The Effect of Acute Ingestion of a Large Dose of Alcohol on the Hemostatic System and Its Circadian Variation

Heikki Numminen, MD; Martti Syrjälä, MD, PhD; Günther Benthin, PhD; Markku Kaste, MD, PhD; Matti Hillbom, MD, PhD

Background and Purpose—Heavy binge drinking may trigger the onset of embolic stroke and acute myocardial infarction, but the underlying mechanisms are unclear. The effects of binge drinking on the hemostatic system and its circadian variation have not been investigated. We investigated the effects of an acute intake of a large dose of alcohol (1.5 g/kg).

Methods—Twelve healthy, nonsmoking men participated in sessions where they were served ethanol in fruit juice or served fruit juice alone and, lying in a supine position, were followed up for 12 to 24 hours. The treatments were randomized and separated from each other by a 1-week washout period. Blood and urine were collected for hemostatic measurements.

Results—The urinary excretion of the platelet thromboxane A₂ metabolite 2,3-dinor-thromboxane B₂ was significantly (P<0.05) greater during the night after an evening intake of alcohol than during the control night. A smaller increase was observed during the daytime after an intake of alcohol in the morning. The effects on the endothelial prostacyclin metabolite 2,3-dinor-6-ketoprostaglandin F₁α excretion were negligible. A 7-fold increase in plasminogen activator inhibitor 1 activity was observed after both morning (P<0.05) and evening (P<0.01) intakes of alcohol.

Conclusions—This is the first study to suggest that acute ingestion of a relatively large but tolerable dose of alcohol transiently enhances thromboxane-mediated platelet activation. The observations also demonstrate alcohol-induced changes in the normal circadian periodicity of the hemostatic system in subjects not accustomed to consumption of alcohol. (Stroke. 2000;31:1269-1273.)

Key Words: alcohol ■ circadian rhythm ■ hemostasis ■ prostaglandins ■ thrombolysis

Many studies indicate that regular light-to-moderate alcohol drinking protects against coronary heart disease.¹ Some studies also suggest that alcohol protects against ischemic stroke of atherothrombotic origin² and peripheral arterial disease.³ These effects have been ascribed to the antiatherogenic and antithrombotic actions of ethanol or some other components of alcoholic beverages. However, some investigations also suggest that heavy alcohol consumption promotes the development of carotid arterial atherosclerosis and that heavy binge drinking may precipitate fatal myocardial infarction and embolic stroke.⁴–⁶

Atherogenesis and thrombogenesis may both be influenced by drinking patterns. Acute heavy drinking may exert different effects compared with those of moderate regular drinking. The favorable effects of alcohol consumption on the lipid profile were recently observed to be independent of the drinking pattern in moderate-to-heavy drinkers.⁷ However, the effects on thrombogenesis were not reported and have yet to be investigated.

The activities of some parameters of the hemostatic system show significant circadian variation. The onsets of myocardial infarction and ischemic stroke also show circadian variation, with a peak at the time when people wake up and become active.⁸–¹² This phenomenon has been speculated to result from the circadian periodicity of the hemostatic system.⁸–¹² In fact, increased platelet aggregability⁸ and decreased fibrinolytic activity⁹ occur in the morning. The former has been observed in healthy volunteers after getting out of bed but not during steady bed rest, which suggests that the act of getting out of bed is responsible for the effect,⁹ whereas the morning decrease in fibrinolytic activity has been observed to be attenuated by keeping the volunteers in upright posture.¹⁷ The effects of acute alcohol ingestion on platelet aggregability and fibrinolytic activity in relation to the circadian periodicity of the hemostatic system have not yet been investigated.¹⁸

To find out whether acute ingestion of a large dose of alcohol influences the hemostatic system and its circadian pattern, 12 healthy human volunteers were randomly assigned to ingest a large dose of ethanol in fruit juice or fruit juice alone either in the morning or in the evening and were then followed up in a supine position for 12 hours. We measured plasminogen activator inhibitor 1 (PAI-1) activity, excretion in urine of platelet thromboxane and endothelial prostacyclin 2,3-dinor-metabolites,
serum thromboxane B₂, which reflects the capacity of platelets to form thromboxane in vitro and platelet aggregability ex vivo.

