Exposure to Environmental Tobacco Smoke Increases Myocardial Infarct Size in Rats

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Background Exposure to environmental tobacco smoke (ETS) has been epidemiologically linked to death from ischemic heart disease in nonsmokers. In this study, we evaluated the influence of 3 days, 3 weeks, and 6 weeks of ETS exposure on myocardial infarct size in a rat ischemia/reperfusion model.

Methods and Results Sprague-Dawley rats exposed to ETS (four Marlboro cigarettes per 15 minutes, 6 hours per day, 5 days per week) for 3 days (n=24), 3 weeks (n=21), or 6 weeks (n=12) and control rats (n=24, n=21, and n=12, respectively) were subjected to 35 minutes of left coronary artery occlusion and 2 hours of reperfusion. Infarct size and risk area were determined by triphenyltetrazolium chloride and phthalocyanine blue staining, respectively. Air nicotine, carbon monoxide, and total particulate measurements were measured in additional groups (6 to 13 rats each) exposed to 3 days, 3 weeks, or 6 weeks of ETS and controls. Average air nicotine, carbon monoxide, and total particulate concentrations were 1103 μg/m³, 92 ppm, and 60 mg/m³ for the ETS-exposed rats. Infarct size (infarct mass/risk area×100%) increased significantly in the ETS groups compared with the control groups in a dose-dependent manner (P<.023), with longer exposure associated with larger infarct size. Infarct size nearly doubled with 6 weeks of ETS exposure (61±5% versus 34±3% for control, mean±SEM). Plasma COHb, nicotine, and cotinine levels increased significantly in the ETS groups in a dose-dependent manner (all P<.001).

Conclusions Exposure to passive smoking increases myocardial infarct size in a rat model of ischemia and reperfusion. This increase of infarct size exhibited a dose-response relation. These results are consistent with epidemiological studies demonstrating that ETS increases the risk of heart death. (Circulation. 1994;89:1282-1290.)

Key Words • smoking • myocardial infarction • nicotine • carbon monoxide

Environmental tobacco smoke (ETS), the tobacco combustion products inhaled by nonsmokers in the proximity of burning tobacco, is hazardous because it contains high concentrations of ammonia, benzene, nicotine, carbon monoxide, and many other carcinogens and irritants.1-2 The effects of passive smoking on health have been reported to include acute effects, such as exacerbation of asthma and angina, as well as chronic effects, such as increased risk of lung cancer, respiratory tract infection, and atherosclerosis.3-6 ETS also has been linked to death from ischemic heart disease in nonsmokers. Epidemiological studies conducted by a number of investigators demonstrate approximately a 30% increase in risk of death from ischemic heart disease or myocardial infarction in nonsmokers living with smokers.3,4,7,8

Although no one has previously reported the effect of ETS exposure on myocardial infarct size, a number of studies suggest that ETS exposure adversely affects the myocardial oxygen supply-and-demand relation that would predispose the heart to develop ischemia or exacerbate preexisting ischemia. Direct or indirect exposure to tobacco smoke has been shown to increase the hemodynamic determinants of myocardial oxygen demand9,10 at the same time that it potentially reduces both myocardial blood supply and oxygen delivery by enhancing the development of coronary atherosclerosis,10,11 causing coronary vasoconstriction,11,12 and reducing the oxygen-carrying capacity of blood through increased serum carboxyhemoglobin (COHb) levels.9 Furthermore, through an increase in platelet aggregation, the likelihood of coronary thrombosis is enhanced after ETS exposure.13-15 Finally, ETS exposure appears to reduce oxidative phosphorylation in cardiac cells, rendering them less capable of producing ATP.19,20 These changes suggest that ETS exposure not only increases the likelihood of developing an acute myocardial infarction but also may increase the amount of myocardial damage once an acute myocardial infarction has occurred.

In this study, the effects of 3 days, 3 weeks, and 6 weeks of ETS exposure on myocardial infarct size were examined in an in vivo rat model of coronary artery occlusion and reperfusion. In addition, we examined the effects of ETS exposure on serum lipids, bleeding time, plasma COHb, nicotine, and cotinine.

