Twenty-Four-Hour Ambulatory Blood Pressure in Shift Workers

Nguyen Phong Chau, PhD, Jean Michel Mallion, MD,
Regis de Gaudemaris, MD, Emmanuel Ruche, MD, Jean Philippe Siche, MD,
Odile Pelen, MD, and Gerard Mathern, MD

Blood pressure and heart rate of 15 male shift workers were measured every 15 minutes for 24 hours during three work shifts: morning, 4:00 AM to noon; afternoon, noon to 8:00 PM; and night, 8:00 PM to 4:00 AM. For each shift, 24-hour systolic and diastolic blood pressure showed a large “trough” (the low pressure span) and a continuous range of elevated pressure (the high pressure span). Fourier series were used to model the 24-hour blood pressure profiles. A careful examination of the residuals (measured minus predicted pressures) showed that four harmonics were necessary to describe the data accurately. The model enabled localization in each blood pressure profile of the high and low pressure spans that did not coincide with the subject’s work and rest periods. The time and slope of blood pressure entering and leaving these spans could also be individually determined. Mean blood pressure during the high pressure span was the same for the three shifts, but mean blood pressure during the low pressure span was lower when the subject worked in the afternoon. During that shift, the systolic blood pressure slopes entering and leaving the low pressure span were steeper than during the two other shifts. The high pressure span was longest during the night shift and shortest during the afternoon shift. Therefore, a change in the working time profoundly perturbed the 24-hour blood pressure profile. We conclude that internal regulation mechanisms, subjects’ activities, and the circadian rhythm are the three main factors that govern a 24-hour blood pressure level; a complete change in the time of activities modifies the combined effects of these three factors. (Circulation 1989;80:341–347)

Several noninvasive semiautomatic or automatic devices are presently available that enable blood pressure and heart rate to be measured in ambulatory conditions over 24-hour periods at a frequency arbitrarily set in advance. Ambulatory blood pressure is an important parameter because hypertension is a major risk factor of cardiovascular morbidity and mortality. Also, in the healthy subject, repeated observations of blood pressure might provide new insight into the physiology of the cardiovascular system.

It is well known that blood pressure and heart rate fluctuate over a 24-hour period. These fluctuations are due to at least three groups of factors. First, blood pressure and heart rate are regulated by complex internal physiologic mechanisms. In the absence of external stimuli, these autoregulated mechanisms may per se induce periodic blood pressure and heart rate variations. Halberg et al reported “free-running” heart rate in a man isolated for 2 months at 130 m underground. In this subject, heart rate showed a periodic change, with a period somewhat longer than 24 hours. In routine living conditions, blood pressure also oscillates almost periodically, with a period different from 24 hours, when observed over a period of less than 1 week. Over a longer time frame (4 weeks or more), the period approaches, in mean value, 24 hours. Second, it is well known that external stresses and the subject’s activities have direct effects on blood pressure and heart rate. Pressure effects of specific activities, such as reading, mental arithmetic calculation, or exercising, have been evaluated. Third, daytime and nighttime may attempt to synchronize blood pressure to their own circadian rhythm.

Most authors reported that blood pressure during daytime is higher than during nighttime. In previous analyses, daytimes and nighttimes have been chosen arbitrarily at fixed hours and taken as the same
for all subjects. However, the changes in blood pressure from the high pressure span to the low pressure span and vice versa certainly vary among subjects and depend on their activities. In current daily life, because subjects have different activities when awake during the day, when resting, or when sleeping during the night, the separate influences on blood pressure of wakefulness and activities on one hand and of the circadian rhythm on the other could not be evaluated.

In the present study, we examined blood pressure in shift workers who worked either in the morning, the afternoon, or at night. Our purpose was to determine whether the “two-level” blood pressure pattern occurs regardless of work shifts and, if so, how high and low blood pressure spans are distributed over 24 hours under the combined effects of work shifts and the circadian rhythm. The analysis required a mathematic modeling of the 24-hour profile; we used Fourier series.

