Tobacco Use and Urinary Excretion of Thromboxane A₂ and Prostacyclin Metabolites in Women Stratified by Age

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Background. Activated platelets have been implicated in both acute thrombus formation and atherogenesis. Because smoking is a risk factor for cardiovascular disease in men and women and male smokers have biochemical evidence of increased platelet activation, we found it of interest to study whether smoking augments platelet activity in women as well.

Methods and Results. Data on smoking habits and a urinary sample were obtained from 125 healthy female nonsmokers and an equal number of smokers, stratified by age in five groups from 18 to 59 years old. Urinary samples were analyzed with gas chromatography/mass spectrometry for the 2,3-dinor-metabolites of thromboxane A₂ (Tx-M), reflecting platelet activity, and prostacyclin (PGI-M), representing platelet/vessel wall interaction. Urinary Tx-M in smokers was higher than in nonsmokers (p<0.001), increasing with the number of cigarettes smoked per day and with age. In nonsmokers, there was no difference in Tx-M between the age groups. Urinary PGI-M in smokers was higher than that in nonsmokers (p<0.001) and decreased with age in nonsmokers but not in smokers. There was no difference in Tx-M between previous smokers and lifelong nonsmokers.

Conclusions. The elevated Tx-M in women who smoke cigarettes indicates an increased platelet activity that is dependent on smoking intensity. In parallel, PGI-M is augmented, suggesting that platelet/vessel wall interaction is stimulated. Quitting smoking is an effective means to restore platelet function. We propose that the observed increase in platelet activity in women who smoke cigarettes may be related to subsequent development of cardiovascular disease and that quitting smoking should be considered a high-priority medical target also in this sex. (Circulation 1992;86:1495–1500)

KEY WORDS • smoking • platelets • prostacyclin • thromboxanes

Cigarette smoking is a major risk factor for cardiovascular disease, as demonstrated in several prospective investigations.1,2 Although the etiological basis is not settled, population studies appear to indicate both short- and long-term cardiovascular effects of smoking.1,3 In particular, the short-term effect appears to be reversible upon quitting.2,3 Platelet activation is involved in thrombogenesis, with subsequent vascular occlusion and tissue infarction,4 and possibly also in the atherogenic process.5 In several experimental investigations, smokers have been reported to display biochemical evidence of increased activation of platelets in vivo.6–8 These data were recently confirmed in a population study in young male nonsmokers and smokers in our laboratory.9 Consequently, it appears that cardiovascular disease in smokers may be based, at least in part, on platelet hyperactivity.

Thromboxane A₂ (TXA₂) is a derivative of arachidonic acid formed in platelets.10 TXA₂ has platelet proaggregatory and proadhesive properties and is, in addition, a strong vasoconstrictor. The administration of aspirin-like drugs in doses that inhibit cyclooxygenase-dependent formation of TXA₂ in platelets has been shown to be efficient in the primary11 and secondary12 prevention of acute myocardial infarction. These data indicate that TXA₂ formed in platelets is etiologically involved in certain acute cardiovascular disorders, besides being a marker for platelet activity.

The above-mentioned studies on increased platelet activation in smokers were all performed in men.6–9 It was previously thought that cigarette smoking was not related to an increased risk of coronary heart disease in women.13 This is no longer the case, inasmuch as a number of studies have demonstrated an increased incidence of coronary disease in smoking women.13–17 Therefore, we considered it urgent to clarify whether cigarette smoking is related to an increase in platelet activation in women as well. Because cardiovascular disease is not abundant in young women, the protocol was also aimed at elucidating a possible influence of age on platelet activity in nonsmokers and smokers. A urinary metabolite of TXA₂, 2,3-dinor-thromboxane B₂ (Tx-M),18 was taken to reflect platelet formation of TXA₂.19 We also determined the urinary excretion of
2,3-dinor-6-keto-PGF<sub>1α</sub> (PGI-M, metabolite of prostacyclin<sup>20,21</sup>), because it has been proposed that an increased formation of prostacyclin in the vascular endothelium may reflect an increased interaction between the platelets and the vascular endothelium.<sup>22</sup>

