

## Relation Between Tobacco Use and Urinary Excretion of Thromboxane A<sub>2</sub> and Prostacyclin Metabolites in Young Men

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**Background.** Cigarette smoking is a risk factor for cardiovascular disease. The present study addressed the effect of tobacco use on the formation of two eicosanoids, thromboxane A<sub>2</sub> and prostacyclin, which have been implicated in both acute and chronic cardiovascular disorders.

**Methods and Results.** In 577 randomly sampled 18–19-year-old men, the urinary excretion of the 2,3-dinor metabolites of thromboxane A<sub>2</sub> and prostacyclin (Tx-M and PGI-M, respectively) was analyzed and related to the subjects' self-reported use of tobacco. Sixty-five percent of the subjects used no tobacco, 7.5% were cigarette smokers, 22% used wet (oral) snuff, and the rest reported a mixed use of tobacco. The urinary excretion of Tx-M was higher ( $p < 0.001$ ) in cigarette smokers than in those not using tobacco (180 versus 128 pg/mg creatinine) and was correlated ( $r = 0.35$ ,  $p < 0.05$ ) with the daily cigarette consumption. Snuff users had no increase in their urinary excretion of Tx-M, despite urinary cotinine levels comparable to those in the cigarette smokers (1,210 and 1,560 ng/ml, respectively). The excretion of PGI-M did not differ between non-tobacco users, cigarette smokers, and snuff users.

**Conclusions.** We conclude that cigarette smoking, but not the use of snuff, facilitates the formation of thromboxane A<sub>2</sub>. We propose that such an increased formation reflects platelet activation in the absence of vascular injury and that it may be of significance for the subsequent development of cardiovascular disease. (*Circulation* 1991;83:1698–1704)

Prospective studies have identified cigarette smoking as a major risk factor in cardiovascular disease.<sup>1,2</sup> Although the pathogenic mechanism is not settled, epidemiologic data seem to indicate both short- and long-term cardiovascular effects of smoking.<sup>1,2</sup> Several reports suggest that the short-term effect is partly reversible upon quitting.<sup>2,3</sup>

Hemostatic processes (i.e., clotting of the blood and/or platelet activation) are involved in one of the acute manifestations of a vascular disorder (i.e., in thrombogenesis followed by vascular occlusion and tissue infarction)<sup>4</sup> and possibly in the development of atherosclerosis.<sup>5</sup> Information about the effects of smoking on various hemostatic variables may consequently help to clarify the etiology of smoking-related vascular disease. Few epidemiologic data are avail-

able on the relation between smoking and thrombogenic factors. Wilhelmsen and coworkers<sup>6</sup> reported that smoking was strongly correlated with plasma fibrinogen, more weakly correlated with plasminogen, and not correlated with other coagulation factors in middle-aged men. The role of smoking in platelet function, however, has not been studied in samples from defined populations.

Thromboxane A<sub>2</sub> (TxA<sub>2</sub>) is an arachidonic acid derivative formed in activated platelets.<sup>7</sup> TxA<sub>2</sub> is a powerful platelet aggregatory and adhesive agent and a strong vasoconstrictor. In large prospective studies, the administration of aspirin-like drugs in doses known to inhibit the formation of TxA<sub>2</sub> in platelets has proven efficient in the primary<sup>8</sup> and secondary<sup>9</sup> prevention of acute myocardial infarction and myocardial infarction in patients with unstable angina.<sup>10</sup> Hence, platelet TxA<sub>2</sub> seems to play a role in the etiology of at least some cardiovascular catastrophes, besides being a biochemical marker of platelet activity. The present study addressed the relation between smoking and cardiovascular formation of TxA<sub>2</sub> in a random sample taken from a population of 18–19-year-old men. This age group was chosen since we aimed to analyze the primary effect of smoking on

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platelet activity in the absence of clinically evident cardiovascular disease.<sup>11</sup> A urinary metabolite of  $\text{TxA}_2$ , 2,3-dinor-thromboxane  $\text{B}_2$  (Tx-M),<sup>12</sup> was taken to reflect the formation of  $\text{TxA}_2$  in platelets.<sup>13</sup> In parallel, we determined the urinary concentration of 2,3-dinor-6-ketoprostaglandin  $\text{F}_{1\alpha}$  (PGI-M, metabolite of prostacyclin<sup>14,15</sup>) since it has been shown that increased formation of prostacyclin in the vascular endothelium may indicate increased interaction between the platelets and the vessel walls.<sup>11</sup>