Subjects and Methods

Subjects and Study Design

Twelve healthy, nonsmoking men aged 20 to 39 years volunteered for the trial. They were all nonobese (body mass index 23.8±1.6 [mean±SD]), infrequent light drinkers (1 to 5 drinks per week), and they had abstained from alcoholic beverages and drugs for 1 week before the trial. None had arterial hypertension, heart disease, hematological disorders, hyperlipemia, diabetes, or any other serious disease in their history, and none were on regular medication. After a full explanation of the purpose, nature, and risks of the study, written informed consent was obtained from each. The study protocol was approved by the Ethical Committee of the Helsinki University Central Hospital. The investigation conforms with the principles outlined in the Declaration of Helsinki.

Each subject participated in 3 sessions, 2 ethanol sessions and 1 control session. The order of the sessions for each individual was randomized, and there was a 1-week washout period between the sessions. The 3 arrangements of treatment and control were equally distributed. The subjects were not allowed to eat for 6 hours before the start of the session, and they served as their own controls. The alcohol sessions started either at 7:00 PM or at 8:00 AM and lasted for 12 hours. If the session started in the morning, the subjects had to arrive in the hospital on the preceding evening and spend the night sleeping in hospital beds. The control session always started in the evening and lasted for 24 hours, including both the control night and the control day. During every session the subjects were awakened at 7 AM. During the daytime they lay in bed, reading books or watching TV. They were allowed to go to the toilet but not move otherwise.

All the sessions were started by taking baseline blood samples and by voiding the urinary bladder. From 7:30 until 10:00 PM or from 8:30 until 11:00 AM, the subjects received half-hourly drinks containing either fruit juice (not including grapefruit) or, during the ethanol session, an equal volume of a mixture of the same fruit juice and ethanol (2 mol/L solution). The time schedule of drinking and the total amount of ethanol (1.5 g/kg of body weight) were chosen to provoke a tolerable but moderate intoxication. During the control session, the subjects were given carbohydrate-rich snacks together with the fruit juice to balance their calorie intake, but no meals were served. Accordingly, during both the alcohol and the control sessions the total energy intake was kept constant (14.8 kcal/kg per 12 hours).

Blood and Urine Sampling

Blood samples were taken at 3-hour intervals, starting either at 7 PM or at 8 AM. After voiding the bladder, urine was collected for 12 hours. After preparation, the plasma and urine samples were frozen at −70°C for subsequent testing.

Platelet Aggregation

Platelet aggregation was measured by Born’s method. Blood was collected with minimal stasis via a plastic cannula into tubes containing 3.8% sodium citrate. To obtain platelet-rich plasma (PRP) and platelet-poor plasma (PPP), the blood samples were centrifuged at 330 g for 10 minutes and at 1500 g for 10 minutes. PPP was used to adjust the platelet count of PRP to 250×10⁹/L. Platelet aggregations were measured with a dual-channel aggregometer (Chrono-Log, Coulter Electronics Ltd), starting 45 minutes after the blood sampling. Arachidonic acid (AA; 1 mmol/L, Sigma Chemical Co) and collagen (5 μg/mL, Hormon Chemie GmbH) were used to stimulate platelets in PRP. The reaction was stopped by 1 mmol/L HCl after 5 minutes. The aggregation results were expressed as the maximum percentage change in light transmittance during the 5-minute period.

Determination of Plasminogen Activator Inhibitor Activity

Citrated plasma was used to analyze PAI-1 activity (units per milliliter). The blood samples were immediately put into crushed ice and centrifuged for 20 minutes at 4°C and at 1500 g. PAI-1 was measured as duplicate determinations with a colorimetric assay (Stachrom PAI, Diagnostica Stago) in the kinetic mode using an automatic coagulometer (Thrombolyzer, Behnk Elektronik GmbH & Co) equipped with a chromogenic channel.

