A Generalized Mantel-Haenszel Analysis of the Regression of Blood Pressure on Blood Lead Using NHANES II Data

by J. Richard Landis* and Katherine M. Flegal†

This paper proposes an alternative analysis of the statistically significant blood pressure/blood lead relationship reported for males, ages 12 to 74, based on data from the second National Health and Nutrition Examination Survey. Because of the substantial decline, both for blood lead levels and blood pressures, during the 4-year survey period, there is considerable interest in the extent to which this association can be attributed to concurrent secular trends. The statistical methods illustrate the use of a randomization model-based approach to testing the statistical significance of the partial correlation between blood lead level and diastolic blood pressure, adjusting for age, body mass index, and the 64 sampling sites.

The resulting analyses confirm that the significant linear association between blood lead levels and diastolic blood pressures cannot be dismissed as a spurious association due to concurrent secular trends in the two variables across the 4-year survey period. In a conservative approach to this investigation, a randomization model-based test statistic, using the actual level of the natural log of blood lead and diastolic blood pressure, remained statistically significant at the 5% level, even when averaging the association across 478 subgroups formed by the cross-classification of age, body mass index, and the 64 sampling sites.

Introduction

Recent investigations of the relationship between blood lead levels and blood pressures resulted in reports of a direct, statistically significant effect of blood lead on both systolic and diastolic pressures for men (1,2). These findings were based on multiple regression models in which blood lead provided a statistically significant effect, after adjustment for age, body mass index, race, nutritional factors, and blood biochemistries, using data from the second National Health and Nutrition Examination Survey (NHANES II). Because the NHANES II data were obtained from a complex, multistage probability sample, final hypothesis testing in those multiple regression analyses needed to incorporate the sampling weights and the complex sample survey design effects. These analytic strategies for fitting predictive models to weighted, complex sample survey data are described in more detail in Landis et al. (3). Briefly, the variability in the estimated parameter vector across the 32 pairs of sampling sites was used to produce an estimated covariance matrix for the model parameters. Special-purpose computer programs that incorporate sampling weights and complex sample design effects were used to fit these multiple regression models (4–6).

An unexpected complexity of the NHANES II data resulted from a striking secular decline in blood lead levels during the survey period from 1976 to 1980. This significant decrease in blood lead levels and its relationship to the declining use of leaded gasoline nationally is described in more detail elsewhere (7). The 64 sampling sites were visited sequentially throughout this 4-year period using three mobile examination centers. Typically, data were being collected at two sites concurrently for about 4 to 6 weeks, while the third mobile unit was in transit to the next sampling site. Thus, unexpected secular trends, such as this decline in blood lead levels, introduced additional variation in the data from these 64 sampling sites. The impact of this decline on the multiple regression analyses is not straightforward to investigate due to potential multicolinearity problems and the basic cross-sectional design assumptions of the replication procedures for variance estimation.

Several modifications of these multiple regression methods could be considered; however, none is entirely satisfactory. On one hand, we could develop an ex-
panded multiple regression model that includes site parameters as an attempt to adjust for the site to site variability. However, this would add 63 additional parameters to the model, posing several difficulties, in addition to instability of parameter estimates. From a conceptual perspective there is no intrinsic interest in these individual sites parameters, since the sites were selected in order to be combined to produce nationally representative estimators. In addition, many of these site parameters may be nonsignificant, but taken as a set of 63 different effects in the model, they would tend to mask any decline across the survey period due to the sequential nature of the sampling by sites. Finally, by including the site parameters in the multiple regression model, it would not be possible to estimate the covariance matrix for the parameter vector based on the complex sample survey design.

In contrast to a model with 63 site parameters, we could incorporate a time effect in a multiple regression model. This would add only one additional parameter to the predictive model, but would introduce serious multicollinearity problems with any variable exhibiting a secular trend during the survey period. Either this approach or the expanded model with 63 site parameters poses serious limitations to a rigorous assessment of the blood pressure on blood lead regression relationship in these data, since the additional model parameters will tend to mask that effect.

Our objective here is to test the significance of the partial correlation between blood pressure and blood lead after adjusting for the relevant covariables and the sampling sites, but without adding site parameters to a multiple regression model. Essentially, we will use a statistical procedure that targets all the statistical power at the linear regression relationship between blood pressure and blood lead levels, averaging across the sampling sites, age, and body mass subgroups. Thus, this method not only will adjust for the two primary covariables in characterizing the variation in blood pressure, but also will adjust for any secular trend indirectly through stratification on the 64 sampling sites.