**Methods**

**Subjects and Blood Pressure Measurements**

Fifteen healthy male subjects (age, 25–40 years; mean±SEM, 30±1) participated in the study. The subjects are employees of a chemical factory, and their work activities consisted of surveying various chemical processes. Work shifts were 4:00 AM to noon (morning), noon to 8:00 PM (afternoon), and 8:00 PM to 4:00 AM (night). The subjects worked in 8-day shifts, permuting schedules every 2 days and resting 2 days after working a cycle of three shifts. Workload was the same for all shifts. All subjects were shift workers for at least 1 year before beginning the study. Informed consent to participate in the protocol was given by each subject.

Within a month, 24-hour blood pressure of each subject in each of the three shifts during a different 8-day period was recorded (Spacelabs 5200 system). The first shift was selected at random, and recordings were determined by cyclic permutation (morning, afternoon, or night). Pressure recordings were taken every 15 minutes. If a recording failed, a second one was automatically taken 2 minutes later. Data with diastolic pressure higher than systolic pressure were rejected. Also, if two successive pressure recordings (diastolic or systolic) differed by more than 50% without a concomitant increase in heart rate, the second measurement was rejected. About 1–5% of data were discarded by this method.

**Modeling of Blood Pressure Profile**

The data show that for each subject and each shift, 24-hour blood pressure showed a two-level pattern, including a time span where blood pressure was high (high pressure span) and a “basin” where blood pressure was low (low pressure span). In general, the high pressure time span was longer than the low pressure time span. We modeled the 24-hour blood pressure profile to localize mathematically the two spans and to define blood pressure “slopes” entering and leaving each of these spans.

The Fourier sum

\[
P(t)=a_0+\sum_{k=1}^{k=n} a_k \cos kwt + b_k \sin kwt \tag{1}\]

where \(P(t)\) is blood pressure measured at time \(t\), \(w = 2\pi/T\), and \(T\) is 24 hours used for modeling. Coefficients \(a_0\), \(a_k\), and \(b_k\) were calculated by linear regression (this method provides, if desired, statistical inference for the parameters).

We used Equation 1 to define the minimal number of harmonics necessary. To do this, we used the run-test and required that the residuals of predicted from measured values be randomly distributed below and above zero when these residuals were recorded from 0 to 24 hours. To perform the run-test, a z transformation is calculated (Siegel et al., Equation 4.7), which is approximately normally distributed under the hypothesis that the residuals are randomly positive or negative from 0 to 24 hours.

**Determination of Low and High Blood Pressure Spans**

The data modeled by Equation 1 \((n=4)\) showed that each profile included a large convex branch that always corresponded to the portion where predicted blood pressure was the lowest over 24 hours. To define the low and high pressure spans, we proceeded as follows. Let \(t_0\) be the time corresponding to the predicted minimal pressure. On the left and right of \(t_0\) (i.e., for \(t\leq t_0\) and \(t> t_0\)), predicted blood pressure increased after a convex curve. From \(t_0\), we determined the first inflection points encountered (i.e., the points corresponding to a nil second derivative). These points (one on the left and one on the right of \(t_0\)) were taken as limit points for the low pressure span. The high pressure span was taken as the remaining times of the day. Where \(u\) is uphill and \(d\) is downhill, let \(t_u\) and \(t_d\) be the times of transit from the low to high and from the high to low pressure spans, respectively. The slopes of predicted blood pressure at \(t_u\) and \(t_d\), denoted by \(s_u\) and \(s_d\), were determined. Mean values of observed blood pressure during the high and low spans were also calculated.

