Daily rates of cardiovascular mortality and morbidity have been associated with daily variations in fine particulate matter (aerodynamic diameter ≤ 2.5 μm, PM$_{2.5}$), but little is known about the influences of the individual source-related PM$_{2.5}$ categories or the temporal lags for the effects. We investigated heart rate (HR) and HR variability (HRV) data collected during a 5-month study involving 6 hr/day, 5 day/week exposures of normal (C57) mice and a murine model for atherosclerotic disease (ApoE–/–) in Sterling Forest (Tuxedo, New York, USA). The mice were exposed to concentrated ambient particles (PM$_{2.5}$) concentrated 10-fold, producing an average of 113 μg/m$^3$. Daily 6-hr PM$_{2.5}$ air samples were analyzed by X-ray fluorescence, permitting attribution to major PM source categories [secondary sulfate (SS), resuspended soil (RS), residual oil (RO) combustion, and other, largely due to motor vehicle traffic]. We examined associations between these PM$_{2.5}$ components and both HR and HRV for three different daily time periods: during exposure, the afternoon after exposure, and late at night. For HR there were significant transient associations for RS during exposure, and for SS in the afternoon after exposure. For HRV, there were comparable associations with RO in the afternoon after exposure and for both SS and RS late at night. The biological bases for these associations and their temporal lags are not known but may be related to the differential solubility of the biologically active PM components at the respiratory epithelia and their access to cells that release mediators that reach the cardiovascular system. Clearly, further research to elucidate the underlying processes is needed.

Key words: concentrated ambient particulate matter, heart rate, heart rate variability, motor vehicle pollution, PM$_{2.5}$, residual oil, resuspended soil, secondary sulfate, source apportionment.

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stage, a time-varying model estimated daily crude effects. In the second stage the true mean of the estimated crude effects was modeled with a polynomial function of time for chronic effects, a linear term of daily CAP exposure concentrations for acute effects, and a random component for unknown noise. A Bayesian framework combined these two stages.

For the analyses of HRV, the times in milliseconds of occurrence of two consecutive R waves in the ECG waveform (RR) were calculated on a beat-to-beat basis. Because of limitation in data storage capacity, the RR intervals were recorded consecutively for 5 sec in every 15-min interval for all mice during 10–27 April 2003, and for ApoE\(^{-/-}\) mice in the control group during 22 April through July 2003. The rest of recordings were taken consecutively about 10 sec in every 5-min interval for the mice. There are about 34–64 and 100 RR intervals recorded in 15- and 5-min intervals, respectively. For the analysis, we decided to work on fluctuations of RR intervals on every 15-min basis. To match the data in the 15-min recordings, we used only the first 60 RR intervals in the last of 3 consecutive 5-min intervals. The two HRV indices that we used were the standard deviations of the RR intervals (SDNNs) and the square root of the mean squared differences (RMSSD) of successive RR intervals in 5 sec. The nonparametric method identified the 0000- to 0500-hr period during which the two groups had the largest HRV differences within each day. To match the HR analyses of effects with the HRV changes, we used the same period (0130–0430 hr) for calculating mean log SDNN and log RMSSD to represent daily HRV responses for this period for each mouse. In the analysis of effects on HR, we also calculated daily responses for the 1100- to 1300-hr period during exposure for examining acute effects. However, because the number of normal RR intervals recorded during the exposure period was small because of interference from the perforated metal chamber, we instead used the 1600- to 1800-hr interval after exposure as an alternate for calculating daily HRV response. Daily changes in HR during this period, which were not reported in the previous study, were also calculated for this analysis.

To examine whether variations of concentrations in major sources are correlated with short-term changes of cardiac functions in exposed mice, we adopted the following approach:

Let \( X_{ijkd} \) be the average cardiac function measurement for mouse \( j \) in the \( i \)th group at a given period on the \( d \)th day of the \( k \)th week, where
\[
i = 0 \text{ (control), } 1 \text{ (exposure)}
\]
\[
j = \begin{cases} 
1–9 & \text{when } i = 0 \\
1–10 & \text{when } i = 1
\end{cases}
\]
\[
k = 1 \text{ (Saturday, 12 April, through Friday, 18 April), 2 (Saturday, 19 April, through Friday, 25 April), . . . , 6 (Saturday, 6 September, through Wednesday, 10 September)}
\]
\[
d = 1 \text{ (Saturday), 2 (Sunday), 3 (Monday), 4 (Tuesday), 5 (Wednesday), 6 (Thursday), 7 (Friday)}
\]

