Analytical Bias for Cholesterol and the Percent of the Population Deemed at Risk for Coronary Heart Disease

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The effect of methodological bias on the population at risk is dependent on the location of the reference value in the distribution of the population. We fitted the cumulative distribution for cholesterol to a rational function and calculated the apparent reference values for four biased methods: Technicon SMAC (2.6%), DuPont aca (4.0 to 4.6%), Kodak DT-60 (−2.0 to −5.5%), and BMD Reflotron (−7.4 to −7.8%). With the true and apparent reference values for cholesterol and the rational function, we determined the percentage increase or decrease in the population deemed at risk for coronary heart disease. The population at risk increased by as much as 48% for methods with positive bias, and decreased by as much as 54% for methods with negative bias. If we restrict the percentage of the population incorrectly diagnosed to 3% and use reference values (cut points) recommended by the National Cholesterol Education Program, the maximum allowable methodological bias would be 1.6% for positive bias and −1.55% for negative bias. Therefore, an absolute methodological bias of 3% (as recommended by the Laboratory Standardization Panel) may be too liberal.

Prevention of coronary heart disease by lowering concentrations of blood cholesterol is a strategy that has recently received increased emphasis. This strategy became widely publicized and officially recognized by the National Institutes of Health's Consensus Conference (1), which recommended that patients with a serum cholesterol concentration above the 90th percentile (high risk) for their age and sex be vigorously treated by diet, and, if necessary, by drugs. Patients with values between the 75th and 90th percentiles (moderate risk) should be treated by diet. The National Cholesterol Education Program of the National Institutes of Health slightly modified the program. They recommended that all adults with cholesterol concentrations ≥2.4 g/L be considered to have high cholesterol, and those with cholesterol concentrations between 2.00 and 2.39 g/L be considered to have borderline-high cholesterol (2). Patients with high cholesterol or with borderline-high cholesterol and two other risk factors for coronary heart disease are urged to have their low-density lipoprotein (LDL) cholesterol determined. If their LDL cholesterol is ≥1.60 g/L, or ≥1.30 g/L with coronary heart disease or with two other risk factors, they are then directed to lower their cholesterol by following a prudent diet. Drug therapy must be considered if their LDL cholesterol is ≥1.90 g/L (or ≥1.60 g/L with coronary heart disease or with two other risk factors). Other risk factors are male sex, family history of premature coronary heart disease, cigarette smoking, hypertension, low concentrations of high-density lipoprotein cholesterol, diabetes mellitus, definite cerebrovascular or peripheral vascular disease, and severe obesity. Furthermore, the Consensus Conference recommended that all adults have their cholesterol concentration measured.

Measurement of total cholesterol, as the initial step in the screening procedure, must be accurate. Several of the commercial methods for cholesterol were shown to be biased compared with the Reference Method used at the Centers for Disease Control (CDC) (3–5). The Reference Method at the Centers for Disease Control was the reference for the Lipid Research Clinics and Multiple Risk Factor Intervention Trial studies (6, 7). The reference values chosen by the Consensus Conference and the National Cholesterol Education Program are based on these studies. The Laboratory Standardization Panel of the National Institutes of Health's National Cholesterol Education Program advocates that the methodological bias be limited to ≤5% now, decreasing to 3% in five years (8). The effect of the methodological bias on the population at risk has not been evaluated. In this study, we determined the effect of bias on the percentage of the population classified as having either high or borderline-high cholesterol concentrations.

Materials and Methods

Correction for the bias of a method. The bias of a method, compared with the Reference Method, can be derived from linear regression. The concentration of cholesterol determined by a biased method can be corrected to that which would be obtained by the Reference Method by adjusting with the formula

\[ x = \frac{(z - b)}{m} \]

where \( x \) is the concentration of cholesterol as determined by the method, \( m \) is the slope of the regression (proportional bias), \( b \) is the y intercept (systematic bias), and \( z \) is the value of cholesterol as would be obtained by the Reference Method. \((z - x) / x\) is the total bias. Using the reference values (cut points) of 2.0 and 2.4 g of cholesterol per liter for \( z \) and previously determined values for \( m \) and \( b \), we corrected the concentrations of cholesterol determined by SMAC (Technicon Instrument Corp., Tarrytown, NY), aca (Du Pont, Wilmington, DE), DT-60 (Kodak, Rochester, NY), and Reflotron (Boehringer-Mannheim Diagnostics, Indianapolis, IN) systems to what would have been obtained with the Reference Method.