**List of Parameters**

Our procedure defined a set of parameters. They are \(a_0\) (mm Hg) (see Equation 1), 24-hour mean pressure; \(t_u\) (hours), time of passage of blood pressure from the low pressure span to the high pressure span; \(t_d\) (hours), time of passage of blood pressure from the high pressure span to the low pressure span; \(t_u-t_d\) (hours), high blood pressure time spans; \(s_u\) (mm Hg/hr), blood pressure slope at \(t_u\); \(s_d\) (mm Hg/hr), blood pressure slope at \(t_d\); \(BP_u\), observed mean blood pressure during the high pressure span; and \(BP_d\), observed mean blood pressure during the low pressure span.
Statistical Tests

Parameters obtained for morning, afternoon, and night shifts were compared by a multivariate linear model with repeated measures using the SYSTAT programs written by Wilkinson.18

Results

Observed Ambulatory Blood Pressure in Three Work Shifts

Figure 1 (left) displays systolic blood pressure hour profiles (mean ± SEM) of the 15 subjects in each of the three shifts. The profiles clearly show the existence of a convex low pressure portion. Outside this convex portion, blood pressure showed small oscillations but overall remained high. The high pressure time span did not entirely coincide with the working time of the subject. For example, the high span of blood pressure corresponding to the morning shift (4:00 AM to noon) lasted far beyond midday, until late in the afternoon. Mean profiles for diastolic blood pressure are also depicted (Figure 1, right). Again, a large basin and small oscillations in the span of high values can be recognized.

Determination of Number of Harmonics in Fourier Modeling

Figure 2 gives an example of Fourier modeling, with zero, one, two, three, and four harmonics (systolic blood pressure in a subject on the night shift). Observed data (75 points), predicted curves, time distribution of residuals, and histograms of residuals are shown. The figure highly suggests that a model with one harmonic cannot predict the data accurately. In fact, with one harmonic, the residuals show systematic oscillations from 0 to 24 hours. Moreover, these residuals are clearly not normally distributed (their histogram is skewed to the right).

Table 1 displays the run-test z values for systolic blood pressure and diastolic blood pressure. For each Fourier model, with one to five harmonics, maximal, minimal, and mean values of z over 45 calculations (15 subjects; for each subject, three shifts) are given. As the level z = ±2 corresponds to a probability of ~0.05, we also display (Table 1) the number of cases where z values were more than 2 or less than −2. It can be seen that a Fourier model with one harmonic is not satisfactory because for systolic and diastolic blood pressure, the time distributions of residuals are not random in 35 and 32, respectively, of 45 cases. The table suggests that n=4 or n=5 be chosen because they were almost equivalent; we chose n=4.

Model Analysis of Systolic and Diastolic Blood Pressures

Table 2 displays model parameters calculated for systolic blood pressure in the three shifts using Fourier models with four harmonics. Mean systolic blood pressure over 24 hours was different (p=0.02) among workers on the three shifts, with the lowest mean observed during the afternoon shift. During the morning shift, high systolic blood pressure lasted from ~5:00 AM to 10:00 PM, while during the afternoon and night shifts, it lasted from 9:00 AM to midnight and from 1:00 PM to 5:00 AM of the next day, respectively. The duration of these spans was significantly different among the three shifts (p<0.02). Working on the night shift induced the longest high blood pressure span (20 hours), while working on the afternoon shift induced the shortest span (14 hours). Systolic blood pressure slopes entering and leaving these pressure spans were also different (p<0.02) among the three shifts. The highest slopes corresponded to the afternoon shift (slo, 22 mm Hg/hr; slo, −20 mm Hg/hr) and the lowest to the morning shift (slo, 15 mm Hg/hr; slo, −15 mm Hg/hr). Note that systolic blood pressure during the high pressure span was the same among the three shifts (~126 mm Hg). By contrast, systolic blood pressure during the low pressure span was different (p<0.03); it was the lowest during the afternoon shift (108 compared with 116 and 113 mm Hg during the morning and night shifts, respectively).

Table 3 displays the results for diastolic blood pressure. Again, the mean pressure was different among the three shifts and was the lowest during the afternoon shift (79 compared with 83 mm Hg during the morning and night shifts). Moreover, the difference was only observed during the low pressure span. The duration of the high pressure span was significantly different (p<0.02) among the three shifts. It was longest during the night shift (19.5 hours) and shortest during the afternoon shift (13.5 hours).
High and Low Blood Pressure Spans and Working Times

Figure 3 displays predicted systolic and diastolic blood pressure values during the three shifts. To obtain Figure 2, individual predicted curves were calculated. Then, mean values of these 15 predicted curves were displayed. Figure 3 also depicts the times of occurrence of high and low pressures as given in Table 1.