**Methods**

**Study Population**

We recruited 250 women, 50 from each of the age groups 18–19, 20–29, 30–39, 40–49, and 50–59 years. All women were working in wards or laboratories in the hospital. The staff in a particular ward or laboratory was collectively informed about the nature of the study, its purpose, and inclusion and exclusion criteria. Inclusion criteria were willingness to answer a questionnaire on previous and present health state, gynecological data, and smoking habits and to deliver a urinary sample. Exclusion data were symptoms or clinical signs of major cardiovascular disease (past acute myocardial infarction, past stroke, overt atherosclerosis) and medication with nonsteroidal anti-inflammatory or otherwise platelet-active drugs. Those who were willing to participate reported this to the investigator, and later a verbal interview was completed. The final decision to include the subject in the study was based on this interview. The recruitment was continued accordingly until 25 nonsmokers and 25 smokers in each of the age groups had been included. The background data of the subjects are presented in Table 1.

Twenty-nine of the nonsmokers were previous cigarette smokers and had given up smoking 1–25 years (median, 9 years) before participating in the study. The remaining subjects in this group had never smoked cigarettes. Regarding exposure to secondhand smoke, it may be noted that smoking is limited to special rooms or outdoor facilities in Swedish hospitals. Smoking also is prohibited in public transportation systems. Hence, the remaining possibility of passive smoking among the present nonsmokers was at home or during social activities. Such possible exposure was not controlled for in the study. Data on daily cigarette consumption and total (lifetime) cigarette consumption among smokers are presented in Table 2.

The study protocol was approved by the local human investigations committee.

**Protocol**

All subjects were carefully instructed not to take any form of nonsteroidal anti-inflammatory or otherwise platelet-active drugs during the week preceding the urine sampling. Compliance was checked verbally by interview in association with the urine sampling. In parallel, the subjects were interviewed on previous and present health state, gynecological data, and smoking habits.

Urine collection was performed in the middle of the day. For the fertile subjects, who all had regular menstrual cycles with a length of 24–30 days, the day of urine collection was located to the preovulatory phase. Smokers were instructed to maintain their ordinary smoking habits immediately before urine collection. Current abstinence, i.e., the period of time between the last cigarette smoked and the urine sampling, was noted.

A 50–100-ml urine sample was collected without additives. It was immediately frozen at −20°C and transferred to storage at −80°C within 1 week. Analysis of the samples was performed within 4 weeks after collection.

**Analyses**

Tx-M was analyzed by a stable-isotope dilution assay using gas chromatography/negative ion–chemical ionization mass spectrometry as previously described.<sup>23</sup> Briefly, 2 ng of a tetradeuterated internal standard was added to aliquots of urine. The samples were then adsorbed onto a phenyl boronic acid column, eluted, and further purified on a reverse-phase Sep-Pac (Waters, Milford, Calif.). The dried eluate was put on a straight-phase thin-layer chromatography plate and converted to its pentafluorobenzyl ester. This material was further purified by another straight-phase thin-layer chromatography and derivatized to its trimethylsilyl ether. Quantitative analysis was done with a Finnigan Incos 50 mass spectrometer coupled to a Varian 3400 gas chromatograph.

PGI-M was also analyzed by a stable-isotope dilution assay. In summary, 1 ng of a tetradeuterated internal standard was added to aliquots of urine. The sample was subsequently subjected to extraction and reextraction procedures performed under alkaline and acidic conditions.<sup>24</sup> After formation of the methoxime pentafluorobenzyl ester and further purification by thin-layer chromatography, the derivatization was completed by formation of the trimethylsilyl ether. Quantitative analysis was done with the same instrument as for analysis of Tx-M.<sup>25</sup>

Creatinine was determined by a standard liquid chromatography method.

