Effect of Cigarette Smoking and Nicotine on Serum Free Fatty Acids

Based on a Study in the Human Subject and the Experimental Animal

By Alfred Kershbaum, M.D., Samuel Bellet, M.D., Edward R. Dickstein, M.D., and Leonard J. Feinberg, Ph.D.

Some recent reports have suggested an association between cigarette smoking and high blood cholesterol levels.1-4 Dawber and associates,1 in a follow-up report of the Framingham study, found that cholesterol levels were higher among cigarette smokers than among nonsmokers. Studies by Thomas2 in medical students revealed that more smokers than nonsmokers had high cholesterol levels. Gofman and associates3 noted higher concentrations of serum lipoproteins and cholesterol in 20- to 29-year-old male smokers compared with nonsmokers of the same age group. In a recent report, Blackburn and associates4 observed significantly higher cholesterol levels in a group of male smokers studied in Finland and slightly higher levels in a group in the United States. Page and associates5 found no effect on serum cholesterol or lipoprotein concentrations during a 30-minute period after smoking two cigarettes.

In experimental studies, Wenzel and associates were able to demonstrate significantly increased plasma cholesterol and lipid phosphorus levels after prolonged oral nicotine administration in rabbits on moderate cholesterol intake,6 but not in cockerels7 or rabbits on high cholesterol intake.8 However, in the rabbits on high cholesterol diets which were given oral nicotine, there were abnormal electrocardiographic changes, an increase in mortality, and pathological evidence of coronary atherosclerosis.

The physiological effects of smoking are primarily nicotine effects mediated through the sympathetic nervous system and the adrenal glands. It seemed important to us to investigate how these effects of nicotine influence the metabolism of lipids, particularly since recent studies9-11 have shown that free fatty acids (FFA) are rapidly released into the circulation from tissue fat depots following the administration of epinephrine and norepinephrine and following stress effects on the sympathetic nervous system.14-16 The present study was undertaken to assess the effect of cigarette smoking and nicotine administration on circulating FFA. In some of the subjects, the effects of smoking on serum cholesterol and triglycerides were also determined. Knowledge of these effects should help to improve our understanding of the relationship of smoking and lipid metabolism.

Methods

Human Subjects

The 31 subjects used in this study were selected at random from the Philadelphia General Hospital population. They consisted of ward patients, outpatients, laboratory personnel, and staff personnel. The subjects varied in age from 16 to 72 years and included 7 normal individuals, 7 patients with coronary heart disease, and 17 patients with various other medical diagnoses (tables 1 and 2). Habitual smokers, using both filter- and nonfilter-type cigarettes, and nonsmokers were included (tables 1 and 2). All studies were performed at 9:00 a.m. with no food having been taken after the previous evening meal.

In 17 subjects, the procedure was as follows: Two cigarettes were smoked in fairly rapid succession within a 10-minute period. Blood samples were taken before and immediately after the smoking period, and 10, 20, and 40 minutes after the cessation of smoking. Determinations of serum FFA levels were made using a modification of the methods of Gordon17 and Davis.18 Serum cholesterol19 and triglycerides20 concentrations were also obtained in all samples.

Another group of nine subjects served as
controls to determine what effect the experimental procedure itself (fasting, preparation, venipuncture, etc.) would have on the FFA levels as a result of coincidental stress. The same procedure was followed as in the original group except for the elimination of smoking. Several of these control subjects inhaled deeply to simulate smoking during the first 10-minute interval. In five of the control subjects, the same experimental conditions were used, and the experiment was repeated with smoking.

To determine the effect of repetitive or "chain-smoking," each of five subjects smoked six cigarettes over a period of 40 minutes. FFA concentrations were determined before smoking and at 10-minute intervals during and after the smoking period for a total period of 60 minutes.

The range of serum FFA in normal subjects in our laboratory in the fasting state is 555 to 1,732 μEq./L. The intrinsic standard error of the chemical method used is ±5 per cent.

**Animal Studies**

To observe whether the smoking effects on FFA would also occur after nicotine administration, 15 experiments were performed on five dogs anesthetized with morphine and a mixture of Dial-urethane and pentobarbital. Nicotine was given by infusion at a dosage rate of 20 μg./Kg./min. for a 20-minute period. Serum FFA concentrations were determined in arterial, venous, and coronary sinus blood samples, which were obtained before infusion, after 10 minutes and after 20 minutes when the infusion was completed. Arterial blood samples were obtained from a femoral artery; venous blood samples were obtained from...
Cigarette Smoking and Nicotine

Figure 1

Effect of smoking six cigarettes consecutively within a 40-minute period on serum free fatty acids. The subject was a 58-year-old male with angina pectoris who was receiving an anticoagulant. He usually smoked 20 to 40 cigarettes daily. Nonfilter type cigarettes were used in this experiment.