Methods

Experimental Groups Sprague-Dawley rats (225 to 250 g) were randomly divided into the experimental and control groups described below. The rats were housed in separate cages in 1.92×1.92×0.97 m (3.58 m³), well-mixed ETS exposure chambers (model H 5500, BioClean, Duo Flo, Lab Products Inc) as previously described.6 The interior volume of the exposure chamber approximates that of a Mazda 626 sedan (3.7 m³).21 The rats were
houses in a room maintained at a constant temperature and kept on a 12-hour light-dark cycle. The 3-day study included two groups: group ETS-3 (n=21) and group ETS-6 weeks (n=21). Animals were placed in a separate room for 3 or 6 weeks. All rats were killed as described above. After the snare occluder was visually tested during a brief period of occlusion and reperfusion, the thoracotomy was closed. The animals were then subjected to the experimental protocol.

**Experimental Protocol**

All six groups (12 to 24 rats each) were subjected to 35 minutes of left coronary artery occlusion followed by 120 minutes of reperfusion. The occlusion/reperfusion protocol was performed 10 to 20 minutes after the 6-hour ETS exposure period. The bleeding time was measured before the rats were killed as described below. At the conclusion of the protocol, infarct size was measured as described below.

**Infarct Sizing**

Infarct sizing was performed as described previously. The left coronary artery was reoccluded, and phalothane blue dye was injected into the left ventricular cavity, allowing normally perfused myocardium to stain blue. The heart was then excised, rinsed of excess dye, and sliced transversely from apex to base into sections 2 mm thick. The tissue samples were incubated in a 1% solution of triphenyltetrazolium chloride for 10 to 15 minutes until viable myocardium was stained brick red. Infarcted myocardium fails to stain with triphenyltetrazolium chloride. Tissue samples were then fixed in a 1% formalin solution and weighed. Color photographs of both sides of each transverse slice were obtained with an Olympus OM-2 camera with a 90-mm Macro lens and a 2X teleconverter. The regions showing blue-stained (nonischemic), red-stained (ischemic but uninfarcted), and unstained (infarcted) tissue were outlined on each color photograph and measured by planimetry in a blinded fashion. On each side, the fraction of left ventricular (LV) area representing infarcted tissue (average of two photographs) was multiplied by the weight of that section to determine the absolute weight of infarcted tissue. The infarct size for each heart was expressed as

\[
\text{Infarct size/LV mass (\%) = } \frac{\sum \text{infarct weight in each slice}}{\text{total LV weight}} \times 100\%
\]

The risk area unstained by blue dye was expressed as

\[
\text{Risk area/LV mass (\%) = } \frac{\text{total weight of unstained section}}{\text{total LV weight}} \times 100\%
\]

Infarct size as a percentage of risk area was then calculated as

\[
\text{Infarct size/risk area (\%) = } \frac{\Sigma \text{infarct weight in each slice}}{\Sigma \text{risk area weight of each slice}} \times 100\%
\]

Four rats in group ETS-3 weeks were excluded because of technical problems with staining.

**Histopathological Evaluation**

Histological sections through the left coronary artery were made in the three control rats and three 6-week ETS-exposed rats. All sections were stained by hematoxylin and eosin (HE) and elastin van Giesen eosin (EVG) separately. Histopathological findings under the light microscope were determined by two investigators blindly.

**Hematological and Biochemical Analysis**

Determination of bleeding times was performed as described previously by warming the rat tail for three minutes in a normal saline bath maintained at 37°C. The tail was then removed from the bath, and a standard 1/2-cm longitudinal incision was made into the distal tail, avoiding any macroscopically obvious vessels, with a carbon steel blade. The incised tail was then immediately placed into the saline bath, which was gently agitated with a magnetic stirrer. The bleeding time was taken as the time required for blood flow to the incision to cease.

Total serum cholesterol, triglycerides, high-density lipoprotein (HDL) cholesterol, plasma COHb, nicotine, and cotinine were measured in additional rats after 3 days, 3 weeks, and 6 weeks of ETS exposure and their controls (6 to 13 rats per group). The plasma concentrations of nicotine and cotinine were determined by gas chromatography with nitrogen-phosphorus detection. This method has been modified for simultaneous extraction and determination of cotinine by use of capillary gas chromatography. Total serum cholesterol and triglyceride levels were determined by automated enzymatic methods (Coulter DART cholesterol reagent and the DACOS and DACOS XL analyzers), and HDL cholesterol concentrations were measured after precipitation of other lipoprotein classes with dextran and magnesium ions (HDL cholesterol precipitant [catalog No. 236141], Ciba Corning Diagnostic Corp, Oberlin, Ohio).