**Statistical Analysis**

The data on urinary excretion of Tx-M and PGI-M were not normally distributed; therefore, they are expressed as median and 75th percentile. Correlations and comparisons of proportions were performed by Pitman’s test.<sup>26</sup> Multiple regression analysis was performed to elucidate the importance of different variables. Regression coefficients were compared by a t test. All tests were two-tailed.

**Results**

Table 1 displays height and weight, gynecological data, past or present disease, and use of drugs in the study subjects, all age groups pooled and stratified into nonsmokers or smokers. As seen from the table, there was a significant difference (p < 0.05) in height between nonsmokers and smokers.

The smoking habits in the smokers are presented in Table 2. As evident from the table, there were no major differences in the number of cigarettes smoked per day in the different age groups. Hence, the total (lifetime) consumption of cigarettes was mainly a function of the total time the subjects had smoked (duration of smoking).

**Urinary Excretion of Tx-M**

The median urinary excretion of Tx-M in nonsmokers was 192 pg/mg creatinine (75th percentile, 300 pg/mg creatinine) and in smokers was 243 pg/mg (75th percen-
TABLE 1. Body Dimensions, Gynecological Data, and Occurrence of Disease/Intake of Drugs in the Study Groups

<table>
<thead>
<tr>
<th></th>
<th>Nonsmokers (n=125)</th>
<th>Smokers (n=125)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height (cm)</td>
<td>167±0.5</td>
<td>165±0.5*</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>64±0.8</td>
<td>62±0.8</td>
</tr>
<tr>
<td>Contraceptives, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oral</td>
<td>26 (21)</td>
<td>26 (21)</td>
</tr>
<tr>
<td>Intrauterine device</td>
<td>14 (11)</td>
<td>18 (14)</td>
</tr>
<tr>
<td>Previous parturition, n (%)</td>
<td>58 (46)</td>
<td>62 (50)</td>
</tr>
<tr>
<td>Postmenopausal, n (%)</td>
<td>21 (17)</td>
<td>25 (20)</td>
</tr>
<tr>
<td>Diseases, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>2 (1.6)</td>
<td>2 (1.6)</td>
</tr>
<tr>
<td>Dermatological</td>
<td>2 (1.6)</td>
<td>3 (2.4)</td>
</tr>
<tr>
<td>Endocrine</td>
<td>9 (7.2)</td>
<td>5 (4.0)</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>2 (1.6)</td>
<td>1 (0.8)</td>
</tr>
<tr>
<td>Gynecological</td>
<td>4 (3.2)</td>
<td>1 (0.8)</td>
</tr>
<tr>
<td>Neurological</td>
<td>1 (0.8)</td>
<td>2 (1.6)</td>
</tr>
<tr>
<td>Respiratory</td>
<td>0</td>
<td>2 (1.6)</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>2 (1.6)</td>
<td>3 (2.4)</td>
</tr>
</tbody>
</table>

Values are mean±SEM (height and weight) or actual numbers and percent.
*p<0.05 different from nonsmokers.

Figure 1 shows the excretion separated into different age groups. The difference in excretion of Tx-M between smokers and nonsmokers is significant (p<0.001).

To reveal whether the increased Tx-M excretion in smokers could be explained by other factors related to both smoking and urinary excretion of Tx-M, a multivariate, nonparametric procedure not based on assumptions about linear relations, in contrast to usual multivariate analysis, was applied (Pitman’s test). The increased excretion of Tx-M in smokers was independent of body mass index, presence of past or current disease, parity, and a set of gynecological variables (including use of an intrauterine device). It was also independent of the use of contraceptive pills (Table 3).

Because the excretion of Tx-M in smokers was higher than in nonsmokers and in addition seemed to increase with age, a multiple regression analysis was conducted to further analyze the individual impact of these factors. It was found that the expected value of the urinary excretion of Tx-M in smokers could be expressed by the equation

$$\text{Tx-M} = 61.3 + 3.42 \times \text{age} + 10.0 \times \text{number of cigarettes per day}$$

The confidence intervals for the regression coefficients (3.42 and 10.0) were (1.25, 5.60; p<0.01) and (5.0, 10.0; p<0.001), respectively. Hence, in a smoking subject the excretion of Tx-M increased significantly.