We have seen that daily cardiac function measurements changed over the 5 months. Such changes may be caused by the cumulative effects of aging, exposure, and other unknown environmental factors. To examine the association between exposed level and acute cardiac function change on exposure days, we generated baseline adjusted measurements for each mouse on the exposure days by subtracting averaged measurement on the previous weekend from each measurement on weekdays. Presumably, the daily series of these baseline-adjusted measurements \( Y_{ijkd} = X_{ijkd} - (X_{ij1} + X_{ij2})/2 \) will have little cumulative effect. To see whether the idea worked or not, we explored the data. Figure 1 shows two series of daily averaged baseline adjusted measurements of HR at the 1100- to 1300-hr period for mice in the control and exposure groups. The exposure chamber effects reduced HR in both groups, which also corresponded to the quiescent period of mouse circadian rhythm during the daytime. The two series also share the same quadratic shape. Although it is not clear why this has happened, some common factors have strong effects on measurements of mice in both control and exposure groups. Instead of searching for a smooth curve for modeling the pattern caused by common factors, we can simply use the baseline-adjusted measurements of the nine mice in the control group to calculate an average for each exposure day. That is the darker curve plotted in Figure 1. If there is no exposure effect, the darker curve and lighter curve of averaged measurements for mice in the exposure group will not differ. In fact, the difference between two curves shown in Figure 2 indicates that CAP exposure had the effect of reducing HR. The difference series in the plot also show no trend over the 5 months, indicating that cumulative effects have been removed. Hence, we may construct a model to fit the baseline-adjusted measurements for examining whether the short-term cardiac function changes are related to exposure levels of the identified source factors F1, F2, F3, and F4. A linear model is given by

\[
Y_{ijkd} = \mu_{kd} + \beta_1 \cdot (F_{1kd} - F_{1c}) \cdot (i = 1) + \beta_2 \cdot (F_{2kd} - F_{2c}) \cdot (i = 1) + \beta_3 \cdot (F_{3kd} - F_{3c}) \cdot (i = 1) + \beta_4 \cdot (F_{4kd} - F_{4c}) \cdot (i = 1) + \epsilon_{ijkd},
\]

where \( \epsilon_{ijkd} \) is an autoregressive process of order one. If the estimate of \( \beta_j \) differs significantly from zero, we may claim that the \( j \)th source factor is associated with the acute changes of HR and HRV.

**Results**

**Associations between sources and short-term HR changes.** Using the source apportionment factors from Maciejczyk and Chen (2005), we have the following four source classes: SS, RS, RO, and MV. There were no significant associations between these four source categories and HR in the C57 normal mice at any of the three intervals. However, as shown in Table 1, there were highly significant associations between PM2.5 and the RS source factor and decreases in HR for the ApoE\(^{-/-}\) mice during the daily CAP exposures but no associations with the other source factors. By contrast, Tables 2 and 3 indicate that there was no residual association of HR with PM2.5 or the RS factor later in the afternoon or late that night.

In the afternoon, there was a significant association between decreases in HR and the SS factor for the ApoE\(^{-/-}\) since that had not been present during exposure and did not persist into the nighttime period. It is also of some interest that the MV traffic and other source category was not significantly associated with HR during any of the three time periods.
For the C57 mice, there were no significant associations of HR with PM_{2.5} or any of its component source classes during any of the three daily time periods.

**Associations between sources and short-term HRV changes.** It is unfortunate that there was too much signal noise during the exposures to permit reliable analyses of HRV changes during the hours of CAP exposure. We therefore cannot tell whether the transient effect of PM_{2.5} or its RS source component on HR was also present for HRV. For C57 mice, the only significant association was between the MV and other source factor and a decline in RMSSD during the afternoons after the exposures ($p = 0.00$; data not shown). For the ApoE^{−/−} mice (Table 4), there were very strong associations of HRV with the RO source factor in the afternoon. These decreases in HRV did not persist at night (Table 5) and had not been seen for HR at any time period. Finally, there were strong associations between HRV during the nighttime hours and both the SS source category and the RS source category that were not seen for HR at the other intervals, or for HRV at the other time periods. However, it must be noted that although the SS source factor was associated with decreased HRV, the RS source category was associated with an increase in HRV. For PM_{2.5}, there was a significant ($p = 0.03$) decrease in RMSSD and a nearly significant ($p = 0.07$) decrease in SDNN for the 0330- to 0430-hr interval but no such an association during the 1600- to 1800-hr period.

**Discussion.** Interpretation of the various significant ($p < 0.05$) associations between source factors and the HR and HRV variables in CAP-exposed mice at this time would be speculative at best, especially because three of the source factors showed some association at one interval or another, and the fourth (MV traffic and other category) showed a strong association ($p = 0.00$) with RMSSD in the afternoon after exposure in the C57 mice. The strongest associations for the ApoE^{−/−} mice are summarized in Table 6.

For the evaluation of the changes on HR and HRV in the last column of Table 6, we have calculated the changes in the measured parameters over the interquartile range of concentrations as is commonly done in epidemiology. For HR, the changes are for exposures at the third quartile to the first quartile of the measured concentrations. The results show about 3–4 beats/min (bpm) changes. For HRV, the interquartile change is the ratio of RMSSDs between the third quartile and first quartile of the concentrations. The results show about 2–6% changes. These are relatively small changes, but they may have played some role in the progressive changes in HR that we observed during the course of the 5 months of exposure that were described by Hwang et al. (2005), and the changes in HRV that were reported by Chen and Hwang (2005).