Results

Human Subjects

The results of the first series of experiments in which two cigarettes were smoked in a 10-minute period are shown in Table 2. In all 17 subjects, the FFA levels rose at the end of the smoking period, and the maximal elevation usually occurred 10 minutes later. Twenty and 40 minutes after smoking, the FFA levels were still above the presmoking level in most instances. The maximal rise in FFA ranged from 98 to 840 μEq./L., with a mean increment of 351 μEq./L. In each subject, the maximal rise was greater than twice the standard error of the method used to determine the serum FFA. The FFA response did not appear to be related to age, sex, smoking habits, type of cigarette used, or presmoking level of FFA. There was essentially no change in the serum total cholesterol or triglycerides during the period of the experiment.

In the control group who did no smoking, there was an average maximal increase of FFA of 9.8 μEq./L. (range of 0 to 44 μEq./L.) over a 20- to 40-minute period (Table 1). Since fasting alone will usually cause a rise in FFA of approximately 5 μEq./L. per five minutes, there was appar-

a femoral vein. Coronary sinus samples were obtained in the intact animal by catheterization of the coronary sinus with a special catheter (modified Morawitz cannula) inserted via the external jugular vein under fluoroscopic guidance.21
### Table 2

**Effect of Smoking Two Cigarettes on Serum Levels of Free Fatty Acids, Cholesterol, and Triglycerides**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age</th>
<th>Sex</th>
<th>Time (min.)</th>
<th>Free fatty acids (μEq./L.)</th>
<th>Cholesterol (mg./100 ml.)</th>
<th>Triglycerides (mg./100 ml.)</th>
<th>Type of cigarette used</th>
<th>Cigarette smoking history (daily)</th>
<th>Clinical diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>43</td>
<td>F</td>
<td>0*</td>
<td>685</td>
<td>143</td>
<td>103.7</td>
<td>nonfilter</td>
<td>nonsmoker</td>
<td>pyelonephritis</td>
</tr>
<tr>
<td>2</td>
<td>56</td>
<td>M</td>
<td>0</td>
<td>1453</td>
<td>179</td>
<td>135.5</td>
<td>nonfilter</td>
<td></td>
<td>duodenal ulcer</td>
</tr>
<tr>
<td>3</td>
<td>16</td>
<td>F</td>
<td>0</td>
<td>1221</td>
<td>239</td>
<td>76.2</td>
<td>nonfilter</td>
<td></td>
<td>hypopituitarism (suspected)</td>
</tr>
<tr>
<td>4</td>
<td>61</td>
<td>M</td>
<td>0</td>
<td>1732</td>
<td>188</td>
<td>89</td>
<td>filter</td>
<td></td>
<td>hypertensive cardiovascular disease</td>
</tr>
<tr>
<td>5</td>
<td>57</td>
<td>M</td>
<td>0</td>
<td>1304</td>
<td>148</td>
<td>78</td>
<td>filter</td>
<td></td>
<td>lung carcinoma</td>
</tr>
<tr>
<td>6</td>
<td>44</td>
<td>M</td>
<td>0</td>
<td>830</td>
<td>234</td>
<td>—</td>
<td>filter</td>
<td>subsiding pneumonia</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>50</td>
<td>M</td>
<td>0</td>
<td>742</td>
<td>235</td>
<td>74.2</td>
<td>filter</td>
<td>subsiding pneumonia</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>49</td>
<td>M</td>
<td>0</td>
<td>1530</td>
<td>120</td>
<td>54.6</td>
<td>nonfilter</td>
<td></td>
<td>cirrhosis of liver</td>
</tr>
<tr>
<td>9</td>
<td>56</td>
<td>M</td>
<td>0</td>
<td>797</td>
<td>249</td>
<td>100.9</td>
<td>filter</td>
<td></td>
<td>diabetes mellitus</td>
</tr>
<tr>
<td>10</td>
<td>34</td>
<td>M</td>
<td>0</td>
<td>861</td>
<td>—</td>
<td>—</td>
<td>nonfilter</td>
<td></td>
<td>rheumatoid arthritis</td>
</tr>
<tr>
<td>11</td>
<td>51</td>
<td>F</td>
<td>0</td>
<td>1266</td>
<td>237</td>
<td>104.3</td>
<td>filter</td>
<td>nonsmoker</td>
<td>diabetes mellitus</td>
</tr>
<tr>
<td>12</td>
<td>32</td>
<td>F</td>
<td>0</td>
<td>928</td>
<td>152</td>
<td>77.4</td>
<td>nonfilter</td>
<td></td>
<td>syphilis endocarditis</td>
</tr>
</tbody>
</table>
**Table 2 (continued)**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age</th>
<th>Sex</th>
<th>Time (min.)</th>
<th>Free fatty acids (μEq./L.)</th>
<th>Cholesterol (mg./100 ml.)</th>
<th>Triglycerides (mg./100 ml.)</th>
<th>Type of cigarette used</th>
<th>Cigarette smoking history (daily)</th>
<th>Clinical diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>30</td>
<td>F</td>
<td>0</td>
<td>1452</td>
<td>215</td>
<td>94.6</td>
<td>nonfilter</td>
<td>20</td>
<td>diabetes inaplasus</td>
</tr>
<tr>
<td>14</td>
<td>64</td>
<td>M</td>
<td>0</td>
<td>2391</td>
<td>130</td>
<td>53.0</td>
<td>nonfilter</td>
<td>15</td>
<td>cirrhosis of liver</td>
</tr>
<tr>
<td>15</td>
<td>72</td>
<td>M</td>
<td>0</td>
<td>939</td>
<td>119</td>
<td>102.6</td>
<td>nonfilter</td>
<td>20</td>
<td>lung carcinoma</td>
</tr>
<tr>
<td>16</td>
<td>38</td>
<td>M</td>
<td>0</td>
<td>1102</td>
<td>---</td>
<td>---</td>
<td>filter</td>
<td>40</td>
<td>arteriosclerotic heart disease</td>
</tr>
<tr>
<td>17</td>
<td>34</td>
<td>M</td>
<td>0</td>
<td>690</td>
<td>174</td>
<td>115.1</td>
<td>nonfilter</td>
<td>20</td>
<td>subsiding pneumonia</td>
</tr>
</tbody>
</table>