### Table 1: z Value for Run-Tests (Tests Performed on Residuals of Fourier Models)

<table>
<thead>
<tr>
<th>n</th>
<th>Lowest</th>
<th>Highest</th>
<th>Mean</th>
<th>Number of cases where z&gt;2 or z&lt;-2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>z values</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Modeling of systolic blood pressure</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>-5.05</td>
<td>0.48</td>
<td>-2.29</td>
<td>31</td>
</tr>
<tr>
<td>2</td>
<td>-4.30</td>
<td>1.45</td>
<td>-1.52</td>
<td>15</td>
</tr>
<tr>
<td>3</td>
<td>-3.99</td>
<td>1.68</td>
<td>-1.01</td>
<td>11</td>
</tr>
<tr>
<td>4</td>
<td>-2.60</td>
<td>1.29</td>
<td>-0.65</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>-2.53</td>
<td>2.05</td>
<td>-0.43</td>
<td>3</td>
</tr>
</tbody>
</table>

Modeling of diastolic blood pressure

| 1   | -5.13   | 0.48    | -2.59 | 32                                |
| 2   | -5.45   | 0.23    | -1.96 | 20                                |
| 3   | -3.95   | 1.03    | -1.20 | 6                                 |
| 4   | -4.41   | 1.74    | -0.42 | 2                                 |
| 5   | -2.70   | 3.05    | -0.08 | 4                                 |

n, number of harmonics used.

Tables 2 and 3 (these points did not necessarily coincide with the inflection points of the depicted curves). The grid areas correspond to working times.

**Discussion**

**Determination of Blood Pressure**

Blood pressure is controlled by complex mechanical, cardiac, neural, and hormonal mechanisms. These mechanisms are autoregulated and ensure a steady-state pressure level. By interaction, they might also generate a periodic change in blood pressure. Other factors also play major roles to define blood pressure level. Among them, the circadian rhythm and the rhythm of our activities are probably the most important factors. Depending on their times of occurrence, the composition of these factors may generate quite different blood pressure profiles.

In the present study, we measured blood pressure and heart rate of shift workers on three different days while the subjects had three different work shifts (although data on heart rate are available, we have focused our attention on blood pressure). Because our subjects have been shift workers for more than 1 year, we considered that their blood pressures have been synchronized to the overall sequence of three shifts and two resting days. The data did not allow for the discernment of the trend
TABLE 2. Modeling of Ambulatory Systolic Blood Pressure by Fourier Model With Four Harmonics

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Work shift</th>
<th>Global</th>
<th>1–2</th>
<th>1–3</th>
<th>2–3</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP24 (mm Hg)</td>
<td>123±7</td>
<td>120±7</td>
<td>124±8</td>
<td>0.02</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td>tu (hr)</td>
<td>4.8±2.5</td>
<td>9.4±1.3</td>
<td>12.8±1.5</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>sls (mm Hg/hr)</td>
<td>14.9±5.6</td>
<td>22.3±6.7</td>
<td>16.1±6.7</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>tu (hr)</td>
<td>21.6±1.5</td>
<td>23.6±0.9</td>
<td>4.5±1.7</td>
<td>&lt;0.001</td>
<td>NS</td>
</tr>
<tr>
<td>sls (mm Hg/hr)</td>
<td>−14.6±6.5</td>
<td>−20.3±5.4</td>
<td>−19.1±5.9</td>
<td>&lt;0.02</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>tu–tu (hr)</td>
<td>16.8±2.3</td>
<td>13.7±1.5</td>
<td>20.3±1.9</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SBPd (mm Hg)</td>
<td>125.0±8.0</td>
<td>126.0±8.0</td>
<td>127.0±8.0</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>SBPp (mm Hg)</td>
<td>116.0±10.0</td>
<td>108.0±8.0</td>
<td>113.0±8.0</td>
<td>0.03</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