Population curve fitting. We used the percentiles and concentrations of cholesterol reported by the National Center for Health Statistics (9) to derive our formula relating percentile to the concentration of cholesterol. Because the fit of a cumulative gaussian distribution is optimized near its center, and not at its extremities, we had difficulty obtaining an accurate fit of the higher percentiles by that distribution. We achieved a better fit by using a rational function (ratio of two polynomials) and heavily weighting (10:1) the points above the 24th percentile. Using a SAS (Statistical Analysis System Institute, Inc., Cary, NC 27511) curve-fitting program, we fit the data to the formula:

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The equation is: \[ y = [(Ax^3 + B)/(1 + Cx + Dx^3)] + E \]

where \( y \) is the percentile, \( x \) is the concentration of cholesterol in g/L, and \( A, B, C, D, \) and \( E \) are various constants. We determined these constants for each subgroup by sex and age, and then we examined the residuals, rejecting the regression if any residual was greater than two percentiles.

### Effect of methodological bias on the population at risk.

We calculated the percentage of the population deemed to have high or borderline-high concentrations of cholesterol with our formula with the true reference values and the corrected values for results from the four biased methods. The difference between these two percentages (true minus corrected) represents the increase or decrease in the percentage of the population who would be deemed to have high or borderline-high cholesterol concentrations.

### Acceptable bias.

We calculated the magnitude of acceptable methodological bias with an iterative computer program. We assumed that the maximum error (inauthentic assignment to the borderline-high and high groups) would be 3% for positive bias and -3% for negative bias.

### Results

We obtained regression coefficients meeting our criteria for white men and women, ages 20–54 years. The effect of methodological bias on the population at risk is presented in Table 1 for the various analytical systems considered. The percentage of the population in either the high or borderline-high group, as assessed with the unbiased method, we designate as the "true" estimate. For example, the true percentage for white men, ages 25 to 34, with high cholesterol (2.4–4.0 g/L) is 14.2% (Table 1). A 2.4–g/L concentration of cholesterol determined with the Du Pont acc would be measured with the Reference Method as 2.3 g/L (Table 2), which would be encountered in 19.1% of white men 25 to 34 years old (Table 1). Thus, the acc’s positive methodological bias of 4.3% causes 4.9% more of the population (19.1% – 14.2%) to be considered as having high cholesterol. Correspondingly, the percentage of this population with borderline-high cholesterol (2.0–2.39 g/L) is 28.9% for an unbiased method and 34.1% for the acc, a difference of 5.2%. Addition of the percentages found for borderline-high and high cholesterol yields the total percentage at risk: 43.1% for the unbiased method and 53.2% for the acc. The difference (10.1%) is the percentage of the population inappropriately diagnosed as having borderline-high or high cholesterol. Thus, the magnitude of this error is greater than that of the methodological bias.

The effect of the methodological bias is similar for males and females, but varies considerably by age group. The percentage diagnosed as having high cholesterol increases with age for the Technicon SMAC and Du Pont acc (Table 1), for both sexes. The percentage misdiagnosed as having borderline-high cholesterol increases up to the 35–to 44-year-old age group for women and to the 25–to 34-year-old age group for men. The failure of the older age groups to show an increased percentage misdiagnosed as having borderline-high cholesterol results from the heightened increase in the high-risk cholesterol group, because the total percentage at risk increases for all age groups. A similar but opposite phenomenon is observed for negative bias, as seen with the Kodak DT-60 and BMD Reflotron (Table 1). The percentage of the population diagnosed as having high cholesterol, as based on results with these two instruments, is always lower than the true percentage. The percentage with borderline-high cholesterol for these instruments with negative bias, however, is greater than the true value for women ages 45–54 years and for men (DT-60 only) ages 35–54 years. In all cases, the negative bias decreases the total percentage of the population diagnosed as having borderline-high or high cholesterol. The augmented decrease in the high cholesterol group causes an apparent increase in percentage with borderline-high cholesterol for these subpopulations.

