Use of Serum Lipid and Apolipoprotein Concentrations to Predict Extent of Diet-Induced Atherosclerotic Lesions in Aortas and Coronary Arteries and to Demonstrate Regression of Lesions in Individual Rhesus Monkeys

Douglas A. Eggen, Ashim K. Bhattacharyya, Jack P. Strong, William P. Newman III, Miguel A. Guzman, and Carlos Restrepo

In most studies, the assessment of lesion "regression" is based on comparisons of group means between the "progression" and regression groups. This comparison depends on the assumption that the extent and distribution of lesions produced by the end of the lesion-induction period in the regression animals are equal to those observed in the progression group. To determine whether significant regression of lesions occurs in an individual regression animal, it is necessary to obtain a measure of the lesions produced in these animals at the end of the lesion-induction period. We achieved this goal by developing models using multiple stepwise regression analysis that related steady-state serum cholesterol and apolipoprotein B and A-I concentrations measured during a lesion-induction period in 27 rhesus monkeys fed an atherogenic high-saturated-fat/high-cholesterol diet for 2 years. The models were developed to estimate the percent of intimal surface with lesions, the esterified cholesterol content (µg/cm²) for the artery segments, and three histomorphometric measures (mean intimal thickness, mean maximal intimal thickness, and mean percent stenosis) for the coronary arteries. In these models, multiple $R^2$ ranged from 0.42 to 0.74 for the aortas and peripheral arteries, indicating that approximately one half to three fourths of the variance in lesions was accounted for. For the three histomorphometric measures in coronary arteries, however, the multiple $R^2$ was 0.27 or 0.28, indicating that only approximately one fourth of the variance in lesions was accounted for. These models were used to predict the percent of intimal surface with lesions and esterified cholesterol content (µg/cm²) for the aortas and four peripheral artery segments and for the three histomorphometric measures in the coronary arteries of 18 animals fed the atherogenic diet for 2 years and then a low-cholesterol regression diet for 30 or 52 weeks. The results indicated that for all arteries except the coronaries, a statistically significant lesion regression occurred in these animals, especially in those with the most extensive lesions. Nonparametric statistics, however, indicated that a borderline significant regression also occurred in the coronary arteries. We suggest that this method for assessing regression of experimentally induced atherosclerotic lesions in individual animals might be a useful alternative to the usual comparison by group and an additional tool for studies of regression of experimental atherosclerosis. (Arteriosclerosis and Thrombosis 1991;11:467-475)
gression” group was then killed to assess the extent of diet-induced lesions. Changes in diet, administration of drugs, or other treatments were then introduced for various periods to other groups of animals (regression groups) before the lesions were examined. Assessment of regression, defined as a decrease in the extent of lesions in the “regression” animals, was based on group means and thus depended on the assumption that the mean extent and distribution of diet-induced atherosclerotic lesions at the end of the induction period in these animals were equal to those in the progression group.

We recently reported a study in which rhesus monkeys were fed an atherogenic diet for 2 years. After sampling the animals for baseline “induced” lesions (progression group), dietary cholesterol was withdrawn from the surviving animals (regression group), which were then divided into two groups for 30 or 52 weeks of additional treatment. By comparing the mean extent of visually estimated lesions in the progression group with those in the regression group, we showed that in both the thoracic and abdominal aortas and in three of four peripheral arteries (but not in the coronary arteries), a partial regression of diet-induced atherosclerotic lesions occurs. Our study involved killing a large group (n = 27) of monkeys at the end of the progression period. We expected that a group of such size would enable us to develop models using serum lipid and apolipoprotein (apo) concentrations measured during the lesion-induction period to predict the extent of lesions induced in individual animals at the end of their lesion-induction period and that then underwent a lesion-regression regimen. These predicted values might then be used to show whether a significant regression occurred in each of the regression animals.

This article presents data on the extent of lesion regression in individual animals in the regression group for six artery segments and the coronary arteries from the 2-year study. The prediction models were based on the association between steady-state serum cholesterol, apo B, and apo A-I concentrations during the high-cholesterol-diet period and the percent of visually estimated total arterial intimal surface with atherosclerotic lesions as determined by visual estimation in each of the six artery segments, and in histomorphometric measurements in three major branches of the coronary arteries in 27 monkeys of the progression group fed a high-cholesterol diet for 2 years.