**Monitoring Smoke Exposure Inside the Chambers**

Air concentrations of carbon monoxide, total particulates, respirable suspended particulates, and nicotine inside the exposure chambers were measured weekly. Average carbon monoxide concentrations during the 6-hour exposure period were determined by a model L15 CO Personal Exposure System (Langan Products, Inc, San Francisco, Calif). The average daily value was taken from 2160 samples during the 6-hour exposure period. The total particulate concentration was measured using a Miniram PDM-3 optical scattering particle monitor (MIE, Inc, Bedford, Mass), which monitored particulate concentration every 10 seconds and computed the average total particulate concentration during the 6-hour exposure period. Respirable suspended particulates were measured with a piezobalance respirable aerosol mass monitor (model 3500, Thermo-System, Inc, St Paul, Minn). In addition, air nicotine levels were measured with a passive diffusion monitor located in the middle of the exposure chamber during the 6-hour exposure period.
Statistics

The text, tables, and figures list data as the mean ± SEM. We tested for changes in infarct size and other variables over time and with duration of ETS exposure using the regression model

\[ y = b_0 + b_1 t + b_2 E + b_3 E t \]

where \( t \) is length of ETS exposure (in hours) for exposed groups and the corresponding control groups and \( E \) is a dummy variable set to 1 if the rat is exposed to ETS and 0 if the rat is in the control group. The interpretation of the coefficients in the regression model is as follows: \( b_0 \) is the estimated value of \( y \) at the beginning of the experiment in the control rats (i.e., \( t = 0 \) and \( E = 0 \)); \( b_1 \) is the change in \( y \) per hour among the control rats (\( E = 0 \)); a value of \( b_1 \) significantly different from zero indicates that there were changes in \( y \) over the 6 weeks of the study in the control rats. \( b_2 \) is the difference in \( y \) between the ETS exposure (\( E = 1 \)) and control rats at the beginning of the experiment (\( t = 0 \)); a significant value of \( b_2 \) indicates a constant difference in the value of \( y \) between the control and ETS-exposed rats during the 6-week study. \( b_3 \) measures the difference in the response over time between the ETS-exposed (\( E = 1 \)) and control rats (\( E = 0 \)); a significant value of \( b_3 \) indicates the presence of a dose-response relation between the duration of ETS exposure and \( y \). For example, suppose that \( b_1 \) and \( b_2 \) are not significantly different from zero and \( b_3 \) is positive. This result would indicate that \( y \) remains constant over time among the control rats but increases significantly with increasing exposure to ETS, indicating a significant dose-response relation between \( y \) and the duration of ETS exposure.

Data on air nicotine, carbon monoxide, total particulate, and respirable suspended particulate concentrations in ETS-contaminated air and clear air were compared by Student's \( t \) test. Differences in mortality rate between the ETS and control animals were assessed by the Fisher Exact Test.

Linear regression was used to relate infarct size to bleeding time, air nicotine, carbon monoxide, total particulate concentrations, plasma COHb, nicotine, and cotinine levels. All computations were done with Minitab Version 7.2 or Primer of BIOSTATISTICS: THE PROGRAM, Version 3.03. Statistical significance was defined as a value of \( P < .05 \).

Results

Smoke Exposure Inside the Chamber

The average air nicotine, carbon monoxide (Fig 1), total particulate, and respirable suspended particulate concentrations were significantly increased in the ETS groups compared with controls during the exposure period (Table 1).

Mortality

None of the rats died before the initial 35-minute ischemia, whereas 21 rats died with ventricular fibrillation during the 35-minute ischemia and 120-minute reperfusion periods. The mortality rate was higher in the ETS groups than in their controls; however, this difference was not statistically significant (23%, \( n = 57 \) versus 14%, \( n = 57 \), ETS versus control; \( P = .33 \)).