It is also interesting that the reduction in HR during the daily exposures associated with PM_{2.5} (−4.1 bpm) may have been due entirely to the influence of the RS factor (−4.5 bpm) and that there was an increase in HR (+2.6 bpm) in the afternoons after the exposures in the same source factor. This appears to have been compensated by the decrease in HR in the afternoon after the exposures (−2.5 bpm) associated with the SS factor. Such a compensation would be consistent with the lack of any association of HR with PM_{2.5} in this interval.

The RO combustion factor, which did not have any significant association with HR, appears to have had the effect of increasing RMSSD by 6.2% during the afternoons after the exposure but not at the other intervals. The other observed statistically significant changes in RMSSD were associated with opposite effects during the late night period by the SS and RS source components, with the RS factor perhaps accounting for the significant association in the same direction for the association of RMSSD with PM_{2.5} during the same period. It is also of interest that the effects reported here for HR and HRV were occurring at relatively low concentrations of outdoor PM_{2.5} and its component source-related factors. The average PM_{2.5} CAPs during the 6-hr exposures was only 113 μg/m³. Thus, the 24-hr average...
exposures were only 28.3 µg/m³ because the mice were breathing air that was filtered of the outdoor air components during the balance of the day. Outdoor PM2.5 does not have much diurnal variation, and it infiltrates indoors with a high degree of penetration. People are there-
diurnal variation, and it infiltrates indoors with
ambient origin at near ambient concentrations
exposures to both cigarette smoke and sulfuric
clearance. In addition, subchronic inhalation
whereas higher levels of exposure retarded the
lungs and found that low-level exposures accel-
14 days of sulfuric acid inhalation on particle
clearance. In another study in this laboratory,
particle clearance in donkeys (Lippmann et al.
smoke from 10 or more cigarettes slowed the
ation of the fresh smoke from two cigarettes
retarded such clearance. Similarly, the inhala-
in the epidemiologic literature. It may well be
some, if various, effects on cardiac physiology,
with various lag structures, and that some com-
ponents mitigate the effects produced by other
components. Also, we do not know at this
time about the short-term effects, and their
temporality, of inhaled PM2.5 on other organ
systems. However, if most of the major com-
ponents of PM2.5 produce some short-term
biologic responses, then the commonly used
integral measure of PM2.5 mass concentration,
that is, 24-hr average PM2.5, may be serving as
a reasonable integrating index for at least some
of the short-term health risks. In any case, the
results reported in this article provide us and
others with additional factors to consider in the
planning of our future laboratory and field
studies of PM health effects.

As noted above, Maciejczyk and Chen (2005) reported that in vitro NFκB expression of BEAS-2B cells exposed to CAPs collected
during the daily 6 hr in vivo exposures was sig-
ificantly increased in association with the RO,
but not with the other source categories of the
CAPs. The NFκB expression is an index of cel-
lular oxidative stress and the release from the
cells of mediators affecting systemic inflamma-
tion. This mechanism for biologic response is
consistent with short lag times between respira-
tory tract particle deposition and cardiac func-
tion changes. However, because the NFκB
index of biologic response to CAP exposure
provides no within-day temporality, it is not
possible to make a direct comparison with the
lagged HR and HRV responses reported in
this article. The different lag structures of the
responses reported in this article may be related
to the solubility of the biologically active com-
ponents in each source category.

We plan to pursue the issues raised by the
results reported here in our future subchronic
exposure studies in mice. In terms of compara-
bale investigations in humans, a study would
need access to a population that is being con-
tinuously monitored for cardiac function as
well as time-resolved PM2.5 compositional
data. The only study we are aware of to date
looking for cardiac responses to ambient air
PM was by Sullivan et al. (2005), in which they
examined the relation between PM2.5
exposure (measured by nephelometry) and
the number of hours preceding the onset of
myocardial infarction (MI). They found no
significant associations between MI and the
nephelometry data. It is possible that neph-
thelometry measurements may not be repre-
sentative of the active components of ambient
PM mix or that the nephelometry measurements
 correlate with outcomes other than MI.

Conclusions

The availability of data on HR and HRV over a
5-month period during subchronic expos-
ures of mice to the regional anthropogenic
CAPs at New York University’s Sterling
Forest laboratory in Tuxedo, New York, and
during the afternoon and nighttime periods after
the daily exposures, as well as elemental
composition data for each day’s exposure
enabled us to examine daily source apportion-
ments of the major source categories during
the exposures and their association with HR
and HRV during each of the three time peri-
ods. The RS component was strongly associ-
ated with a transient decrease in HR during
exposure, comparable with that of the whole
PM2.5. The SS component was strongly associ-
ated with a transient HR decrease in the
afternoon after the day’s exposure. The RO
component was strongly associated with
increases in HRV in the afternoon after the
day’s exposure. The SS and RS components
were strongly associated with HRV in the
nighttime period, with decreased HRV for the
SS component and increased HRV for the RS
component. These effects were occurring after
exposures at daily average PM2.5 concentra-
tions occurring frequently in the United States
and may be relevant to the subpopulation
with atherosclerotic disease.

The biologic bases for these various associ-
atations and their temporal lags are not known
at this time but may relate to the differential
solubilities of the PM components at the res-
piratory epithelia and their access to cells
that release mediators that reach the cardiovas-
cular system. Further research that can elucidate
the underlying processes is clearly needed.

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