Materials and Methods
Survey Description
The NHANES II study was conducted from February 1976 to February 1980 on a representative sample of the civilian noninstitutionalized U.S. population from the ages of 6 months to 74 years. A total of 20,322 persons were examined. Details of the complex sample survey design, nonresponse adjustments, and the examination and laboratory measurement procedures have been published (8,9).

Blood pressure measurements were obtained from each individual using an appropriate size cuff on three separate occasions during the examination: a) with the examinee seated early in the examination, b) recumbent midway through the examination, and again c) seated near the end of the examination. The American Heart Association recommendations on blood pressure recording were followed. The first- and fifth-phase Korotkoff sounds were taken as systolic and diastolic pressures, respectively. The second seated blood pressure was used for the present analysis, although previous analyses demonstrated that similar results are obtained when using the first seated blood pressure, the recumbent blood pressure, and the mean of the first and second seated blood pressures (1,2).

Blood samples were obtained by venipuncture and shipped to the Clinical Chemistry Division, Center for Environmental Health, Centers for Disease Control, Atlanta, GA, where the samples were analyzed. Blood lead concentrations were analyzed by atomic absorption spectrophotometry on odd-numbered samples from the examinees aged 12 to 74 years. Specimens were analyzed in duplicate, and an extensive quality control system was used to ensure accurate assessment without laboratory drift (10).

Statistical Methods
The randomization model methods outlined in this section provide an alternative strategy for multiple regression modeling when the main focus is on the primary relationship between a factor and a response variable averaged across the strata formed by the covariables, rather than on a predictive model that includes effects for each of the levels of the covariable set. When both the factor and the response variable are continuous, the actual measurements may be used as scores, so that no loss of information in their levels occurs. Furthermore, this level of measurement is directly analogous to that used in the corresponding multiple regression model.

In our application here, this generalized randomization model approach will be used to test whether the linear correlation between the factor, the natural logarithm (ln) of blood lead measured in ug/dL units, and the response variable, diastolic blood pressure measured in mm Hg, remains statistically significant after adjusting for the sampling sites and the other relevant covariables. For practical and substantive reasons, these adjustments are made for the two main covariables in the variation of blood pressure, age at three levels and body mass at three levels, in addition to the 64 sampling sites.

Many research investigations involve the analysis of data in which the primary focus is directed at the association between a factor, with levels indexed by \( i = 1, 2, ..., s \) and a response variable, with levels indexed by \( j = 1, 2, ..., r \), while controlling for \( t \) distinct levels of a set of relevant covariables, indexed by \( h = 1, 2, ..., t \). In principle, the data can be arrayed as frequencies in a set of \( t \) two-way contingency tables, as summarized in Table 1, where \( n_{ijh} \) denotes the number of subjects in the sample who are jointly classified as belonging to the \( i \)th row and the \( j \)th column in the \( h \)th subtable. Fre-
Table 1. Observed contingency table for cross-classification of \( \ln \) blood lead level and diastolic blood pressure at level \( h \) of the stratification variables.

| \( \ln \) Pb levels | \( y_1 \) | \( y_2 \) | \ldots | \( y_r \) | Total |
|---------------------|--------|--------|-------|--------|-------|
| \( x_1 \)           | \( n_{h11} \) | \( n_{h12} \) | \ldots | \( n_{h1r} \) | \( N_{h1} \) |
| \( x_2 \)           | \( n_{h21} \) | \( n_{h22} \) | \ldots | \( n_{h2r} \) | \( N_{h2} \) |
| \( x_s \)           | \( n_{hs1} \) | \( n_{hs2} \) | \ldots | \( n_{hsr} \) | \( N_{hs} \) |
| Total               | \( N_{h1} \) | \( N_{h2} \) | \ldots | \( N_{hr} \) | \( N_h \) |

This probability distribution forms the basis for such familiar procedures as Fisher’s exact test in a single \( 2 \times 2 \) table and other exact conditional tests resulting from natural extensions to larger tables.