1, morning work shift (4:00 AM to noon); 2, afternoon work shift (noon to 8:00 PM); 3, night work shift (8:00 PM to 4:00 AM); SBP24, mean value of systolic blood pressure (SBP) over 24 hours; tu, time of passage from low pressure span to high pressure span; sls, slope of SBP at tu; tu, time of passage of from low pressure span to high pressure span; sls, slope at tu; SBPd, mean SBP during the high pressure span; SBPp, mean SBP during the low pressure span.

p values correspond to an F test (multivariate general linear model with repeated measures18). Comparison between two schedules were performed by testing profile difference.

Effect of previous shifts on the blood pressure of a given day. The study of trend effects requires special organization of working times for our subjects (working in one given shift for several days), and this was not possible. Also, we could not, from the observations, discern the intrinsic role of internal regulation mechanisms, of daytimes and nighttimes, and of the subjects’ activities. However, we have drawn some conclusions from the data.

Modeling

A model was necessary to analyze a blood pressure profile. We used Fourier series. Fourier equations have been used by many authors.7,8,16,19–21 Most of them considered only one harmonic (the cosinor model). Figure 1 shows that with monitored ambulatory blood pressure, one harmonic is insufficient to describe the 24-hour pressure profile. First, the residuals of this model were not normally distributed. Second, a systematic oscillation of these residuals from 0 to 24 hours could be observed. Third, this model led to a symmetric high and low pressure level with high and low pressure spans of 12 hours each. Our data suggested that the high pressure span was longer than the low pressure span, especially for blood pressure observed during the night shift. Therefore, more harmonics must be added to the cosinor model.

The most difficult problem with Fourier analysis is to determine the minimal number of harmonics to be used. There are many possible ways to deal with this problem. One method consists of performing a stepwise linear regression with classic criteria to enter or remove the harmonics. However, we do not believe that automatic programming would be a good method for our purpose. For example, with the data of Figure 1, the stepwise regression program suggests that the cos wt term should be entered first in the equation, followed by cos3wt, by sin2wt, by sin wt, and so on. It is very difficult to interpret such a result.

Another method is to examine the residuals of predicted from measured values. One generally requires these residuals to be normally distributed and the normalcy of the distribution to be tested by χ². However, χ² is a rather unpractical test. We

TABLE 3. Modeling of Ambulatory Diastolic Blood Pressure by Fourier Model With Four Harmonics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Work shift</th>
<th>Global</th>
<th>1–2</th>
<th>1–3</th>
<th>2–3</th>
</tr>
</thead>
<tbody>
<tr>
<td>DBP24 (mm Hg)</td>
<td>83±7.5</td>
<td>79±7</td>
<td>83±6.5</td>
<td>0.005</td>
<td>&lt;0.002</td>
</tr>
<tr>
<td>t₀ (hr)</td>
<td>3.7±0.7</td>
<td>9.1±1.3</td>
<td>12.3±1.4</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>sls (mm Hg/hr)</td>
<td>19.2±4</td>
<td>17.8±5.3</td>
<td>12.2±7</td>
<td>&lt;0.02</td>
<td>NS</td>
</tr>
<tr>
<td>t₀ (hr)</td>
<td>20.7±2</td>
<td>22.6±0.7</td>
<td>4.9±0.8</td>
<td>&lt;0.001</td>
<td>NS</td>
</tr>
<tr>
<td>sls (mm Hg/hr)</td>
<td>−12.8±4.6</td>
<td>−17.6±4.5</td>
<td>−16.5±4.8</td>
<td>NS</td>
<td>...</td>
</tr>
<tr>
<td>t₀–t₀ (hr)</td>
<td>17±1.8</td>
<td>13.5±1.4</td>
<td>19.4±1.8</td>
<td>&lt;0.001</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>DBPp (mm Hg)</td>
<td>85.0±7.0</td>
<td>85.0±7.8</td>
<td>86.0±6.0</td>
<td>NS</td>
<td>...</td>
</tr>
<tr>
<td>DBPp (mm Hg)</td>
<td>73.0±7.0</td>
<td>70.0±6.0</td>
<td>73.0±6.0</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