TABLE 3. Excretion of Tx-M and PGI-M in Smokers and Nonsmokers in the Pooled Age Groups 18–49 Years, Stratified Into Those Using Contraceptive Pills and Those Using No Contraceptives (Including Intrauterine Device)

<table>
<thead>
<tr>
<th></th>
<th>Contraceptive pills</th>
<th>No contraceptives</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Smokers</td>
<td>Nonsmokers</td>
</tr>
<tr>
<td>Number</td>
<td>26</td>
<td>26</td>
</tr>
<tr>
<td>Median age, years</td>
<td>19</td>
<td>25</td>
</tr>
<tr>
<td>Tx-M (pg/mg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>75th percentile</td>
<td>297</td>
<td>275</td>
</tr>
<tr>
<td>Median</td>
<td>196</td>
<td>179</td>
</tr>
<tr>
<td>25th percentile</td>
<td>160</td>
<td>131</td>
</tr>
<tr>
<td>PGI-M (pg/mg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>75th percentile</td>
<td>251</td>
<td>242</td>
</tr>
<tr>
<td>Median</td>
<td>221</td>
<td>151</td>
</tr>
<tr>
<td>25th percentile</td>
<td>153</td>
<td>91</td>
</tr>
</tbody>
</table>

*Data on total cigarette consumption given in thousands of cigarettes.
with both age and the number of cigarettes smoked per day.

Despite the positive correlation with age, the median urinary excretion of Tx-M appeared to be lower in the age group 50–59 than in the age group 40–49 (Figure 1), possibly suggesting a decrease in Tx-M excretion after the menopause. However, stratification of the pooled age groups 40–49 and 50–59 into premenopausal and postmenopausal women revealed no difference in excretion of Tx-M.

To reveal the influence of age on the excretion of Tx-M in nonsmokers, the same type of multiple regression analysis as above was performed in this group as well. The resulting equation for estimated Tx-M in nonsmokers was

\[ \text{Tx-M} = 228.4 - 0.078 \times \text{age} \]

The confidence interval for the regression coefficient \((-0.078)\) was \((-1.94, 1.79; \text{NS})\). The regression coefficient for the influence of age on Tx-M excretion in nonsmokers \((-0.078)\) differs from the corresponding coefficient in smokers \((3.42)\) \((p<0.05)\). Hence, age did not significantly affect the excretion of Tx-M in nonsmokers, in contrast to the finding in smokers.

### Urinary Excretion of PGI-M

The median urinary excretion of PGI-M in nonsmokers was 136 pg/mg creatinine (75th percentile, 216 pg/mg creatinine) and in smokers was 178 pg/mg (75th percentile, 246 pg/mg). Figure 2 shows the excretion separated into different age groups. There was a significant difference between nonsmokers and smokers with respect to the urinary excretion of PGI-M \((p<0.001)\).

The dependence of the increased excretion of PGI-M in smokers on other factors related to both smoking and urinary excretion of PGI-M was tested with Pitman’s test as described above for Tx-M. The increased excretion of PGI-M was independent of body mass index, presence of disease, parity, and a set of gynecological variables (including use of an intrauterine device). It was also independent of the use of contraceptive pills (Table 3).

The expected value for the urinary excretion of PGI-M in smokers was estimated with multiple regression analysis as a function of age and number of cigarettes smoked per day, in analogy to the procedure performed above for Tx-M. The estimated equation for excretion of PGI-M was

\[ \text{PGI-M} = 214.8 - 0.89 \times \text{age} + 1.44 \times \text{number of cigarettes per day} \]

The confidence intervals for the regression coefficients \((-0.89)\) and \((1.44)\) were \((-2.07, 0.29; \text{NS})\) and \((-1.28, 4.16; \text{NS})\), respectively. Hence, the excretion of PGI-M in smokers was not significantly affected either by age or by the number of cigarettes smoked per day. However, age significantly affected the excretion of PGI-M in nonsmokers, in contrast to the finding in smokers. The equation for estimated PGI-M in nonsmokers was

\[ \text{PGI-M} = 219.8 - 1.63 \times \text{age} \]

The confidence interval for the regression coefficient \((-1.63)\) was \((-2.72, -0.55; p<0.01)\).