## Methods

### Study Population

Sweden has a compulsory military service requiring that every man aged 18–19 years be screened for enrollment in the national defense system. The screening procedure comprises mental and physical fitness tests and is performed at one of five centers in the country. The center at S ve outside Gothenburg annually tests about 10,000 subjects, comprising about 20% of the year class, recruited from the southwest of Sweden. The present study group was selected in connection with this military test procedure. The study protocol was approved by the local institutional review board and by the military authorities.

All 756 subjects tested on 17 randomly selected Tuesdays during 1 year were eligible to participate. The subjects were asked to complete a questionnaire and to deliver a urine sample. Questionnaire completion and urine sampling were performed in the early afternoon throughout the study. Compliance to answering the questionnaire was 100%, but 64 subjects failed to deliver a urine sample. Exclusion criteria were medication with aspirin-like drugs during the week preceding the urine sampling, incomplete questionnaires, and acute or chronic disease. After review of the questionnaires, 97 subjects were excluded due to drug intake, seven due to incomplete questionnaires, and two due to diabetes. The urine samples from the remaining subjects were analyzed for Tx-M, PGI-M, and creatinine. In addition, samples collected during the latter part of the study were analyzed for cotinine ( $n=185$ ), norepinephrine ( $n=194$ ), and epinephrine ( $n=194$ ). All samples were frozen within 20 minutes after collection and kept at  $-80^\circ\text{C}$  until analysis. Nine subjects reporting no use of tobacco displayed high urinary concentrations of cotinine upon analysis and were excluded for this reason. The number of subjects finally included in the study was 577.

### Biochemical Analyses

The Tx-M concentration was analyzed by a stable isotope dilution assay using gas chromatography/negative ion-chemical ionization mass spectrometry (GC/MS) as previously described.<sup>16</sup> Briefly, 2 ng of a tetradeuterated internal standard was added to 5-ml aliquots of urine. The samples were then treated with methoxyamine hydrochloride, and the resulting dinor-TxB<sub>2</sub> methoxime was adsorbed onto a phenylboronic acid column. After elution, the dinor-TxB<sub>2</sub>

methoxime was further purified on a reversed-phase Sep-Pac column (Waters Chromatography Division, Millipore Corp., Milford, Mass.) and eluted into ethyl acetate. The dried residue was applied to a straight-phase thin-layer chromatography (TLC) plate and developed in the organic layer of ethyl acetate:acetic acid:hexane shaken with water (54:12:25:100 by vol). The area corresponding to 2,3-dinor-TxB<sub>2</sub> methoxime was scraped and eluted in ethyl acetate. The organic layer was transferred to another tube, dried, and converted to its pentafluorobenzyl ester. This material was further purified by another straight-phase TLC/organic layer of isooctane:ethyl acetate shaken with water (65:85:100 by vol) followed by scraping and elution of the appropriate area on the TLC plate. The derivatization was completed by formation of the trimethylsilyl ether. Quantitative analysis was accomplished using a Finnigan MAT 4500 mass spectrometer (San Jose, Calif.) coupled to a Varian Vista 6000 gas chromatograph (Walnut Creek, Calif.) or a Finnigan Incos 50 mass spectrometer coupled to a Varian 3400 gas chromatograph. The instruments were operated in the negative ion-chemical ionization mode using methane as the reactant gas and selective monitoring of  $m/e$  586 for endogenous Tx-M and  $m/e$  590 for the tetradeuterated internal standard. Prior to GC/MS, the trimethylsilyl derivatization mixture was dried under a stream of dry nitrogen gas and the sample was dissolved in 10  $\mu\text{l}$  hexane, of which 2–5  $\mu\text{l}$  were injected into a splitless injector operated at  $250^\circ\text{C}$ . The column oven was kept at  $280^\circ\text{C}$ .

