(219)</td>
<td>(196)</td>
</tr>
<tr>
<td></td>
<td>P &lt; 0.01‡</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL</td>
<td>59 ± 23</td>
<td>58 ± 22</td>
<td>59 ± 24</td>
</tr>
<tr>
<td></td>
<td>(421)</td>
<td>(219)</td>
<td>(196)</td>
</tr>
<tr>
<td></td>
<td>P &lt; 0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VLDL</td>
<td>13 ± 9</td>
<td>14 ± 10</td>
<td>12 = 9</td>
</tr>
<tr>
<td></td>
<td>(258)</td>
<td>(199)</td>
<td>(59)</td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglyceride</td>
<td>40 ± 17</td>
<td>39 ± 16</td>
<td>40 ± 17</td>
</tr>
<tr>
<td></td>
<td>(603)</td>
<td>(307)</td>
<td>(296)</td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Mean ± standard deviation.
†Number of subjects.
‡Comparison of men and women for each lipid fraction.

| Abbreviations: NS = not significant; LDL = low density lipoprotein cholesterol; HDL = high density lipoprotein cholesterol; VLDL = very low density lipoprotein cholesterol.

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*Here and elsewhere, all values are expressed as mg per 100 ml ± the standard deviation.
Table 2

| Lipid Levels for Men and Women of Black or White Races (mg per 100 ml) |
|----------------|----------------|----------------|----------------|
|                | Black men     | White men     | Black women    | White women    |
| Cholesterol    | 146 ± 41      | 144 ± 41      | 144 ± 36       | 133 ± 28       |
| Total          | (49)          | (252)         | (50)           | (241)          |
| LDL            | 70 ± 52       | 86 ± 48       | 84 ± 41        | 71 ± 18        |
|                | (39)          | (181)         | (31)           | (162)          |
| HDL            | 62 ± 22       | 57 ± 22       | 59 ± 17        | 59 ± 26        |
|                | (39)          | (181)         | (31)           | (162)          |
| VLDL           | 12 ± 6        | 14 ± 10       | 7.1 ± 5        | 13 ± 9         |
|                | (31)          | (168)         | (11)           | (48)           |
| Triglyceride   | 37 ± 13       | 40 ± 16       | 43 ± 14        | 40 ± 17        |
|                | (49)          | (252)         | (50)           | (241)          |

This table excludes 11 subjects who were of Oriental and other races.

*Comparison of white men and white women.
†Comparison of black men and white women.
All other comparisons are not significant.

Discussion

The primary purpose of this investigation was to study a group of healthy Air Force recruits for asymptomatic hyperlipoproteinemia. However, a major challenge lies in the definition of cut-off points above which an individual may be regarded as clearly abnormal. Fredrickson, Levy, and Lees emphasized that not only is the setting of "limits of normal" for biologic quantities often arbitrary, but also what is usual for one population may not be for another and is not necessarily healthy for either. Additionally, a variety of factors such as age, sex, diet, underlying disease, laboratory methods, and preparation of the patient may influence the results of lipoprotein analysis.

Although other studies suggest "normal" limits for plasma lipids in healthy subjects around age 20, the differences in laboratory methodology, patient preparation, and selection of study groups raises considerable doubt about the applicability of these cut-off points to the results obtained in this study. For example, Keys et al. reported a mean plasma cholesterol of 173 mg% for individuals between ages 17 and 20 who were engaged mainly in business, scholastic, and professional pursuits and lived in a metropolitan area. Thomas, Murphy, and Bolling found a mean plasma cholesterol of about 210 mg% in a survey of medical students who were primarily white males. Lewis et al. reported the mean plasma cholesterol to be about 188 mg% at age 18, but in their large population study, most of the data were collected from men 40 to 59 years of age. Even the study done by Clark, Allen, and Wilson on West Point graduates, in which mean plasma cholesterol was found to be 249 mg% in subjects around age 20, is not totally comparable to ours since their group was composed of men only. Many of these and other population studies have been performed on nonfasting subjects which, although not an important factor in the determination of plasma cholesterol, exerts a critical influence upon plasma triglyceride concentrations. Hence, our subjects who were studied in the fasting state and are relatively homogeneous in age and physical condition, but of widely divergent genetic, geographic, and socioeconomic backgrounds, differ in important aspects from each of these other populations studied.

It is generally accepted that plasma cholesterol levels are very low at birth and rise slowly until age 30, then increase more rapidly to age 50, when they tend to plateau. Plasma triglyceride concentrations also increase with age. These changes reflect the
slow ascent in both very low density lipoproteins (VLDL) and low density lipoproteins (LDL) which are observed in the apparently healthy American population. The mean plasma cholesterol values found in our study are, however, substantially lower than those found by most investigators for subjects around the age of 20.16-19 When our results are compared to those reported for normal subjects by Fredrickson and Levy20 (table 3), the difference in total plasma cholesterol appears to be due mainly to the lower LDL cholesterol values observed in our subjects. Our subjects are a select group of healthy individuals who were under the psychologic stress of entering a new environment which could tend to lower plasma cholesterol levels. Also, the low mean plasma triglyceride concentration (40 ± 17 mg%) which we observed most likely reflects the lean habitus and good health of this group of young individuals.

When the influences of sex and race upon plasma lipids were examined in this group, women were found to have significantly lower total and LDL cholesterol levels than men. There were no important differences in plasma lipid concentrations between the races, except as influenced by sex.