### Effect of Quitting Smoking

The median urinary excretion of Tx-M in lifelong nonsmokers was 184 pg/mg creatinine (75th percentile, 324 pg/mg creatinine) and in previous smokers was 210 pg/mg (75th percentile, 272 pg/mg).

The median urinary excretion of PGI-M in lifelong nonsmokers was 142 pg/mg creatinine (75th percentile, 224 pg/mg creatinine) and in previous smokers was 135 pg/mg (75th percentile, 190 pg/mg).

There was no significant difference between lifelong nonsmokers and previous smokers with respect to the urinary excretion of Tx-M or PGI-M. Neither was there any significant correlation between time interval since quitting smoking and excretion of Tx-M among previous smokers. In an attempt to elucidate in more detail the effect of quitting smoking, two regression analyses were performed, applying the excretion of Tx-M as dependent variable. In the first of these regression analyses, age and time interval since quitting were the nondependent variables. In the second analysis, the square of the time interval since quitting was added. It was found that the correlation was not substantially improved by adding the square of the time interval since quitting (correlation coefficient, 0.15 compared with 0.14 without that term). There was, however, a slow change with time interval since quitting, with an estimated reduction in excretion of Tx-M amounting to 1.7 pg/mg creatinine per year. That result indicates a more immediate reduction in excretion of Tx-M shortly after quitting, because the observed negligible difference between lifelong nonsmokers and previous smokers would not have been reached with such a very low annual reduction after quitting.

### Discussion

The urinary excretions of the cardiovascular eicosanoid metabolites Tx-M and PGI-M were higher in smoking than in nonsmoking women in the present study. In addition, the excretion of Tx-M was related to
The median urinary excretion of Tx-M in the present female smokers was higher than that in nonsmokers, as previously demonstrated in men.6-9 Thus, the present study is the first to demonstrate that women who smoke cigarettes, in analogy with men, excrete more Tx-M than those who do not smoke. Note, however, that in women 18–19 years old, there was no difference in the median excretion of Tx-M, whereas in men of the same age group, an excretion almost 50% higher in smokers than in nonsmokers was reported previously.9 Statistical analysis revealed that the current excretion of Tx-M was positively related to the number of cigarettes smoked per day. We have earlier demonstrated that in young, apparently healthy men, the excretion of Tx-M is related to the daily cigarette consumption.9 The present study extends this observation to women who smoke cigarettes.

Although the cross-sectional design applied in the present investigation does not permit any conclusions regarding the mechanism behind the increased urinary excretion of Tx-M, some considerations may be justified. Being a metabolite of TXa2,18 the excretion of Tx-M into the urine reflects mainly the formation of this platelet eicosanoid.9 Also, the excessive excretion of Tx-M in smokers is mainly of platelet origin, as demonstrated by Nowak et al.7 These authors reported that a 20-mg dose of aspirin, which is known to selectively inhibit platelet cyclooxygenase, abolished the difference in excretion of Tx-M between smokers and nonsmokers. Also, after intake of aspirin, the excretion of Tx-M returned to the levels observed before treatment in parallel to the recovery of the cyclooxygenase activity in the platelets.7 In analogy with those observations, we propose that the excessive excretion of Tx-M found in currently smoking women, which is as much as 100% higher than the corresponding excretion in the nonsmokers, is also of platelet origin. Hence, platelet activity seems to be higher in smoking than in nonsmoking women.

The higher urinary excretion of Tx-M in old smokers than in young smokers may indicate that the platelets increase their sensitivity to smoking with age. Other factors, e.g., a slower elimination of the product(s) responsible for platelet activation in older than in young smokers, may also be considered in this respect. The increase in Tx-M excretion with age in smoking women is of particular interest in relation to the development of cardiovascular disease. Cardiovascular disease is not abundant in young women. The currently observed age dependence in the excretion of Tx-M in women who smoke cigarettes is compatible with an augmented impact of smoking on the development of cardiovascular disease in older women.