Myocardial Infarct Size

Myocardial infarct size (infarct mass/risk area \( \times 100\% \)) was stable over the 6-week experimental period in the control rats (\( P \) for \( b_1 = .901 \)) but significantly increased with increasing ETS exposure (\( b_2 = 0.148 \pm 0.064\% \) per hour, \( P = .023 \)) (Fig 2 and Table 2). The significant value of \( b_2 \) indicates a significant dose-response relation between infarct size and the duration of ETS exposure. Infarct size was nearly doubled after 180 hours of ETS exposure, to 61 ± 5% compared with 34 ± 3% in the control group.

Histopathological Evaluation

There was no evidence of left coronary artery atherosclerosis on EVG and HE stain in both control rats \( (n = 3) \) and 6-week ETS-exposed rats \( (n = 3) \). Within the infarcted zone there was evidence of contraction band necrosis in both control and ETS-exposed rats.

COHb, Nicotine, and Cotinine Levels in Plasma

As demonstrated in Figs 3 and 4 and Table 3, COHb, nicotine, and cotinine levels in plasma significantly

<table>
<thead>
<tr>
<th>Chamber</th>
<th>Air Nicotine, µg/m³</th>
<th>Air CO, ppm</th>
<th>Air TP, mg/m³</th>
<th>Air RSP, mg/m³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.5 ± 0.5</td>
<td>2.0 ± 0.4</td>
<td>0.06 ± 0.05</td>
<td>0.07 ± 0.02</td>
</tr>
<tr>
<td>ETS exposure</td>
<td>1103 ± 214</td>
<td>91.7 ± 2.7</td>
<td>59.8 ± 3.2</td>
<td>18.1 ± 1.3</td>
</tr>
<tr>
<td>P</td>
<td>.027</td>
<td>.01</td>
<td>.001</td>
<td>.001</td>
</tr>
</tbody>
</table>

CO indicates carbon monoxide; TP, total particulates; RSP, respirable suspended particulates; ETS, environmental tobacco smoke; and \( n \), number of samples. Values are mean ± SEM. For nicotine, CO, and TP, each of the \( n \) samples represents the average value observed during the exposure period (6 hours of ETS) on \( n \) different days. For RSP, the sample size represents the average concentrations during \( n \) 10-minute sample periods taken while the smoke levels were at steady state.
increased in ETS-exposed rats (\( b_E \) significantly greater than zero, \( P < .001 \) for all variables) and continued to increase over time in a dose-dependent manner (\( b_E \) significantly greater than zero, \( P < .001 \)) in ETS-exposed animals compared with controls. There was a slight increase in COHb over time in the control rats (\( P \) for \( b_E = .25 \)), but change was much less in the ETS-exposed rats. There was no change over time in plasma nicotine or cotinine in the control rats, with the levels below the level of detection.

**Alterations in Lipids**

As demonstrated in Table 4, the average serum total cholesterol, HDL cholesterol, and triglycerides did not differ significantly between ETS-exposed and control groups or change significantly over time. Serum total cholesterol tended to increase slightly over time in the control rats (\( b_E = .11 \pm .03 \text{ mg} \cdot \text{dL}^{-1} \cdot \text{h}^{-1}, P = .003 \)) but not in the ETS-exposed rats (\( b_E = -.15 \pm .05 \text{ mg} \cdot \text{dL}^{-1} \cdot \text{h}^{-1}, P = .002 \), which cancels out the \( b_t \) effect).

**Bleeding Times**

The bleeding times in the ETS-exposed rats were significantly shortened (\( P \) for \( b_E = .002 \)) compared with their respective control groups (Table 3 and Fig 5). There was a similar reduction in bleeding time in all ETS-exposed rats, regardless of duration of ETS exposure (\( P \) for \( b_E = .215 \)).

**TABLE 2. Effects of Passive Smoking on Infarct Size**

<table>
<thead>
<tr>
<th>Group</th>
<th>Infarct Mass/ Risk Area, %</th>
<th>Infarct Size/ LV Mass, %</th>
<th>Risk Area/ LV Mass, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control-3 days (n=21)</td>
<td>35±4</td>
<td>15±2</td>
<td>43±1</td>
</tr>
<tr>
<td>ETS-3 days (n=16)</td>
<td>37±6</td>
<td>17±3</td>
<td>45±2</td>
</tr>
<tr>
<td>Control-3 weeks (n=17)</td>
<td>34±5</td>
<td>16±3</td>
<td>44±2</td>
</tr>
<tr>
<td>ETS-3 weeks (n=15)</td>
<td>43±5</td>
<td>17±2</td>
<td>39±2</td>
</tr>
<tr>
<td>Control-6 weeks (n=11)</td>
<td>34±3</td>
<td>16±2</td>
<td>48±2</td>
</tr>
<tr>
<td>ETS-6 weeks (n=9)</td>
<td>61±5</td>
<td>26±3</td>
<td>42±2</td>
</tr>
</tbody>
</table>