Methods

The detailed description of the study design has been previously reported. Briefly, 54 of 60 male rhesus monkeys (Macaca mulatta), 3–6 years of age, were fed a high-saturated-fat/high-cholesterol diet for 2 years. The other six animals served as the control group (group C); these animals were fed an identical diet but without the added cholesterol. One animal died of an undetermined cause, and groups of four high-responding and four low-responding animals were reallocated for use in other studies. Twenty-seven animals were killed for assessment of diet-induced lesions (the progression group, group P); then, cholesterol (but not fat) was removed from the diet of the remaining 18 animals. Six of these animals (group R1) were killed after 30 weeks, and the remaining 12 animals (group R2) were killed after 52 weeks of feeding on the high-saturated-fat but essentially cholesterol-free diet.

Details of the diet, description of the termination procedures, and methods for assessing the extent of atherosclerotic lesions have been described in the earlier publication. Briefly, the experimental atherogenic diet contained protein at 15% and fat (polyunsaturated/saturated = 0.35) at 38% of calories with cholesterol at 0.35 mg/kcal during lesion induction and at 0.02 mg/kcal during the regression period. The right sagittal half of the descending aorta and the right member of bilaterally paired arteries were used for chemical analyses, and the left half of the aorta and left members were used for gross evaluation after staining with Sudan IV. The perfusion-fixed coronary arteries were cut into sections perpendicular to the lumen axes, and the mean intimal thickness, the maximal intimal thickness, and the percent area within the “circularized” internal elastic lamina occupied by the intimal lesion were measured morphometrically as described earlier.

Chemical Analyses

Total serum cholesterol was measured by the Autoanalyzer II (Technicon Instruments Corp., Tarrytown, N.Y.) using a modified Lieberman–Burchard reagent. Methods for analyses of serum apos, fractionation of serum lipoproteins, and analyses for apo in lipoprotein fractions have been described earlier. Esterified cholesterol content was determined in the intima–media preparation of thoracic and abdominal aortas and in the arterial walls of the proximal 5 cm of the brachial and iliac-femoral arteries, the common carotid artery, and the carotid sinus as described earlier.

Statistical Analyses

The relations between steady-state serum cholesterol and apo concentrations during the lesion-induction period and the extent of atherosclerotic lesions induced by the high-saturated-fat/high-cholesterol diet were examined in the 27 animals of group P using multiple regression analyses. For these analyses, the following measures were used as dependent variables.

1) Gross measure. The percent arterial intimal surface with atherosclerotic lesions as determined by visual estimation in each of the six artery segments, that is, the left sagittal half of the thoracic and abdominal aortas, the proximal 5 cm of the left brachial and left iliac-femoral arteries, the left common carotid artery, and the left carotid sinus that included the regions 5 mm proximal and 5 mm distal to the flow divider.
2) Chemical measure. The esterified cholesterol content (\(\mu g/cm^2\)) of the intima–media preparation of the arterial walls of the six artery segments described above.

3) Morphometric measures of coronary arteries. The mean intimal thickness measured in 12 histological cross sections from the three major branches of the coronary arteries, the mean maximal intimal thickness in each of the 12 cross sections, and the mean percent of coronary lumen occupied by the intimal lesion, that is, percent stenosis.

A number of potential independent variables or predictors were measured during the lesion-induction period. These included rank by age, body weight, and concentrations of serum total cholesterol, triglycerides, apo B, and apo A-I. Preliminary examination of the relations using correlation and regression methods indicated that only the following three variables, obtained for each animal between the 18th and 102nd week of the high-cholesterol diet, were significantly related to lesion induction: 1) mean steady-state serum total cholesterol concentration (the mean of 20 determinations), 2) mean steady-state serum total apo B concentration (the mean of five to nine determinations), and 3) mean steady-state serum total apo A-I concentration (the mean of seven or eight determinations). In addition, the squares of each of these three measures were included in the analyses to allow for nonlinearity in the relations.

The model for each dependent variable was selected using the RSQUARE and STEPWISE procedures (with MAXR option) of the SAS statistical package.23 The model selected for each dependent variable was the one having the most parameters that contributed significantly \((p<0.10)\) to the reduction in the sum of squares. Where necessary, the square-root transformation of the dependent variable was used to obtain an approximate uniform distribution of residuals over the range of predicted values. By this procedure, the model selected is based solely on the data set for the 27 animals in the progression group. No variables were forced into the model based on other considerations.