The nonsmokers in the various age groups in the present study did not differ in urinary excretion of Tx-M. The age dependency of the excretion of Tx-M and PGI-M has previously been studied by others. Reilly and FitzGerald27 also reported that the excretion of Tx-M was higher in an apparently healthy group of volunteers (10 men and 10 women) 50–88 years old than in 16 healthy volunteers (12 men and four women) 21–39 years old. However, they did not report sex data separately. Fischer et al6 reported data more consistent with those observed here. Thus, they found no difference in Tx-M between young women and postmenopausal women, in accordance with the observations in the nonsmoking group in the present study. However, the group of women studied by Fischer et al6 consisted of both smokers and nonsmokers (number of each unspecified), which makes a more detailed comparison difficult. The older age group studied by Reilly and FitzGerald27 was recruited from a geriatric clinic, but Fischer et al6 did not report selection criteria for their study group. Our subjects were recruited from one community only, suggesting that they constituted a more homogeneous group. We are therefore inclined to claim that the excretion of Tx-M in nonsmoking women 18–59 years old is independent of age.

The median urinary excretion of PGI-M was also elevated in smokers compared with nonsmokers in the present study. In contrast to the excretion of Tx-M, however, that of PGI-M was not related to the number of cigarettes smoked per day. The basis for this lack of dose dependence in excretion of PGI-M is not obvious. Previous studies have reported conflicting results with respect to the excretion of PGI-M in smokers. In our earlier population study of 18–19-year-old men,9 the excretion of PGI-M was not elevated in smokers compared with nonsmokers. Similar data were reported by Fischer et al,6 who studied men 20–35 years old. In contrast, Nowak et al7 found PGI-M in urine to be elevated in six male smokers 24–46 years old, and Barrow et al9 reported a similar elevation of PGI-M in 30 healthy smokers 18–39 years old. As mentioned earlier, an increased excretion of PGI-M indicates a facilitated interaction between activated platelets and the vessel walls.22 Such a change in platelet/vessel wall interaction is probably a consequence of functional and/or morphological degeneration of the vascular endothelium, possibly in the direction of atherosclerosis. The present data on an increased excretion of PGI-M in smokers, taken together with those reported earlier on this topic,6-9 support the hypothesis that smoking leads to an accelerated degeneration of the vascular endothelium, which in turn promotes platelet/vessel wall interaction and thereby enhances the excretion of PGI-M.

Reilly and FitzGerald27 also reported increased urinary excretion of PGI-M in their older subjects, in contrast to Fischer et al,6 who found lower PGI-M in women after the menopause. Either of these observations may accord with the findings in the present study inasmuch as we found a lower excretion of PGI-M at higher age in the group of nonsmokers but not in smokers. It may be speculated that the lack of age dependence of PGI-M in smokers was based on a continuous smoking-induced facilitation of the vascular formation of prostacyclin, the magnitude of which is sufficient to meet the normal age-dependent attenuation seen in nonsmokers. Such a compensatory increase in prostacyclin formation in smokers should not be regarded as disadvantageous. It may rather be considered a consequence of increased platelet/vessel wall interaction in the older smokers.

The excessive urinary excretion of Tx-M in smokers appeared completely reversible, inasmuch as people who had quit smoking did not excrete more Tx-M than those who had never smoked. This reversibility probably indicates that newly synthesized platelets in people who...
have stopped smoking do not differ in activity from those in nonsmokers.

In conclusion, this study provides the first evidence of an increase in platelet activity and platelet/vessel wall interaction in women who smoke cigarettes. The defect in platelet function is related to the number of cigarettes smoked per day. Quitting smoking appears to be an effective means to restore platelet function. The data support the concept that smoking may lead to cardiovascular dysfunction and disease in women and that quitting smoking should be considered a high-priority medical target in this sex as well.

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References


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