Regression model results

| \( b_0 \), %          | 34.9±4.3                     | 15.3±2.1                  | 42.5±1.6               |
| \( b_1 \), %/h         | -0.006±0.042                 | 0.006±0.021               | 0.026±0.016            |
| (\( P = .901 \))       | (\( P = .759 \))             | (\( P = .108 \))          |
| \( b_E \), %           | -1.9±6.5                    | -0.8±3.16                 | 1.43±2.49              |
| (\( P = .774 \))       | (\( P = .801 \))            | (\( P = .567 \))          |
| \( b_{Et} \), %/h      | 0.148±0.064                 | 0.048±0.031               | -0.050±0.024           |
| (\( P = .023 \))       | (\( P = .130 \))            | (\( P = .045 \))          |

ETS indicates environmental tobacco smoke. Plus/minus values are SEM.
Correlations Between Infarct Size and Air Nicotine, Carbon Monoxide, Total Particulate Concentrations, Plasma COHb, Nicotine, and Cotinine

There were positive correlations between infarct size and air nicotine, carbon monoxide, and total particulate concentrations multiplied by the hours of exposure ($r=.45$ to $.50$, all $P<.001$). There were also positive correlations between infarct size and plasma COHb, nicotine, and cotinine levels ($r=.35$ to $.41$, all $P<.01$). There was no correlation between infarct size and bleeding time ($r=.077$, $P=.485$).

Discussion

Epidemiological studies concluded that heart disease is an important consequence of exposure to ETS and estimated that the excess risk of heart disease for nonsmokers living with smokers was 30% and that the public health burden from ETS exposure is likely to be much greater for heart disease than for lung cancer. Our results demonstrate that exposure to passive smoking significantly increases myocardial infarct size in a rat model of ischemia and reperfusion, with longer ETS exposure producing a larger effect on infarct size. ETS exposure also decreases bleeding time regardless of the length of ETS exposure. There were positive correlations between the infarct size and air nicotine, carbon monoxide, total particulate concentrations, and plasma COHb, nicotine, and cotinine levels.

Of the thousands of chemicals in ETS, those that are suspected to contribute to passive smoking–induced cardiovascular disease include nicotine, carbon monoxide, polycyclic aromatic hydrocarbons, and tobacco glycoproteins. The present study showed that air nicotine, carbon monoxide, and total particulate concentrations increased with ETS exposure and that this increased exposure in air led to a continuous buildup of plasma COHb, nicotine, and cotinine levels in ETS-exposed rats. Possible mechanisms whereby these changes could increase infarct size are described below.

Coronary Hemodynamics and Coronary Vasospasm

Exposure to cigarette smoke alters hemodynamics, resulting in an increase in myocardial oxygen demand. Previous investigators have shown that direct exposure to tobacco smoke increases the resting heart rate, systolic and diastolic blood pressure, rate-pressure product, cardiac output, and maximal first derivative of left ventricular pressure (LV dp/dt). These effects are probably secondary to adrenergic stimulation by nicotine. There is also evidence that ETS has the potential to reduce myocardial oxygen supply by atherosclerotic narrowing of the coronary arteries, by vasoconstriction of the coronary arteries, by...

Table 3. Regression Model Results in Plasma Carboxyhemoglobin, Nicotine, Cotinine, and Bleeding Times