For the selected model for each lesion measure, the REG procedure of the SAS package was then applied to the data for the 27 animals of group P to determine the “predicted” value of the dependent variable for the 18 animals in the two regression groups, R1 and R2. This value was taken as the estimate of the extent of atherosclerotic lesions that were induced by feeding the high-saturated-fat/high-cholesterol diet at the time the animals of group P were killed. The residuals, obtained by subtracting this predicted value from the extent of lesion actually measured at the time the animals were killed, thus gave an estimate of the extent of regression (if negative) or progression (if positive) for the lesion measure for that particular animal. The REG procedure also provided the standard deviation of the residual, which, together with the mean square for error, was used to calculate the 95% confidence limits for testing the hypothesis that the residual, that is, lesion regression or progression in an individual animal, was zero.

Results

Table 1 shows the standardized coefficients of the models derived from the data for the 27 animals of group P that were used for predicting the extent of lesions induced in the 18 animals of the regression groups. These standardized coefficients are distributed as the Student’s \(t\) statistic for the hypothesis that the coefficient is zero. The level of statistical significance for this hypothesis is indicated in the table footnote.

It is clear from the data in Table 1 that steady-state serum total cholesterol concentration is the dominant parameter in the models for measures of lesion induction. In this study as in others (D.A. Eggen, unpublished observations), we have observed a highly significant correlation between serum total cholesterol and total apo B concentrations. Thus, to a large extent, these predictors represent the same underlying properties of the two parameters. The fact that both serum total cholesterol and total apo B concentration entered many of the models indicates that this correlation is not perfect. Most models have a quadratic term, with a negative coefficient for one of these predictors indicating that the rate of increase in the extent of lesion decreases with an increase in the magnitude of the predictor. Serum total apo A-I concentration contributed significantly to the models for the two measures in the brachial artery and for the esterified cholesterol content of the iliac-femoral artery, in all cases with a negative coefficient.

Table 1 also shows that the multiple regression coefficients \((R^2)\) for both measures of lesions in the aortas and the four peripheral artery segments varied between 0.42 and 0.74, indicating that approximately one half to three fourths of the variance in lesions was accounted for by the prediction models. For the three histomorphometric measures in the coronary arteries, however, the \(R^2\) was 0.27 or 0.28, indicating that only about one fourth of the variance in the lesions was explained by the prediction models.

In Figure 1 for individual animals of the regression groups, we show the relation between the change in the percent of surface with lesion during the regression diet period and the percent of surface predicted to have lesions induced during the atherogenic diet period. For the thoracic aorta, 11 of 18 animals in the two regression groups (two of six in group R1 and nine of 12 in group R2) show statistically significant regression of lesions. For the abdominal aorta, 10 animals (three in group R1 and seven in group R2) show statistically significant regression of lesions. For the carotid bifurcation, a total of seven animals (two in group R1 and five in group R2) show statistically significant regression. For the common carotid or the iliac-femoral arteries, only one animal in group R1 shows significant regression. No animal showed a
TABLE 1. Standardized Coefficients in Models for Predicting Extent of Lesions Induced After Feeding Atherogenic Diet for 2 Years

<table>
<thead>
<tr>
<th>Measure</th>
<th>Predictor</th>
<th>C</th>
<th>C²</th>
<th>B</th>
<th>B²</th>
<th>A</th>
<th>A²</th>
<th>R²</th>
</tr>
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<tbody>
<tr>
<td>Percent of surface with lesion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thoracic aorta</td>
<td></td>
<td>4.7†</td>
<td>-3.2†</td>
<td>-2.1†</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>0.68</td>
</tr>
<tr>
<td>Abdominal aorta</td>
<td></td>
<td>3.2†</td>
<td>...</td>
<td>2.5‡</td>
<td>-3.2†</td>
<td>...</td>
<td>...</td>
<td>0.53</td>
</tr>
<tr>
<td>Carotid bifurcation</td>
<td></td>
<td>2.7†</td>
<td>-1.7</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>0.57</td>
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<tr>
<td>Common carotid artery</td>
<td></td>
<td>4.0†</td>
<td>...</td>
<td>...</td>
<td>-2.0</td>
<td>...</td>
<td>...</td>
<td>0.54</td>
</tr>
<tr>
<td>Brachial artery</td>
<td></td>
<td>2.4‡</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>-2.1‡</td>
<td>...</td>
<td>0.50</td>
</tr>
<tr>
<td>Iliac-femoral artery</td>
<td></td>
<td>3.6†</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>-3.0†</td>
<td>...</td>
<td>0.52</td>
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<td>Esterified cholesterol content*</td>
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<td>...</td>
<td>...</td>
<td>0.67</td>
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<tr>
<td>Thoracic aorta</td>
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<td>...</td>
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<td>Abdominal aorta</td>
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<td>0.42</td>
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<tr>
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<td>5.6†</td>
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<td>...</td>
<td>...</td>
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<td>...</td>
<td>0.56</td>
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<tr>
<td>Common carotid artery</td>
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<td>-2.0</td>
<td>...</td>
<td>-3.0†</td>
<td>...</td>
<td>0.74</td>
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<tr>
<td>Brachial artery</td>
<td></td>
<td>3.5†</td>
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<td>-3.1†</td>
<td>-2.6‡</td>
<td>...</td>
<td>...</td>
<td>0.61</td>
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<td>Morphometric measures of coronary arteries</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean intimal thickness</td>
<td></td>
<td>2.4‡</td>
<td>-2.0</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>0.27</td>
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<tr>
<td>Maximal intimal thickness</td>
<td></td>
<td>2.3‡</td>
<td>-1.9</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>0.27</td>
</tr>
<tr>
<td>Percent of lumen occluded</td>
<td></td>
<td>2.5‡</td>
<td>-2.1</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>0.28</td>
</tr>
</tbody>
</table>