We determined the methodological bias when the acceptable error in the population with borderline-high or high cholesterol is restricted to a maximum of 3%. The allowable bias ranged from 1.4 to 1.9% (mean, 1.6%; SD, 0.15%) for positive bias and from -1.4 to -1.9% (mean, -1.55%; SD, 0.14%) for negative bias.

Overall, the relationship between the change in the population considered to have borderline-high or high cholesterol vs the methodological bias is not linear (Figure 1). There is essentially a linear relationship between a positive methodological bias and the increase in the percentage of the population; however, for a negative methodological bias, the relationship is curved, with the percentage of the population considered to have borderline-high or high cholesterol decreasing to zero as the negative bias increases.

### Discussion

The National Institutes of Health’s Consensus Conference has altered our concept of the reference interval for laboratory tests (1). Instead of establishing reference intervals with statistics based on the cholesterol distribution of a “normal” population, the Consensus Conference advocated

### Table 1. Percentage of the Population* Deemed at Risk Based on the Cholesterol Education Program Guidelines*

<table>
<thead>
<tr>
<th>Age, y</th>
<th>Prog*</th>
<th>SMAC</th>
<th>ACS</th>
<th>DT-60</th>
<th>Reflo.</th>
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<td></td>
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<td>16.1</td>
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<td>45–54</td>
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<td>34.5</td>
<td>33.7</td>
<td>40.5</td>
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<tr>
<td>Men</td>
<td></td>
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</tr>
<tr>
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<td>37.8</td>
<td>40.4</td>
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</tbody>
</table>

*For white adults; see ref. (9).

*High cholesterol, >2.4 g/L; borderline-high, from 2.0 to 2.39 g/L.

*Cholesterol Education Program guidelines, i.e., the unbiased results.

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using reference values associated with the risk of developing coronary heart disease. Reference values chosen by this new approach are no longer located in the low-frequency "tails" of the distribution of all values. This shift alters the relationship between methodological bias and the population deemed at risk for coronary heart disease. To establish criteria to assess the effect of methodological bias on the number of patients deemed healthy or at risk, we related the effect of methodological bias to the populations with borderline-high or high cholesterol values. These new reference values are not near the 97.5th percentile, but near the 25th, 50th, and 75th percentiles, where a methodological bias may create a larger error for the percentage of the population at risk. Thus, if we are to keep the magnitude of error for the new reference values near the same value as that for statistically determined reference intervals, the determination of cholesterol requires greater analytical accuracy than is currently advocated (8).

The current methods for cholesterol are biased in comparison with the Reference Method, as has been demonstrated with CAP surveys (10) and in direct comparisons with the Reference Method; the biases range between -7% and +5% (2-4). A bias changes the number of patients considered at risk, and it is the change in this number that is important. The cut points selected by the National Cholesterol Education Program are based on serum cholesterol concentrations determined with the CDC Reference Method. These cut points are fixed, i.e., independent of any methodological bias of whatever cholesterol method is used.

The direction of the bias is important, too. Positive bias shifts the distribution to the right, and the number of patients deemed at risk for coronary heart disease will be greater than if an unbiased method is used (Table 1). Moreover, this change in the number of patients deemed at risk varies with their sex and age.

We used the observed biases for the Du Pont aco, Technicon SMAC, Kodak DT-60, and BMD Reflotron, because they are near the 5% and 3% goals of the National Institutes of Health's Cholesterol Standardization Panel (8). Even with the SMAC, which has only a 2.6% methodological bias, more than 3% of the population will be wrongly classified.

We determined that the maximum, average, absolute methodological bias that can be permitted (to limit the percentage of the population misdiagnosed as being at risk) is 1.6%, considerably below the 3% recommended by the Laboratory Standardization Panel (8). In the central region of the density distribution, small errors in the value for cholesterol affect more patients than in extremities of the distribution. Here we established an approach to assess the effect of bias, which may be used to find the appropriate allowable bias once the limit for allowable error has been agreed upon.

References