<table>
<thead>
<tr>
<th>Regression Model Results</th>
<th>Carboxyhemoglobin, %</th>
<th>Nicotine, ng/mL</th>
<th>Cotinine, ng/mL</th>
<th>Bleeding Times, s</th>
</tr>
</thead>
<tbody>
<tr>
<td>$b_0$</td>
<td>0.661±0.331</td>
<td>0.453±3.218</td>
<td>4.53±18.74</td>
<td>76.865±4.468</td>
</tr>
<tr>
<td>($P=.055$)</td>
<td>($P=.889$)</td>
<td>($P=.810$)</td>
<td>($P&lt;.001$)</td>
<td></td>
</tr>
<tr>
<td>$b_1$</td>
<td>0.007±0.003</td>
<td>0.0001±0.025</td>
<td>0.0006±0.147</td>
<td>0.119±0.044</td>
</tr>
<tr>
<td>($P=.025$)</td>
<td>($P=.998$)</td>
<td>($P=.997$)</td>
<td>($P=.008$)</td>
<td></td>
</tr>
<tr>
<td>$b_E$</td>
<td>3.348±0.468</td>
<td>18.452±4.386</td>
<td>216.33±25.54</td>
<td>-21.213±6.746</td>
</tr>
<tr>
<td>($P&lt;.001$)</td>
<td>($P&lt;.001$)</td>
<td>($P&lt;.001$)</td>
<td>($P=.002$)</td>
<td></td>
</tr>
<tr>
<td>$b_{E1}$</td>
<td>0.023±0.004</td>
<td>0.254±0.038</td>
<td>1.406±0.220</td>
<td>-0.082±0.065</td>
</tr>
<tr>
<td>($P&lt;.001$)</td>
<td>($P&lt;.001$)</td>
<td>($P&lt;.001$)</td>
<td>($P=.215$)</td>
<td></td>
</tr>
</tbody>
</table>

Plus/minus values are SEM.
and by a reduction in the oxygen-carrying capacity of the blood through the formation of COHb. A large number of clinical and experimental studies demonstrate that chronic exposure to ETS has the potential to cause atherosclerotic narrowing of coronary arteries.\textsuperscript{3,6,29} Furthermore, acute exposure to cigarette smoke causes coronary vasoconstriction through nicotine activation of the sympathetic nervous system and \( \alpha \)-adrenergic stimulation\textsuperscript{1,30} in patients,\textsuperscript{10,11} an effect that can be prevented by calcium blockers and nitroglycerin.\textsuperscript{12} ETS also causes an increase in COHb caused by exposure to carbon monoxide.\textsuperscript{9} The combined effect of reduced oxygen-carrying capacity, presumed coronary vasoconstriction, and increased rate-pressure product resulted in a decrease in anginal threshold in chronic stable angina patients who were exposed to ETS.\textsuperscript{3,21} A recent clinical study\textsuperscript{22} concluded that smoking appears to be the major risk factor associated with coronary vasospasm in patients without significant angiographic coronary stenosis.

### Increased Production of Oxygen Free Radicals and Endothelial and Myocardial Cell Injury

An experimental study\textsuperscript{33} indicated that exposing rats to a low concentration of cigarette smoke increased myocardial sensitivity to ischemia/reperfusion injury and that a free radical mechanism might participate in this injury. Oxygen free radicals are known to produce damage in many biological tissues. The oxygen free radical–producing activity of polymorphonuclear leukocytes is increased by cigarette smoking.\textsuperscript{34} The results suggested that the increased generation of oxygen free radicals by polymorphonuclear leukocytes might be responsible for an enhanced risk of various diseases related to cigarette smoking.

Passive exposure to tobacco smoke damages endothelial cells and increases the number of circulating nuclear carcasses of endothelial cells.\textsuperscript{17} ETS appears to alter cardiac cellular metabolism in such a way as to render the myocyte less capable of producing ATP. Some investigators\textsuperscript{19} demonstrated reduced oxidative phosphorylation in cardiac mitochondrial fractions taken from rabbits that had been exposed to ETS. Further studies indicated that the reduction in mitochondrial respiration secondary to ETS exposure is probably a result of decreased cytochrome oxidase activity.\textsuperscript{19,20} Carbon monoxide increases the COHb level of the blood because its affinity for hemoglobin is much greater than that of oxygen. Therefore, it diminishes oxygen transport capacity and might damage myocardial mitochondria and endothelium.\textsuperscript{35}

In addition, cigarette smoking can cause DNA single-strand breaks in cultured human lung cells.\textsuperscript{36} This DNA strand break formation can be blocked by an inhibitor of endonuclease activation and by BAPTA, an intracellular calcium chelator. A mouse study\textsuperscript{37} also showed that nicotine and cotinine inhibit testosterone production.