The PGI-M concentration was also analyzed by a stable isotope dilution assay. In summary, 1 ng of a tetradeuterated internal standard was added to a 5-ml aliquot of urine. The sample was subsequently subjected to extraction and re-extraction procedures performed under alkaline and acidic conditions.<sup>17</sup> After formation of the methoxime pentafluorobenzyl ester and further purification by TLC, the derivatization was completed by formation of the trimethylsilyl ether. Quantitative analysis was accomplished using the same instruments, mode, and mass numbers as for analysis of Tx-M.<sup>18</sup>

The creatinine concentration was determined by a standard liquid chromatography method.

To assess the degree of nicotine exposure in different groups, urinary cotinine levels were analyzed. Cotinine in the urine was analyzed using capillary column gas chromatography with nitrogen-sensitive detection.<sup>19</sup> The normal upper limit for non-tobacco users was set to 100 ng/ml urine.

The levels of norepinephrine and epinephrine in the urine were analyzed as follows. A portion of the sample was acidified to pH 3 immediately after collection (i.e., before being frozen). Upon analysis, the urine was preliminarily extracted on an ion-exchange resin (Biorex, Bio-Rad Laboratories-Chemical Division, Richmond, Calif.).<sup>20</sup> Catecholamines in the eluate were extracted at pH 8.3 on alumina,<sup>21</sup> and eluted with antioxidant-containing

TABLE 1. Tobacco Consumption and Urinary Cotinine Concentration in Study Groups

Group	No.	%	Daily tobacco consumption		Accumulated tobacco consumption		Tobacco abstinence time (hrs)	Cotinine in urine (ng/ml)	
			Cigarettes	Snuff (g)	Cigarettes ( $n \times 1,000$ )	Snuff (kg)		Median	Range
Non-tobacco users	377	65.3	...	...	...	...	...	...	...
Never used	344	59.6	...	...	...	...	...	5.7	0.6-90
Used previously	33	5.7	...	...	6.6 $\pm$ 2.5	16.6 $\pm$ 4.0	...	5.4	0.8-20
Cigarettes only	43	7.5	12.2 $\pm$ 0.8	...	17.4 $\pm$ 2.0	...	3.1 $\pm$ 1.9	1,560	570-3,450
Snuff only	127	22.0	...	25 $\pm$ 1	...	29.1 $\pm$ 2.7	2.7 $\pm$ 0.9	1,210	3.1-4,280
Cigarettes+snuff	30	5.2	7.8 $\pm$ 1.3	27 $\pm$ 3	9.1 $\pm$ 2.1	43.4 $\pm$ 8.7	2.2 $\pm$ 0.7	1,773	840-2,800

Values are mean  $\pm$  SEM unless noted.

perchloric acid. The eluate was injected into a liquid chromatography system (Nucleosile 5SA column) with electrochemical detection (Bioanalytical Systems, Inc., West Lafayette, Ind.) and corrected for individual recovery using an internal standard of  $\alpha$ -methyldopamine.<sup>22</sup>

#### Cardiovascular Measurements

Data on height and weight, systolic and diastolic blood pressures, maximal heart rate, and maximal working capacity were obtained from the tests performed by the military authorities. Systolic and diastolic blood pressures were measured indirectly after 10-15 minutes of supine rest. The maximal working capacity was tested on an electrically braked bicycle ergometer (Siemens-Elema, Stockholm). The work load on the ergometer was initially set at about 125 W (range 75-150 W, depending on the subject's body dimensions and history of physical fitness). The stable heart rate obtained after 5 minutes of work at this submaximal load was used to calculate the standardized maximal aerobic capacity. Subsequently, the load was increased at a rate of 25 W/min. The load at which the subject was exhausted and unable to continue the work was taken as the maximal working capacity, and the heart rate at the time of interruption was taken from the continuous electrocardiographic recording to represent the maximal heart rate.

#### Statistical Analysis

Normally distributed data are presented as mean $\pm$ SEM, and skewed data are presented as

median and range. The frequency distributions of the cardiovascular measurements differed nonsignificantly from the normal distribution (Kolmogorov-Smirnov test), while the distributions of the urinary concentrations of Tx-M and PGI-M deviated significantly. Hence, differences in blood pressure and work load among groups were tested using the normal distribution, while differences in the urinary concentrations of Tx-M and PGI-M among groups were tested with a nonparametric method (Wilcoxon's rank sum test).