For each subtable indexed by \( h = 1, 2, \ldots, t \), it follows that the expected value of \( n_{hij} \) under \( H_0 \) in Equation 1 is

\[
m_{hij} = N_{h1} N_{h} / N_h.
\]

Furthermore, the covariance between \( n_{hij} \) and \( n_{hij'} \) under \( H_0 \) in Equation 1 is

\[
v_{h,ij,ij'} = \frac{N_{hi} N_{hj} (\hat{\epsilon}_{ii} N_{h} - N_{hi}) (\hat{\epsilon}_{jj} N_{h} - N_{hj})}{N_h^2 (N_h - 1)}.
\]

where \( \hat{\epsilon}_{ii} = 1 \), if \( i = i' \), = 0 otherwise, and \( \hat{\epsilon}_{jj} = 1 \), if \( j = j' \), = 0 otherwise.

There are a number of different situations in which this randomization model structure has been used to test \( H_0 \) as reviewed in Landis et al. (11). In the simplest case, when the factor and response variables have only two levels, the resulting data display simplifies to a set of \( t \) \( 2 \times 2 \) tables and the test statistic for \( H_0 \) is commonly referred to as the Mantel-Haenszel test (12). In the most general context, the test statistic is a quadratic form involving the expected values in Equation 3 and a covariance matrix composed of the elements in Equation 4 which has a chi-square distribution with \( (s-1)(r-1) \) degrees of freedom (11-13). If either the response variable or both the factor and the response variable are ordinaly scaled, the resulting test statistic incorporating these scores can be used to test ordered alternatives of \( H_0 \) (11-14). These methods can be implemented in the computer package, PARCIT documented in Landis et al. (15) and are now available in SAS, Version 5, within PROC FREQ (16).

The correlation test statistic based on the randomization model in Equation 2, using the moments in Equations 3 and 4, simplifies considerably in situations for which the row and column variable are analyzed as continuous variables, with scores \( x_i \) and \( y_j \) as indicated in Table 1. Although this method was described in an early paper by Mantel (14) and illustrated in a recent paper by Mantel et al. (17), none of the available statistical packages can be used to compute this statistic in large data sets, such as the NHANES II sample, without special-purpose programming. The main reason is a practical one in that each unique level of the factor generates a separate row of Table 1 and each unique level of the response variable corresponds to a separate column of Table 1. As a result, the number of cells becomes prohibitively large, requiring excessive space requests in a package such as SAS (16). Furthermore, the 1 df correlation statistic is actually computed in parallel with a total association statistic having \((s-1)(r-1)\) df and a mean score statistic.
having \((s-1)\) df, both of which lead to singularities if certain configurations of cells are empty across all \(t\) subtables. Consequently, we needed to bypass totally the construction of each subtable, but still extract the corresponding components from each stratum necessary to compute the test statistic.

Another important consideration for data arising from complex sample surveys is the use of the sampling weights. As noted previously, the multiple regression models were developed using weighted regression estimators \((1-7)\). The sampling variances of the parameter estimates were obtained by either replication methods or Taylor series linearization across the sampling sites. However, in this application we are conditioning on the marginal distribution of the \(ln\) blood lead levels and the diastolic blood pressure levels in each stratum and computing the moments as in Equations 3 and 4 for the unweighted frequencies.

To summarize this statistical procedure, let

- \(y_{hu}\) denote the observed response (column) variable value for the \(u\)th individual in the \(h\)th stratum;
- \(x_{hu}\) denote the observed predictor (row) variable value for the \(u\)th individual in the \(h\)th stratum, for \(h = 1, 2, ..., t\).

Then the pivot function for the test statistic from the \(h\)th stratum simplifies to

\[
F_h = \sum_u x_{hu} y_{hu},
\]

with expected value under \(H_0\) given by

\[
E(F_h|H_0) = N_h \bar{x}_h \bar{y}_h,
\]

where \(\bar{x}_h\) and \(\bar{y}_h\) are the respective sample means within the \(h\)th stratum. The contribution to the numerator of the test statistic from the \(h\)th stratum is

\[
G_h = F_h - E(F_h|H_0) = \sum_u x_{hu} y_{hu} - \left( \sum_u x_{hu} \right) \left( \sum_u y_{hu} \right) / N_h,
\]

which has expected value of 0 under \(H_0\), and the contribution to the denominator of the test statistic from the \(h\)th stratum simplifies to

\[
\text{Var}(G_h|H_0) = \frac{\left( \sum_u y_{hu}^2 - N_h \bar{y}_h^2 \right) \left( \sum_u x_{hu}^2 - N_h \bar{x}_h^2 \right)}{N_h - 1},
\]

using standard results provided in references \((11,14,15,17)\).