### Table 4. Effects of Passive Smoking on Serum Lipids

<table>
<thead>
<tr>
<th>Group</th>
<th>Total Cholesterol, mg/dL</th>
<th>High-Density Lipoprotein Cholesterol, mg/dL</th>
<th>Triglycerides, mg/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control-3 days</td>
<td>61±2</td>
<td>57±2</td>
<td>64±5</td>
</tr>
<tr>
<td>ETS-3 days</td>
<td>61±3</td>
<td>53±2</td>
<td>75±8</td>
</tr>
<tr>
<td>Control-3 weeks</td>
<td>62±4</td>
<td>50±3</td>
<td>81±9</td>
</tr>
<tr>
<td>ETS-3 weeks</td>
<td>56±5</td>
<td>53±4</td>
<td>130±16</td>
</tr>
<tr>
<td>Control-6 weeks</td>
<td>80±3</td>
<td>54±5</td>
<td>61±9</td>
</tr>
<tr>
<td>ETS-6 weeks</td>
<td>53±1</td>
<td>47±2</td>
<td>99±13</td>
</tr>
</tbody>
</table>

Regression model results

- \( b_0 \), mg/dL: 56.5±3.2 (P=.003)
- \( b_1 \), mg · dL\(^{-1} \) · h\(^{-1} \): 0.11±0.03 (\( P =.277 \) )
- \( b_2 \), mg/dL: 4.90±4.45 (\( P =.897 \) )
- \( b_3 \), mg · dL\(^{-1} \) · h\(^{-1} \): -0.15±0.05 (\( P =.212 \) )

ETS indicates environmental tobacco smoke. Plus/minus values are SEM.

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**Fig 5.** Bar graph showing average bleeding time (in seconds) for each rat group. C-3d indicates control group for 3 days; ETS-3d, exposure to environmental tobacco smoke (ETS) for 3 days (18 hours); C-3w, control group for 3 weeks; ETS-3w, exposure to ETS for 3 weeks (90 hours); C-6w, control group for 6 weeks; and ETS-6w, exposure to ETS for 6 weeks (180 hours). Values are mean±SEM.
and verapamil significantly reversed this inhibition. These results suggest that nicotine and cotinine either affect intracellular calcium content or block the effects of calcium on steroidogenesis in the mouse.

**Thrombosis**

ETS also has been shown to increase platelet aggregation, which might increase the likelihood of coronary thrombosis or vasoconstriction after acute exposure or the development of coronary atherosclerosis after chronic ETS exposure.\(^3,13,14\) Passive smoking also increases platelet-activating factor, platelet factor 4, \(\beta\)-thromboglobulin, and fibrinogen concentration, which provides a marker of its effect on coronary heart disease.\(^30-40\) Davis and coworkers\(^5-18\) demonstrated that as little as 20 minutes of ETS exposure resulted in significantly increased platelet aggregability in patients. We found similar increases in platelet activity, reflected as a shortened bleeding time. Consistent with our earlier work,\(^6\) we found a large effect associated with even the lowest level of ETS exposure (in this case 18 hours over 3 days).

Nicotine has been shown to inhibit the in vitro release of prostacyclin from the rings of rabbit or rat aorta and human veins. The suggested mechanism is the inhibition of prostacyclin synthesis through the inhibition of cyclooxygenase. Nicotine could also affect platelets by releasing catecholamines, which lead to increased thromboxane A\(_2\).\(^30\) Passive smoking also increases blood viscosity and hematocrit because of relative hypoxia induced by chronic carbon monoxide exposure.\(^30\) Clinical studies\(^41,42\) also showed that after abstention from chronic cigarette smoking, the blood thrombogenic factors and hemorheological parameters will be normalized.

**Catecholamine Release and Arrhythmogenesis**

A clinical study\(^43\) demonstrated that smoking-associated increments in mean (± SEM) plasma norepinephrine (227±23 to 324±39 pg/mL, \(P<.01\)) and epinephrine (44±4 to 113±27 pg/mL, \(P<.05\)) and smoking-associated increments in pulse rate, blood pressure, blood glycerol, and blood lactate/pyruvate ratio were prevented by adrenergic blockade. Therefore, it can be concluded that smoking-associated increments in these hemodynamic and metabolic variables are mediated through adrenergic mechanisms. Cigarette smoking can lead to catecholamine release by nicotine. Increased circulating concentrations of catecholamines enhance platelet adhesiveness and decrease the ventricular fibrillation threshold, which is also affected by carbon monoxide.\(^30,44\) We found a higher mortality from ventricular fibrillation in ETS-exposed compared with control rats (23% versus 14%). This was not statistically significant, however (\(P=.33\)). The lack of significance may be a reflection of the small numbers of rats we studied. The power of these experiments to detect a doubling of the mortality rate is only 36%.