Although some of the characteristics of our group could tend to result in lower than usual mean plasma cholesterol and triglyceride levels, there is little reason to suspect that the selection process would exclude those with asymptomatic inherited or certain acquired forms of hyperlipoproteinemia (e.g., dietary induced).

Of importance in the determination of cut-off points for plasma lipoproteins is the absence of a clearly bimodal distribution which we and others have observed.16,17 The high normal values usually tend to blend imperceptibly with the clearly abnormal to give a distribution skewed to the right. This is probably the result of incomplete genetic expression or polygenic transmission21 in the heritable forms of hyperlipoproteinemia and the result of the influence of diet and other environmental factors on all types of lipoprotein abnormalities.20 This positive skewing may be quite misleading in the parametric calculation of fiducial limits.16,17 However, if the logarithms of the values rather than the natural values are used, the distribution is more nearly normal and the 95% fiducial limits can be calculated with greater reliability.17 Indeed, the range of values for total plasma cholesterol in this study were quite wide, and as depicted in figure 1, skewed to the right. Statistical analysis13 confirmed that these data did not follow a normal distribution (P < 0.01). Both total and VLDL triglyceride values also displayed similar positive skewing, but LDL cholesterol distribution was normal. Calculations of 95% fiducial limits, summarized in table 3, were therefore performed after logarithmic transformation of the values for total cholesterol and total triglyceride to a normal Gaussian distribution pattern.

By our criteria, 26 (4.3%) of the 603 individuals in this study had plasma concentrations above the 95% confidence limits for either one or both total and LDL cholesterol. Nine subjects had elevations of both moieties. Five others with elevated plasma cholesterol values did not have plasma LDL cholesterol determined. In the remaining 12 individuals in whom one or the other of total or LDL cholesterol was above our cut-off point, only two had LDL cholesterol values which we regarded as "normal"; whereas, ten had "normal" total plasma cholesterol levels (but had elevated LDL cholesterol concentrations) by our standards. Of the 26 with abnormal plasma cholesterol values, 15 were men. When the limits suggested by Fredrickson and Levy20 were applied to our subjects, 26 individuals fell into the abnormal range for either one or both total and LDL cholesterol. By both sets of standards, a larger number of subjects were

**Table 3**

*Plasma Lipid Concentrations in Normal Subjects (mg per 100 ml)*

<table>
<thead>
<tr>
<th></th>
<th>This study</th>
<th>Fredrickson and Levy20</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean values</td>
<td>Upper 95% fiducial limits</td>
</tr>
<tr>
<td>Age, yr.</td>
<td>18-22</td>
<td>0-19</td>
</tr>
<tr>
<td>Cholesterol Total</td>
<td>139 ± 30*</td>
<td>175 ± 55</td>
</tr>
<tr>
<td>LDL</td>
<td>86 ± 40†</td>
<td>103 ± 29</td>
</tr>
<tr>
<td>HDL</td>
<td>50 ± 23</td>
<td>51 ± 13</td>
</tr>
<tr>
<td>VLDL</td>
<td>13 ± 9†</td>
<td>12 ± 8</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>40 ± 17</td>
<td>65 ± 55</td>
</tr>
</tbody>
</table>

*Mean ± standard deviation.
†Number of subjects.
‡Calculated from: LDL = bottom fraction cholesterol – HDL (N = 183).
§Calculated from: LDL = total cholesterol – (TG/5 + HDL), where total and HDL cholesterol were measured directly.
¶White females.
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Since this study offers no information about the prognostic significance of the individual lipid components, it is not possible to relate our findings to the risk of future coronary artery disease in our subjects. It does, however, seem clear that hyper-low density lipoproteinemia is an identifiable marker of enhanced tendency toward development of coronary atherosclerosis. Since by age 20 demonstrable coronary atherosclerosis is often present, by inference our results would suggest that the lipid levels we observed represent an important progression from those seen in even younger persons. It would also seem proper to select arbitrarily those younger persons above the 95% confidence limits for total cholesterol or LDL cholesterol for therapy.

No clear instances of hyper-very low density lipoproteinemia emerged from this study. While 30 individuals had plasma triglyceride levels above 83 mg% (95% fiducial limits established for our group), none had levels in excess of 150 mg%. Although we would not suggest that plasma triglyceride values above 90 mg% are abnormal, this is also probably a good reflection of the upper ideal level in healthy persons around the age of 20. How this finding relates to the development of atherosclerosis remains speculative.

Lipoprotein electrophoresis, as noted by others,\textsuperscript{15} is of little value in screening for subtle lipid abnormalities since the density of the lipoprotein bands varies considerably, particularly as the age. Since 30% of our entire group had a clear pre-beta band caused by either normal lipoproteins of beta mobility which were present in the sediment rather than the supranate at density 1.006 ("sinking pre-beta")\textsuperscript{20} or by normal levels of VLDL, lipoprotein electrophoresis was not useful in screening for hyperpre-beta lipoproteinemia.

This study would indicate that about 4% of apparently healthy American persons by age 20 have either total plasma or LDL cholesterol values which can be safely regarded as abnormal. This observation, considered in light of the close association of hyper-low density lipoproteinemia with premature coronary artery disease, reemphasizes the usefulness of instituting screening studies in young, healthy individuals.

Acknowledgments

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