Using these components, the overall test statistic for partial correlation between the factor and the response variable simplifies to

\[
Q = \frac{\left( \sum_{h=1}^t G_h \right)^2}{\sum_{h=1}^t \text{Var}(G_h|H_0)} = \frac{\left( \sum_{h=1}^t \left( \sum_u x_{hu} y_{hu} - \frac{\left( \sum_u x_{hu} \right) \left( \sum_u y_{hu} \right)}{N_h} \right) \right)^2}{\sum_{h=1}^t \left( \sum_u y_{hu}^2 - N_h \bar{y}_h^2 \right) \left( \sum_u x_{hu}^2 - N_h \bar{x}_h^2 \right) / (N_h - 1)}.
\]

Essentially, this is a test of \(H_0\) in Equation 1 against a linear correlation alternative using the actual values for \(X\) and \(Y\), rather than assigning scores to grouped levels of the factor and response variable. It can be noted immediately in Equation 9 that the test statistic is based on weighted sums of the usual components of the simple linear correlation coefficient between \(Y\) and \(X\) across the \(t\) strata. Under \(H_0\), \(Q\) asymptotically follows the chi-square distribution with df = 1. This statistic is directed at the extent to which there is a consistent positive (or negative) linear association between the response variable levels and the factor levels in the respective strata. Thus, it is directed at average partial association alternatives across the \(t\) strata.

**Application to the NHANES II Data**

This generalized randomization model procedure can be used to test the significance of the linear relationship between blood lead on the \(ln\) scale and diastolic blood pressure, adjusting both for the key predictive variables, age, and body mass, and for the 64 sampling sites. An attractive feature of this methodology for the NHANES II design is that it provides adjustments for the potential effects of the sampling sites without requiring a predictive model with 63 site parameters. This statistic, averaged over the strata formed by the cross-classification of age, body mass subgroups, and sampling sites, is potentially more robust than a multiple regression model with indicator variables for the sites.

This analysis was conducted using the sample of 3181 males between the ages of 12 and 74 years for
whom both the second seated diastolic blood pressure measurements and blood lead concentration measurements were available. In order to adjust for age and body mass, we constructed several alternative ordinal factor levels for age and Quetelet index, the conventional body mass index calculated as weight in kilograms divided by the square of height in meters. Although a large number of different possible partitions of these two risk factors could be considered, we investigated two reasonable possibilities and will report results from both.

Let \( A \) denote age and \( Q \) denote Quetelet index as continuous variables. Then define the stratification subclass variables according to

\[
A_1 = \begin{cases} 
1, & A \leq 40 \\
2, & A > 40
\end{cases},
\]

\[
A_2 = \begin{cases} 
1, & A \leq 20 \\
2, & 20 < A \leq 40 \\
3, & A > 40
\end{cases},
\]

\[
Q_1 = \begin{cases} 
1, & Q \leq 22 \\
2, & 22 < Q \leq 26.5 \\
3, & Q > 26.5
\end{cases},
\]

\[
Q_2 = \begin{cases} 
1, & Q \leq 23 \\
2, & 23 < Q \leq 27 \\
3, & Q > 27
\end{cases}.
\]

These subclasses were chosen to represent substantively meaningful divisions, while still maintaining adequate sample sizes within each stratum. The cut points of 22.0 and 26.5 for \( Q_1 \) approximately correspond to 100% and 120% of standard weight for height according to the 1959 Metropolitan Life tables (18). The cut points of 23.0 and 27.0 for \( Q_2 \) correspond to similar values for the 1983 Metropolitan Life weight-for-height tables (19).

### Results

To illustrate the effects of age and body mass on diastolic blood pressure and blood lead levels (In scale), the mean diastolic blood pressure (DBP) and mean blood lead level (In scale) is summarized in Table 2 for each of the nine subgroups formed by the cross-classification of \( A_2 \) and \( Q_2 \). The increase in mean DBP for increasing levels of age and Quetelet index is quite pronounced. The relationship between mean blood lead levels and these two factors are not as clear for these aggregate subgroups here, but these relationships have been discussed in considerable detail elsewhere (1,9,10).