C, serum total cholesterol; B, serum total apolipoprotein B; A, serum total apolipoprotein A-I.

*Square-root transformation used.

Statistical significance for hypothesis that regression coefficient is zero: †p<0.01; ‡p<0.05; all others, p<0.10.

significant reduction in the extent of lesions in the brachial artery.

Figure 2 shows the relation between the change in esterified cholesterol content (µg/cm²) during the regression-diet period and the predicted esterified cholesterol content during the atherogenic diet period for individual animals in the two regression groups. Statistically significant regression of esterified cholesterol for the thoracic and abdominal aortas occurred in eight animals, for the carotid bifurcation in five animals, for the common carotid artery in six animals, for the brachial artery in seven animals, and for the iliac-femoral artery in six animals.

The two figures clearly show that statistically significant regression occurred mainly for those arteries having the highest predicted values for diet-induced atherosclerotic lesions. This does not mean that regression did not occur in other artery segments that did not have a sufficient extent of induced lesions; rather, this means that the method for estimating the change in the extent of lesions may have been too insensitive to show it.

Figure 3 shows the change in the lesions for the three histomorphometric measures in the coronary arteries. For all three measures, 10–13 animals from the two regression groups R1 and R2 had negative residuals; with the exception of one animal in group R2 showing significant regression in maximal intimal thickness, none of the residuals were statistically significant for any animal. However, application of the nonparametric sign test²⁴ for the hypothesis that postulates equal numbers of positive and negative residuals indicated that there was an excess of negative residuals (p=0.048).

**Discussion**

In previous studies, assessment of regression in the extent of lesions and cholesterol content and concentration in the arteries in regression animals has been made by assuming that the group mean extent of diet-induced lesions and cholesterol content and concentration at the end of the lesion-induction period is identical to that of the progression group.¹⁻¹⁹ Thus, the extent of regression was assessed by comparing the group means of progression and regression animals. The objectives of this article have been twofold: 1) to develop models for predicting the extent of induced atherosclerotic lesions and the accumulation of esterified cholesterol that occur in different arteries during a lesion-induction period in individual live animals, using measures of serum cholesterol and apo concentrations during the lesion induction period as predictors, and 2) to use these predicted values for measures of atherosclerotic lesions in the individual animal to show that regression of lesions does occur in a given animal after subjecting it to a regimen expected to cause regression.

We show in this article that steady-state serum cholesterol and apo (apo B and apo A-I) concentrations determined during the lesion-induction period can be used to predict the extent of lesions and the accumulation of esterified cholesterol in different arteries in individual animals that underwent the regression regimen. We also show that this prediction
can be made with sufficient precision to enable assessment of changes (i.e., regression) in the extent of induced lesions and esterified cholesterol content in individual animals after withdrawal of dietary cholesterol. The multiple regression procedures used to obtain the predicted extent of lesions and esterified cholesterol content before removal of dietary cholesterol also provided estimates of the confidence limits for the estimated change in the extent of lesions and esterified cholesterol content. When we applied this method of analysis to the two measures of diet-induced lesions in six artery segments of 18 monkeys that were fed the atherogenic diet for 2 years and were then fed the regression diet for 1 year, we found that individually in most animals there was a decrease in the extent of lesions. As would be expected, the decrease was greatest in those animals with the greatest extent of induced lesions and
reached the level of statistical significance in many of these animals. We emphasize, as stated earlier, that this does not mean that regression did not occur in artery segments with a lesser extent of induced lesions; it is likely that, as applied here, the method for assessing the change in the extent of lesions is too insensitive to show a statistically significant change. These analyses extend and confirm the conclusions based on group means presented in the earlier report that removal of cholesterol while retaining the high-saturated-fat content in the diet results in regression of diet-induced lesions by 30 or 52 weeks.