*Before smoking.
†After smoking two cigarettes.

**Discussion**

The increase in circulating FFA, after smoking, was rapid, consistent, and in some cases marked. The absence of a response in the control subjects indicates that the FFA rise after smoking was not due to the emotional stress of the experiment. This was further emphasized by the significant FFA elevation which developed in five control subjects when they repeated the experiment with smoking (table 1). The FFA response to tobacco smoke, as with other physiological effects of smoking, is presumably due to the nicotine absorbed. The elevation of FFA in the animal experiments after nicotine infusion would tend to substantiate this.

The FFA elevations in the animal studies were observed to be five times greater in the systemic venous blood than in blood samples taken from the coronary sinus. This can be partly explained by the higher concentration of adipose tissue (from which FFA can be released) being drained by the systemic veins than by the coronary sinus. Gordon has shown that plasma from venous sites which drain large fat depots has a higher FFA con-
Table 3

<table>
<thead>
<tr>
<th>Site of blood sampling</th>
<th>Time (min.)</th>
<th>Dog 339</th>
<th>Dog 475</th>
<th>Dog 411</th>
<th>Dog 501</th>
<th>Dog 666</th>
</tr>
</thead>
<tbody>
<tr>
<td>Femoral artery</td>
<td>0</td>
<td>1069</td>
<td>1266</td>
<td>946</td>
<td>1080</td>
<td>1363</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>1124</td>
<td>1504</td>
<td>1131</td>
<td>1091</td>
<td>1439</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>1310</td>
<td>1537</td>
<td>1131</td>
<td>1113</td>
<td>1439</td>
</tr>
<tr>
<td>Femoral vein</td>
<td>0</td>
<td>—</td>
<td>1341</td>
<td>1099</td>
<td>1037</td>
<td>1581</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>—</td>
<td>1908</td>
<td>1349</td>
<td>1222</td>
<td>1733</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>—</td>
<td>1799</td>
<td>1366</td>
<td>1222</td>
<td>1733</td>
</tr>
<tr>
<td>Coronary sinus</td>
<td>0</td>
<td>1058</td>
<td>1091</td>
<td>859</td>
<td>971</td>
<td>1559</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>895</td>
<td>1102</td>
<td>1001</td>
<td>1004</td>
<td>1592</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>906</td>
<td>993</td>
<td>1001</td>
<td>1004</td>
<td>1592</td>
</tr>
</tbody>
</table>

*Nicotine infused at a rate of 20 µg./Kg./min. for 20 minutes.

Changes in Serum Free Fatty Acids in Dogs During Intravenous Nicotine Infusion

NicotineInfused at a rate of 20 µg./Kg./min. for 20 minutes.

tent than that from veins draining areas containing less adipose tissue. Also, the high degree of utilization of fatty acids by myocardial tissue would tend to lower FFA levels in the coronary sinus and to lessen the FFA response to nicotine at this site.