The crucial results to note in Table 2 are the simple linear correlation and regression coefficients between DBP and InPb overall, as well as within each of these nine subgroups.

The overall correlation coefficient of 0.15 is highly significant, but because the levels of blood lead increase both with age and body mass index, this coefficient also reflects that association. More importantly, the magnitude of the regression coefficient, 4.90, suggests a sizable impact of ln blood lead levels on DBP. The subgroup correlation coefficients, ranging from 0.04 to 0.12, show a remarkable consistency of this relationship within each of these 9 subgroups. This reduced strength from the overall coefficient of 0.15 reflects the subgroup adjustments for the age and body mass index effects, as do the separate regression coefficients, predominantly between 2.3 and 3.6. All of these adjustments are incorporated more precisely within the multiple regression models incorporating the sampling weights and the complex sample survey design effects described in more detail elsewhere (1).

It is precisely the stability of this association between blood lead and DBP across the 64 sampling sites, in addition to these adjustments for age and body mass index, that is under investigation here. In other words, we are interested in determining the extent to which this relationship is reflecting a parallel decline of blood pressures and blood lead levels during the 4-

| Age in years \((A_2)\) | Quetelet index \((Q_2)\) | Observed sample size | Unweighted mean DBP | Unweighted mean \(\ln\text{Pb}\) | Coefficient of DBP on \(\ln\text{Pb}\) | Correlation | Regression |
|-----------------------|------------------------|---------------------|---------------------|-------------------|-----------------------------|-------------|-----------|
| \(A \leq 20\)         | \(Q \leq 23\)         | 484                 | 70.9                | 2.55              | 0.10                        | 2.75        |           |
| \(Q \leq 27\)         | 135                    |                     |                     |                   |                             |             |           |
| \(Q > 27\)            | 43                     |                     |                     |                   |                             |             |           |
| \(20 < A \leq 40\)    | \(Q \leq 23\)         | 336                 | 75.4                | 2.78              | 0.12                        | 3.30        |           |
| \(Q \leq 27\)         | 368                    |                     |                     |                   |                             |             |           |
| \(Q > 27\)            | 265                    |                     |                     |                   |                             |             |           |
| \(A \geq 40\)         | \(Q \leq 23\)         | 327                 | 79.9                | 2.77              | 0.11                        | 3.43        |           |
| \(Q \leq 27\)         | 672                    |                     |                     |                   |                             |             |           |
| \(Q > 27\)            | 551                    |                     |                     |                   |                             |             |           |
| Total                 |                        | 3181                | 80.5                | 2.72              | 0.15                        | 4.90        |           |

*Males, 12 to 74 years, NHANES II, United States, 1976 to 1980.*
year survey period, or whether this relationship persists after adjustment for the secular trends.

This issue was investigated using the randomization model statistic summarized in Equation 9. This statistic was computed to test the significance of the linear relationship between ln blood lead levels and diastolic blood pressures, adjusting for the effects due to the 64 sampling sites, Quetelet index and age, both singly and in combination. The resulting test statistics are summarized in Table 3.

These adjustments were performed sequentially to illustrate the relative effects of each covariate on the overall test statistic. For example, as noted in lines 1 and 2 of Table 3, the effect of adjusting only for the 64 sampling sites is to reduce $Q$ from 68.8 to 44.0, still highly significant at $\alpha < 0.01$. Based on the single factor adjustments, these results suggest that the age partition at three levels ($A_2$) produces the largest reduction in the test statistic. Otherwise, the alternative partitions of body mass index produce very similar results.

The test statistics reflecting the simultaneous adjustments for age and body mass index are summarized in Table 3 in lines 7-10. In particular, the adjustment for these factors, as cross-classified into the nine subgroups described in Table 2, yields $Q = 27.99$. This statistic corresponds directly to a test for the significance of the partial correlation between ln blood lead and DBP across these nine subgroups.