Thus, passive smoking, including nicotine and carbon monoxide, may contribute to acute myocardial ischemia or sudden death through reduction of coronary blood flow, increasing myocardial oxygen demands, increasing oxygen free radical generation, enhancement of thrombosis, releasing catecholamines, and arrhythmogenesis. ETS exposure may not only increase the likelihood of having an acute myocardial infarction but also increase the amount of myocardial damage once an acute myocardial infarction does occur.

**Dose and Duration**

This study is the first to show that passive smoking significantly increases myocardial infarct size in a rat model of ischemia and reperfusion, with greater myocardial damage directly related to greater exposure to ETS. There were positive relations between myocardial infarct size and the average concentrations of nicotine, carbon monoxide, and particulates. Compared with average air concentrations of nicotine and carbon monoxide in human heavy smoking environments (50 to 500 \(\mu g/m^3\) and 5 to 50 ppm, respectively),\(^6,45\) the average concentrations of air nicotine and carbon monoxide in our present study were only about twofold higher (1103 \(\mu g/m^3\) and 92 ppm). The duration of exposure, however, was short compared with even a rat’s lifetime. Myocardial infarct size nearly doubled with just 180 hours of ETS exposure spread over 6 weeks.

Recently, Penn and Snyder\(^46\) reported that passive smoking significantly enhanced atherosclerotic plaque in cockerels who were exposed to sidestream smoke for 6 hours a day, 5 days a week, for 16 weeks. This is equivalent to 0.4% of their projected lifespan. Assuming a human lifespan of 74 or 75 years, an equivalent period of exposure to sidestream smoke would be 3 hours a day for 2.4 years.

The exposure chamber we used had an interior volume (3.58 m\(^3\)) similar to that of a Mazda 626 automobile (3.7 m\(^3\)). Recently, Ott et al\(^41\) reported that the air concentration of carbon monoxide in a moving automobile is 63 ppm when a smoker smokes each cigarette for 7 minutes, four cigarettes per hour, and the windows are closed. This real microenvironment was the same size as our exposure chamber and would be similar to our rat study if four passengers each smoked four cigarettes per hour.

**Clinical Relevance**

Previous clinical studies have demonstrated increased cardiovascular morbidity associated with cigarette smoking. In 976 patients with a first myocardial infarction, Robinson et al\(^47\) reported that the smokers had a larger infarct size as estimated by serum peak creatine kinase concentrations (1382 versus 1146 IU/L, \(P=.010\)). This study did not report on the severity of coronary disease. Multivariate analysis of possible risk factors for reinfarction identified at the time of initial infarction\(^48\) showed current cigarette smoking to be the only predictive factor (reinfarction occurred in 12.5% of smokers versus 6.3% of nonsmokers, \(P=.04\)). One other study\(^49\) that followed patients after a first acute myocardial infarction reported that the risk of nonfatal reinfarction correlated with a variety of clinical parameters, including the presence or absence of cigarette smoking. Smoking was found to increase the risk of first acute myocardial infarction in a dose-dependent manner, the risk increasing 2% to 3% for each gram of tobacco smoked daily.\(^50\) Patients with a previous myocardial infarction\(^44\) had a significantly higher plasma carbon monoxide level after exercise in a smoking environment. The cardiac responses, including peak exercise power, time to recovery of preexercise heart rate, and expired concentration of carbon monoxide, were significantly worsened by
passive smoking, especially in those subjects with previous myocardial infarction. There are no previous data regarding the direct effect of passive smoking on myocardial infarct size. The present study is the first study to demonstrate that passive smoking increases experimental myocardial infarct size in rats.

Conclusions

These data indicate that exposure to passive smoking significantly increases myocardial infarct size in a rat model of ischemia and reperfusion with a dose-response relation. These results are consistent with epidemiologic studies demonstrating that ETS increases the risk of heart disease death.

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