1, morning work shift (4:00 AM to noon); 2, afternoon work shift (noon to 8:00 PM); 3, night work shift (8:00 PM to 4:00 AM); DBP24, mean value of diastolic blood pressure (DBP) over 24 hours; t₀, time of passage from low pressure span to high pressure span; sls, slope of DBP at t₀; t₀, time of passage of from low blood pressure span to high blood pressure span; sls, slope at t₀; DBPp, mean DBP during the high pressure span; DBPp, mean DBP during the low pressure span.

p values corresponded to an F test (multivariate general linear model with repeated measures18). Comparison between two schedules were performed by testing profile difference.
prefer the one-sample run-test, which is much easier to perform.\(^7\) As can be seen in Figure 1, when the residuals are randomly positive and negative from 0 to 24 hours (see the cases \(n=3\) and \(n=4\)), then the residuals are also normally distributed. Table 3 depicts the run-test performed over the whole sample and for the three shifts. The result suggests that a Fourier model with \(n=3\), 4, or 5 might be appropriate. We have chosen \(n=4\).

**Blood Pressure Profiles in the Three Shifts**

For each shift, blood pressure essentially showed a basin that most probably corresponded to the sleeping time of the subject and a span of high values with small oscillations due to different activities (Figure 1). The high value span always included the times of professional activities. This observation suggests that professional activities play a central role on blood pressure profile and that trend effect from previous shifts, if it exists, might be dominated by the effect of activities during the day. Note that the high pressure span did not coincide entirely with the times of activities. This result shows that factors other than the subject’s activities do contribute to 24-hour blood pressure oscillations.

Depending on the times of occurrence, the composition of the factors described above might generate a 24-hour high or low blood pressure mean, a short or long high pressure span, or a steep or flat blood pressure slope in the transient time between high and low pressure spans. In our subjects, mean pressure was higher when the subjects worked during the morning or at night than when they worked during the afternoon. On the other hand, the high pressure time span was longer when the subjects worked during the morning or at night than when they worked during the afternoon. Finally, the slopes of systolic blood pressure in the transient time from high to low or low to high pressure spans were steeper when the subjects worked during the afternoon than when they worked during the morning or at night. Overall, our analysis has disclosed many disturbances of blood pressure due to changes in working times.

Working late in the afternoon and at night might generate many psychologic, social, and familial inconvenientes. The present study does not address these important issues. However, our results suggest that shift workers should be observed for a long time to determine whether the disturbances observed in blood pressure profiles might have some repercussion on their cardiovascular system.

The present study has defined a set of parameters, some of them new, that can be used to discuss any blood pressure profile. To define individually the time span of high or low blood pressures might be of interest. Because high office blood pressure is a risk of cardiovascular mortality and morbidity, a long time span of elevated ambulatory blood pressure might be considered potentially harmful. We suggest that the present mathematic approach be applied to blood pressure in subjects of different sex, at different age ranges, and with normotension or hypertension. Hopefully, some new insights into the role of daily activities and of the intrinsic ambulatory blood pressure regulation might be obtained by this approach.

**References**

8. Bingham C, Cornelissen GC, Halberg E, Halberg F: Testing period for single cosinor: Extent of human 24-h cardiovas-
10. Conway J, Boon N, Jones JV, Sleight P: Involvement of the baroreceptor reflexes in the changes in blood pressure with sleep and mental arousal. *Hypertension* 1983;5:746–748

**KEY WORDS**  •  blood pressure determination  •  shift workers
Twenty-four-hour ambulatory blood pressure in shift workers.
N P Chau, J M Mallion, R de Gaudemaris, E Ruche, J P Siche, O Pelen and G Mathern

Circulation. 1989;80:341-347
doi: 10.1161/01.CIR.80.2.341

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1989 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on
the World Wide Web at:
http://circ.ahajournals.org/content/80/2/341