The mechanism by which FFA are increased in the circulation after smoking and nicotine administration is probably similar to that occurring after other stresses, such as fear and psychic stimulation. Nicotine stimulates the ganglia of the sympathetic nervous system with a consequent discharge of impulses along the postganglionic fibers and results in a release of norepinephrine. Additionally, the adrenal gland is stimulated directly and by way of postganglionic sympathetic fibers. The cumulative effect of this activity is an increase in epinephrine and norepinephrine in the circulation. Watts has recently shown that there is an increased urinary excretion of epinephrine after smoking in man and an increase in arterial blood epinephrine in dogs after intravenous nicotine, but not after a ganglionic blocking dose. These increased concentrations of circulating catecholamines rapidly act on the fat stores of the body to effect a mobilization of FFA.

The significance of the elevation of FFA following smoking, as well as after other stresses, is emphasized by the effect of an FFA rise on other serum lipids. Shafrir, Sussman, and Steinberg found that dogs developed a prompt rise in serum FFA after subcutaneous epinephrine in oil and a delayed elevation of serum lipoproteins. After daily injections for six to eight days, the serum cholesterol rose to 91 per cent above control values. It has been suggested that this response may have resulted from the increased circulating FFA giving rise to an increase in liver lipids which stimulated the production of cholesterol and other lipoprotein lipids. Kaplan and associates and Dury also found an elevation in serum lipids in dogs and rabbits after administration of long-acting epinephrine in oil. In our human experiments, there was a prompt rise in FFA but no observed immediate effect on serum cholesterol or triglycerides after smoking. This is similar to the immediate effect of short-acting epinephrine in animals. The fact that cholesterol can be maintained at an elevated level by repeated or long-acting epinephrine administration suggests a mechanism by which repeated daily smoking, as well as psychic stress, can give rise to an increase in blood cholesterol.

The results of the present study indicate that the FFA rise after smoking two cigarettes occurs rapidly and consistently and persists for a period of 20 to 40 minutes. In an individual who smokes continually at the rate of about four to six cigarettes per hour, there is a repeated catecholamine effect on the fat depots of the body with release of FFA. This is not unlike the effect in animals of repeated epinephrine administration. An eventual, delayed increase in serum levels of cholesterol and other lipoprotein lipids, as occurs in animals after epinephrine, is a conceivable result in humans after smoking.
at least, suggests an explanation for the higher cholesterol levels often found in smokers.\textsuperscript{1-4} If, as many investigators believe, a disturbance of lipid metabolism is a factor in the development of atherosclerosis, the effect of cigarette smoking on lipid metabolism should be given attention in considering the pathogenesis of this disorder.

Summary

The effect of cigarette smoking on serum free fatty acids (FFA) was studied in human subjects. After smoking two cigarettes there was an average maximal elevation in FFA of 351 μEq./L. This usually occurred 10 minutes after smoking and, in most instances, there was still some elevation 20 and 40 minutes after smoking. There was essentially no effect on serum cholesterol and triglyceride levels. In subjects who "chain-smoked" six cigarettes, all showed a rise in FFA during a 60-minute period, one showing a three-fold elevation. The effect of intravenous nicotine on serum FFA was studied in dogs. In 13 of 15 observations there was a rise in FFA. The mean maximal elevation of 166 μEq./L occurred after 10 minutes of nicotine infusion. These effects are probably due to sympathetic and adrenal stimulation by nicotine. This results in a rise in circulating catecholamines which rapidly effect a mobilization of FFA from the fat stores in the body.

References

Anatomia Angiografica del Cane. Tecnica, Metodo
dologia, Atlante Iconografico, G. C. Canossi,
M. Dardari, N. Cortesi, B. Brunelli, and C.
Pasquinelli. Torino, Italy, Minerva Medica, 1960,
145 pages, illustrated. 7,000 lire.

On the basis of personal experience during five
years of experimental work, the authors accurately
describe anatomical, surgical, and radiological
details of angiographic techniques in the dog. An
atlas is also included in this monograph, with very
clear angiograms and explanatory anatomical
schemes from all the circulatory regions. The book
should be of great assistance to the investigator
concerned with the circulation of the dog.
Effect of Cigarette Smoking and Nicotine on Serum Free Fatty Acids: Based on a Study in the Human Subject and the Experimental Animal
ALFRED KERSHBAUM, SAMUEL BELLET, EDWARD R. DICKSTEIN and LEONARD J. FEINBERG

doi: 10.1161/01.RES.9.3.631
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1961 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/9/3/631

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at: http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at: http://circres.ahajournals.org/subscriptions/