The final adjustments displayed in Table 3 correspond to the joint cross-classification of each of these pairwise partitions of age and body mass index and the 64 sampling sites. In principle, this leads to $64 \times 6 = 384$ subtables or $64 \times 9 = 576$ subtables, depending on whether $A_1$ or $A_2$ is used for the age partition. In actual fact, as summarized in column 2 of Table 3, the number of usable subtables ranges from 355 out of the 384 when using $A_1$ to 478 out of 576 when using $A_2$. This reduction in subtables results from certain cross-classifications not yielding at least two individuals, the minimum required for the estimation of the variance term in Equation 8. However, the loss of actual individuals in the overall analysis is at most 61, (3181-3120).

Lines 12 and 14 of Table 3 summarize the most stringent adjustments for these covariates. For example, the bottom line corresponds to the same nine subgroups described in Table 2 being formed in each of the 64 sampling sites. For this situation, the test statistic is 4.62, still statistically significantly at $\alpha < 0.05$. In other words, the partial correlation between ln blood lead level and diastolic blood pressure cannot be dismissed as a random phenomenon due to a parallel secular decline in these two variables over the survey period. Even with the adjustments for age, body mass index, and sampling site, the statistical significance of this relationship is substantiated.

### Discussion

The primary objective in the analysis of these data was to investigate the relationship between blood lead levels and diastolic blood pressures, adjusting for other important sources of variation. Because of the strong multicollinearity between age, body mass index, and blood lead levels, the adjustment for age and body mass are also adjusting for some of the lead effect on blood pressure. Moreover, because the sites were visited sequentially over the period of the survey and there was a striking decline (nearly 40% for all relevant socio-demographic subgroups) in blood lead levels over time, there is a statistically significant variation in mean blood lead levels across the sampling sites (7). Blood pressure levels also declined over the time of the survey and there is also a significant variation in mean blood pressure levels among the sites. Thus, adjustment for the sampling sites also reduces the potential that blood lead levels will show a significant association with blood pressures.

The statistical methods used in these analyses represent an extremely conservative approach to the assessment of this linear association between blood lead levels and blood pressures. Essentially, the association must be sufficiently robust to persist across 478 subgroups formed on the basis of factors also having an association with the levels of blood lead and blood pressure. Nevertheless, even with these severe adjustments by sampling sites, age, and Quetelet index, the diastolic blood pressure/blood lead relationship is still statistically significant at the 5% level. In fact, the chi-square statistic of approximately 4 in the analysis with the most stringent adjustments corresponds to the square of the test statistic of 2 in the most conservative multiple regression models (1,2).

In summary, these analyses suggest that the

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**Table 3. Correlation test statistic for diastolic pressure on ln blood lead level based on randomization model adjusted for stratification variables.**

| Strata   | Number of strata with $N_s \geq 2$ | Sample size | Correlation test statistic (df = 1) | Significance level |
|----------|-----------------------------------|-------------|------------------------------------|-------------------|
| None     | 1                                 | 3181        | 68.83                              | < 0.01            |
| Sites    | 64                                | 3181        | 44.04                              | < 0.01            |
| $A_1$    | 2                                 | 3181        | 50.44                              | < 0.01            |
| $A_2$    | 3                                 | 3181        | 24.07                              | < 0.01            |
| $Q_1$    | 3                                 | 3181        | 54.04                              | < 0.01            |
| $Q_2$    | 3                                 | 3181        | 55.27                              | < 0.01            |
| $Q_1A_1$ | 6                                 | 3181        | 39.85                              | < 0.01            |
| $Q_1A_2$ | 9                                 | 3181        | 27.70                              | < 0.01            |
| $Q_2A_1$ | 6                                 | 3181        | 41.61                              | < 0.01            |
| $Q_2A_2$ | 9                                 | 3181        | 27.99                              | < 0.01            |

*Males, 12 to 74 years, NHANES II, United States, 1976 to 1980.*
significant linear association between ln blood lead levels and diastolic blood pressures cannot be dismissed as concurrent secular trends in the two variables across the 4-year survey period. In a conservative approach to this investigation, a randomization model-based test statistic, using the actual level of ln blood lead and diastolic blood pressure, remained statistically significant at \( \alpha < 0.05 \), even when averaging the association across 478 subgroups. Finally, it should be noted that even though these partial correlation are not large, the magnitude of the regression coefficients suggests that elevated blood lead levels may be an important risk factor for elevated blood pressures as developed in considerably greater detail for the restricted group of white men 40 to 59 years of age (2).

Supported in part by NIH Grant No. 2 R01-HL33407-02

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