Previously, we found that the means of the histomorphometric evaluations of the coronary artery lesions among the control, the progression, and the regression animals were not statistically significantly different. This suggested that the coronary artery lesions do not regress to any significant extent. However, examination of the data for individual animals had indicated that a majority of the reduction in the mean value resulted from reductions in fewer animals with severe lesions. Therefore, we suggest that it is likely that some lesion regression occurred in the coronary arteries for those animals with the most severe lesions. This suggestion is supported by the results of the analyses presented in this report (Figure 3). A nonparametric sign test of the data for the two groups combined indicated that lesion regression occurred in the coronary arteries (p=0.048). The data in Figure 3 also suggest that this tendency to regress is seen most easily in those animals with the greatest predicted lesions after the atherogenic diet period. These results are essentially in agreement with those reported by Clarkson and colleagues.

The serum parameters measured during the lesion-induction period that contributed significantly to the prediction of lesion extent and esterified cholesterol...
terol content were serum cholesterol, apo B, and apo A-I concentrations. Serum total cholesterol concentration is one of the best established risk factors for atherosclerosis in humans and animals; therefore, it was no surprise that in all arteries, the steady-state serum cholesterol concentration was the major predictor variable in the models and provided the greatest contribution to the reduction in the sum of squares. Since serum apo B and cholesterol concentrations, as stated earlier, are positively correlated, they are, therefore, to some degree interchangeable in these models. Thus, for most of the models, lesions were found to have a negative-quadratic term with regard to one of these predictors. This indicated that the rate of increase in the extent of induced lesions decreased with an increase in the magnitude of the predictor. Serum apo A-I contributed significantly, with a negative coefficient, to the reduction of the sum of squares for the two measures of lesions in the brachial artery and one measure in the iliac-femoral artery. Serum apo B and apo A-I concentrations were measured only five to nine times, whereas serum total cholesterol was measured 20 times during the steady-state period. It is possible that a more detailed and precise measurement of apolipoproteins over the long lesion-induction period could have provided a greater precision in predicting the extent of lesions, and thus, a statistically significant extent of lesion regression
could also have been demonstrated in those animals with less severe lesions.

Nevertheless, we have demonstrated that in individual live animals, the extent of lesions induced experimentally by feeding of an atherogenic diet can be predicted with precision using serum total cholesterol, apo B, and apo A-I concentrations and that a statistically significant change occurred in the extent of lesions during the regression-diet period. This, we believe, is a significant advance in studies of atherosclerosis regression because previously, the extent of regression has only been assessed by comparing the group means of lesion extent between progression and regression groups.1–19 Furthermore, it is a common experience that within the regression group, not all animals show regression; often, few animals show no regression of lesions, and some even show some degree of progression of lesions on the regression diet, suggesting that among animals there might exist a wide variability in susceptibility to regress. Comparison of the means of progression and regression groups, of course, obscures this fact and is possibly one reason why it has been difficult to show highly significant results of regression in nonhuman primates.

In summary, we have shown that using multiple stepwise regression analyses relating steady-state serum total cholesterol, apo B, and apo A-I concentrations measured during a lesion-induction period to the extent of lesions induced in a group of rhesus monkeys fed an atherogenic diet for 2 years, models were developed that predicted with reasonable accuracy the extent of lesions induced in the aortas, peripheral artery segments, and coronary arteries in individual animals in another group of animals fed the atherogenic diet for the same length of time. This enabled us to assess the change in the extent of lesions occurring in individual animals fed the regression diet with sufficient precision to demonstrate that a statistically significant regression had occurred. The availability of a measure of the extent of lesions induced in individual animals might then make it possible to categorize the animal for purposes of varying the treatment during regression. The measure of the extent lesion regression in an individual animal might also be used to examine associations with other characteristics that may vary among animals. Thus, we believe that the method described here should provide an additional tool for further studies of regression of experimental atherosclerotic lesions.

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lesions in rhesus monkeys consuming a high saturated fat diet. 
*Arteriosclerosis* 1987;7:125–134


**KEY WORDS** • serum cholesterol • apolipoproteins • fatty streaks • regression
Use of serum lipid and apolipoprotein concentrations to predict extent of diet-induced atherosclerotic lesions in aortas and coronary arteries and to demonstrate regression of lesions in individual rhesus monkeys.