#### Results

Among the 577 subjects included in the study, 377 did not use any form of tobacco. The non-tobacco users were stratified into one subgroup who had never used tobacco ( $n=344$ ) and another subgroup who had used tobacco previously ( $n=33$ ). The tobacco users were stratified into one group smoking cigarettes only ( $n=43$ ), one group using wet (oral) snuff ( $n=127$ ), and one group reporting a mixed use of cigarettes and snuff ( $n=30$ ). The latter group contained one subject who also smoked a pipe. Data on tobacco consumption and urinary cotinine concentration in these groups are presented in Table 1. There was no significant difference among tobacco-using groups in the duration of tobacco abstinence at the time of urine collection. Also, there was no difference among tobacco-using groups in urinary cotinine concentration.

Body dimensions, systemic blood pressure, and data on aerobic capacity in the different groups are presented in Table 2, together with data on urinary

TABLE 2. Body Dimensions, Basal Blood Pressure, Working Capacity, and Excretion of Catecholamines in Study Groups

Group	Height (cm)	Weight (kg)	Blood pressure (mm Hg)		Working capacity		Catecholamines (nmol/g creatinine)	
			Systolic	Diastolic	Maximum heart rate ( $\text{min}^{-1}$ )	Maximum work load (W)	Norepinephrine	Epinephrine
Non-tobacco users	180 $\pm$ 0.3	70.6 $\pm$ 0.5	122 $\pm$ 0.5	66.4 $\pm$ 0.5	192 $\pm$ 0.5	300 $\pm$ 2.6	150 $\pm$ 4.8	49.1 $\pm$ 2.7
Cigarettes only	180 $\pm$ 1.0	70.5 $\pm$ 1.6	118 $\pm$ 1.4*	67.8 $\pm$ 1.2	190 $\pm$ 1.6	271 $\pm$ 8.0†	142 $\pm$ 8.9	42.1 $\pm$ 3.9
Snuff only	179 $\pm$ 0.6	69.7 $\pm$ 1.0	122 $\pm$ 0.9	65.3 $\pm$ 0.8	188 $\pm$ 0.9	291 $\pm$ 4.7	148 $\pm$ 8.6	50.0 $\pm$ 4.1
Cigarettes+snuff	179 $\pm$ 1.0	70.0 $\pm$ 1.5	122 $\pm$ 2.3	66.4 $\pm$ 1.6	189 $\pm$ 2.0	277 $\pm$ 9.7*	156 $\pm$ 16.0	28.5 $\pm$ 4.0

Values are mean $\pm$ SEM.

\*† $p < 0.05$  and  $0.001$ , respectively, different from non-tobacco users.

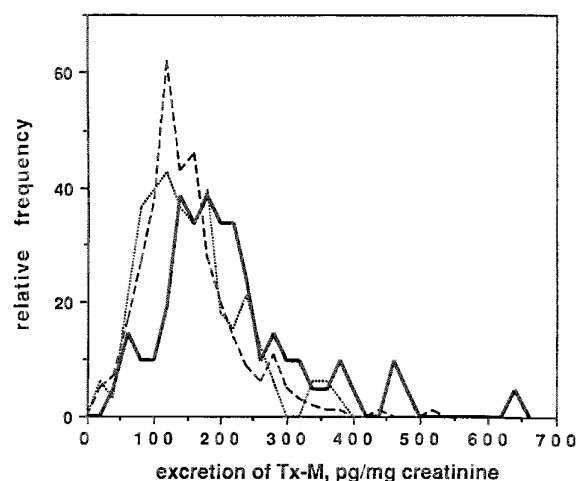


FIGURE 1. Frequency distribution of urinary excretion of 2,3-dinor thromboxane  $B_2$  (Tx-M) in study groups. Solid line, pooled group of cigarette smokers (cigarettes only and cigarettes+snuff); dotted line, those using snuff only; dashed line, non-tobacco users. Distribution of Tx-M in cigarette smokers differs significantly ( $p < 0.001$ ) from distributions among non-tobacco users and those using snuff only.

catecholamine excretion. Body dimensions did not differ among groups. Systolic blood pressure was lower ( $p < 0.05$ ) in those smoking cigarettes only than in the non-tobacco users. The maximal work load reached during the bicycle test was lower in both cigarette smokers ( $p < 0.001$ ) and those with mixed use of cigarettes and snuff ( $p < 0.05$ ) than in the non-tobacco users. Urinary catecholamine concentrations did not differ among groups.

The frequency distribution of urinary Tx-M concentration in the groups is shown in Figure 1. The median excretion of Tx-M in the non-tobacco users was 128 pg/mg creatinine (Table 3), and the 95th percentile for Tx-M excretion was 270 pg/mg creatinine. In the pooled group of cigarette smokers (cigarettes only and mixed use of tobacco groups combined), the distribution of urinary Tx-M concentration was shifted to the right ( $p < 0.001$ ) compared with the non-tobacco users (Figure 1). The median excretion of Tx-M in the group smoking cigarettes only was 180 pg/mg creatinine (Table 3),

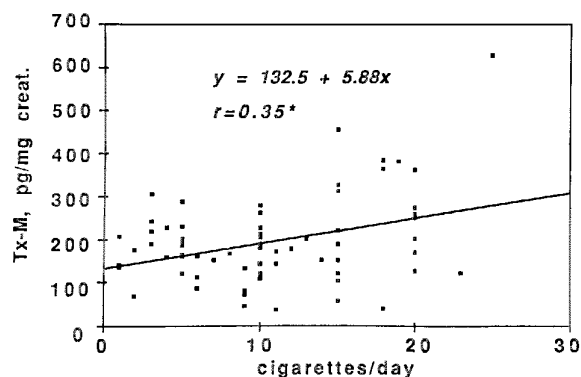


FIGURE 2. Relation between number of cigarettes smoked per day and urinary excretion of 2,3-dinor thromboxane  $B_2$  (Tx-M) in healthy male cigarette smokers aged 18-19 years. Intercept of regression line falls close to median excretion of Tx-M in non-tobacco users from same population (128 pg/mg creatinine). \* $p < 0.01$  different from 0.

and about 20% of the Tx-M excretion figures in the pooled group of cigarette smokers fell above the 95th percentile in the non-tobacco users. The excretion of Tx-M correlated ( $r = 0.35$ ,  $p < 0.05$ ) with the daily consumption of cigarettes (Figure 2) but not the urine level of cotinine or catecholamines. In the group using snuff only, the distribution of urinary Tx-M concentration did not differ from that in the non-tobacco users (Figure 1). The median excretion of Tx-M in the group using snuff only was 126 pg/mg creatinine, similar to that in the non-tobacco users (Table 3). No more than about 6% of the Tx-M excretion figures in the group using snuff only fell above the 95th percentile in the non-tobacco users. There was no correlation between the daily consumption of snuff and the urinary Tx-M concentration on the one hand or the urinary concentrations of cotinine or catecholamines on the other.

The excretion of PGI-M in the non-tobacco users is shown in Figure 3. The median excretion of PGI-M in this group was 129 pg/mg creatinine (Table 3). The 95th percentile for excretion of PGI-M was close to that of Tx-M (i.e., 277 pg/mg creatinine). The excretion of PGI-M in the different groups of tobacco users did not differ from that in the non-tobacco users (Table 3). About 6% of the PGI-M excretion

TABLE 3. Urinary Excretion of Thromboxane  $A_2$  Metabolite (Tx-M) and Prostacyclin Metabolite (PGI-M) in Study Groups

Group	Tx-M (pg/mg creatinine)		PGI-M (pg/mg creatinine)	
	Median	Range	Median	Range
Non-tobacco users	128	16-502	129	20-496
Never used	127	16-502	130	20-496
Used previously	132	42-228	112	49-309
Cigarettes only	180*	37-471	140	40-339
Snuff only	126	13-372	121	43-428
Cigarettes+snuff	187*	47-629	122	56-426

\* $p < 0.001$  different from non-tobacco users.

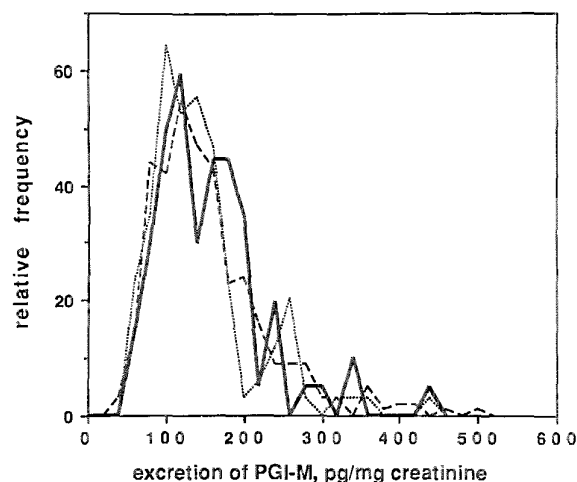


FIGURE 3. Frequency distribution of urinary excretion of 2,3-dinor-6-ketoprostaglandin  $F_{1\alpha}$  (PGI-M) in study groups. Solid line, pooled group of cigarette smokers (cigarettes only and cigarettes+snuff); dotted line, those using snuff only; dashed line, non-tobacco users. There was no significant difference among distributions.

data in the group of cigarette smokers fell above the 95th percentile in the non-tobacco users, while in the group using snuff only about 3% of the data did so. In the group of cigarette smokers the excretion of PGI-M correlated with neither the excretion of Tx-M nor the daily consumption of cigarettes.

### Discussion

The present study demonstrates that, in a population of healthy young men, the excretion of Tx-M is higher in those who smoke cigarettes than in those who do not smoke and that the excretion increases with the number of cigarettes smoked per day.

The relation between cigarette smoking and urinary excretion of Tx-M has previously been investigated in case reports or experimental studies with limited numbers of participants. Vesterqvist and coworkers<sup>23</sup> found that none of three smokers showed any apparently divergent excretion of Tx-M compared with nonsmokers. In contrast, two other studies reported the excretion of Tx-M to be elevated in selected groups of chronic cigarette smokers.<sup>24,25</sup> Nowak and coworkers<sup>26</sup> observed that the excretion of Tx-M in six healthy male habitual smokers aged 24–46 years was higher than that in a group of age-matched controls. The excretion figures reported in that study (230 pg/mg creatinine in smokers and 143 pg/mg creatinine in nonsmokers) are close to those we observed.

The present data also indicate that the excretion of Tx-M in the pooled group of smokers is related to the intensity of smoking. Statistically, each cigarette smoked per day increased the excretion of Tx-M by about 6 pg/mg creatinine above the basal level of about 130 pg/mg creatinine. Hence, although these epidemiologic data do not offer any possibility of

evaluating the etiology of the observed relation between cigarette consumption and facilitation of Tx-M excretion, they indicate that smoking is comparable to any type of drug administration that elicits dose-related changes in body function.

A metabolite of  $TxA_2$ ,<sup>12</sup> the excretion of Tx-M into the urine mainly reflects the formation of  $TxA_2$  in platelets.<sup>13</sup> Also, the excessive excretion of Tx-M in smokers is mainly platelet-derived, as shown by Nowak et al.<sup>26</sup> These authors demonstrated that a low (20 mg) dose of aspirin, known to selectively inhibit platelet cyclooxygenase, abolished the difference in excretion of Tx-M between smokers and nonsmokers. Furthermore, after aspirin ingestion the return of Tx-M excretion to pretreatment levels paralleled the recovery of platelet cyclooxygenase function.<sup>26</sup> We propose, by analogy, that the increased excretion of Tx-M in the young healthy smokers presently investigated is also of platelet origin.

Previous experimental studies have demonstrated differences in platelet aggregability between smokers and nonsmokers. The maximum platelet aggregation response *ex vivo* to a fixed dose of adenosine diphosphate (ADP) in smokers was higher after smoking a cigarette than after sham smoking or smoking lettuce leaf.<sup>27</sup> In another study, the lowest amount of arachidonic acid required to elicit irreversible platelet aggregation *in vitro* did not differ between smokers and nonsmokers.<sup>28</sup> In the same study, aminophylline inhibited ADP-induced platelet aggregation *in vitro* more in nonsmokers than in smokers, suggesting lower endogenous concentrations of antiaggregatory activity in blood from smokers than from nonsmokers. Evidence of increased platelet activation *in vivo* has also been presented previously. In addition to the above-mentioned studies demonstrating increased excretion of Tx-M in smokers,<sup>24–26</sup> platelet survival has been shown to be shortened in apparently healthy chronic smokers.<sup>29</sup> These experimental studies provide evidence that platelet function is affected in chronic smokers. The present data also favor the existence of platelet dysfunction in a young population of apparently healthy smokers.

As to the mechanism behind the apparent platelet activation in smokers, the current data provide no more than provisional evidence. The excretion of Tx-M was elevated in smokers, without any parallel change in the excretion of PGI-M. The latter is a metabolite of prostacyclin,<sup>14</sup> and the urinary excretion of PGI-M accurately reflects the formation of prostacyclin in the vascular endothelium.<sup>15</sup> Several states of acute and chronic cardiovascular disease are characterized by increased urinary excretion of both Tx-M and PGI-M,<sup>11</sup> probably reflecting increased interaction between platelets and the injured vessel walls. The subjects currently studied were young and had no clinically evident history of cardiovascular disease. Hence, it may be assumed that vascular injury was present to only a very limited, subclinical, degree. In harmony with this assumption, the excre-

tion of PGI-M in these subjects was not increased. Hence, it seems that platelet activation is present in young smokers in the absence of an increased platelet-vessel wall interaction. In the study of Nowak et al<sup>26</sup> increased excretion of both Tx-M and PGI-M was observed in smokers aged 24–46 years (i.e., in subjects with a longer history of smoking than those studied here). These authors interpreted their results as consistent with the hypothesis that the platelet defect in chronic smokers largely reflects smoking-induced vascular injury. That assumption is not supported by the present data, which demonstrate an isolated increase in the excretion of Tx-M in young smokers. Rather, our data indicate a primary action of smoking on platelets themselves or on their precursor cells. The concept of a primary action of cigarette smoking on platelet activity finds some support in the dose-response relation between daily cigarette consumption and Tx-M excretion. A similar relation was not present between cigarette consumption and PGI-M excretion; neither was the excretion of PGI-M related to the excretion of Tx-M.

The use of oral (wet) snuff is a popular substitute for cigarette smoking in several countries. In the present study, more than 25% of the subjects reported the use of snuff, alone or in combination with cigarette smoking. Those using only snuff had a median level of cotinine in the urine of 1,210 ng/ml, close to the level in the group using cigarettes only. Consequently, the exposure to nicotine in the group using snuff only was close to that in the group using cigarettes only. Despite these similarities in nicotine exposure, the snuff only group displayed no increase in Tx-M excretion compared with the non-tobacco users. The identity of the factors in cigarette smoke causing the various settings of cardiovascular disease is not known in detail, although nicotine is generally considered responsible for several of them.<sup>1,30</sup> The unaffected excretion of Tx-M in the snuff-only group seems to disfavor the hypothesis that nicotine can elicit platelet activation. Further studies are, however, required to elucidate whether the differences in pharmacodynamics of tobacco constituents administered via the lungs and via the gastrointestinal tract may explain the discrepancy in Tx-M excretion between smokers and snuff users.

Quitting smoking is known to decrease cardiovascular mortality in patients with manifest disease.<sup>2,3</sup> Substratification of the present non-tobacco users revealed that about 6% of them were previous tobacco users, with a substantial accumulated consumption of cigarettes and/or snuff (Table 1). The excretion of Tx-M in the subgroup of previous tobacco users did not differ from that in the entire group of non-tobacco users (Table 3). It consequently appears that the proposed effect of smoking on platelet activity does not persist after quitting smoking. This is in accordance with the observation that the abnormality in thromboxane biosynthesis declines significantly soon after cessation of smoking.<sup>26</sup> Such a rapid effect of quitting smoking on

platelet activity also disfavors the hypothesis<sup>26</sup> discussed above (i.e., that vascular injury in the smoking subject plays a significant role in activating the platelets); it appears unlikely that established vascular lesions would be diminished within a short time after cessation of smoking.

The present data on Tx-M excretion in smokers and nonsmokers bring up the question of whether switching from cigarette smoking to using snuff can be regarded as an adequate approach to reducing the possible risk for cardiovascular disease in subjects who are unable to quit smoking. The increased excretion of Tx-M in smokers highlights the role of platelets in smoking-induced cardiovascular disease, yet the current data do not allow any conclusions in this direction.

In summary, the present study demonstrates that young male cigarette smokers excrete more thromboxane metabolite than nonsmokers, suggesting a primary dysfunction of their platelets in the absence of vascular injury. The thromboxane metabolite excretion is related to the smoking intensity. We propose that platelet dysfunction should be tentatively considered as a possible etiologic link between cigarette smoking and cardiovascular disease.

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