Determination of Prostanoids and Other Laboratory Parameters

Urine was collected for the determination of 2,3-dinor-6-ketoprostaglandin F₁α (2,3-dinor-PGF₁α) and 2,3-dinor-thromboxane B₂ (2,3-dinor-TXB₂). The samples were analyzed by a stable isotope dilution assay with a mass spectrometer (Finnigan MAT 4500) coupled to a gas chromatograph (Varian Vista 6000), as previously described. The urinary excretion of prostanoids was related to the amount of creatinine excreted.

The capacity of platelets to form thromboxane was measured as serum thromboxane B₂ (TXB₂). For the measurement of serum TXB₂, 2 mL of blood was drawn into a glass tube and incubated at 37°C for 60 minutes to allow clotting. The amount of TXB₂ was measured from the serum by ELISA (Cayman Co).

Creatinine in urine and blood ethanol were determined by routine liquid chromatographic and gas-liquid chromatographic techniques, respectively. The ingestion of 1.5 g/kg ethanol in fruit juice within 2.5 hours resulted in peak blood concentrations of 32.3 ± 6.4 mmol/L (95% CI 28.7 to 33.4) in the evening and in the morning, respectively. These values correspond to blood levels that cause a moderately severe but tolerable acute alcoholic intoxication (1.5/1000) in a subject who has not developed central nervous system tolerance to alcohol. The time needed to eliminate the dose of alcohol from the body was more than 12 hours.

Statistical Methods

The results were expressed as mean±SEM, SD, or 95% CI. The time-dependent changes, including the testing of trend during each session, were compared by repeated-measures ANOVA, either as a 1-way analysis for a single session or as a 2-way analysis between the sessions. A paired t test was also used to test the differences in urinary excretion of prostanoids and the Wilcoxon signed rank test to test the differences in plasma PAI-1 values between the control and alcohol sessions. The associations between the different parameters were calculated by simple regression. All the analyses were made with Statview II or SuperAnova (Abacus Concepts).

Results

Alcohol drinking in the evening produced a significant increase in urinary excretion of 2,3-dinor-TXB₂ (Figure 1).
The mean difference was 13.0 pg/μmol creatinine (95% CI 6.2 to 19.8, P<0.01) between the values of the night (from 7 PM to 7 AM) following alcohol ingestion and those of the night following fruit juice ingestion (control night). Alcohol drinking during the daytime also caused an increase. In contrast, the urinary excretion of 2,3-dinor-PGF1a was similar during the day (from 8 AM to 8 PM) and the night, irrespective of whether alcohol was consumed or not. The urinary excretion of 2,3-dinor-TXB2 decreased during the control night compared with the control day (mean difference 3.3 pg/μmol creatinine, 95% CI 1.0 to 5.7, P<0.01). The urinary excretion of 2,3-dinor-TXB2 correlated positively with the serum TXB2 values (r²=0.18, slope 0.20, 95% CI 0.01 to 0.40, P<0.05). The correlation was not significantly influenced by alcohol.

Because alcohol ingestion could have influenced the urinary excretion of creatinine, we also calculated urinary excretion of the prostaglandin metabolites per total volume urine excreted to avoid bias due to a possible effect of alcohol on creatinine excretion (Table). A significant effect of alcohol drinking was confirmed. During the evening drinking session the mean difference in urinary excretion of TXB2 was 78.6 ng/12 h (95% CI 21.1 to 136.0, P<0.05). The urinary excretion of 2,3-dinor-TXB2 expressed in this way also correlated positively with the serum TXB2 values (r²=0.20, slope 0.22, 95% CI 0.1 to 0.35, P<0.01).

The platelet aggregability observed during the daytime (control session) showed significant variation over time when tested with both AA (P<0.05) and collagen (P<0.001). During the control session, platelet aggregability decreased from morning to afternoon, as was expected (Figure 2). It decreased from 8 AM until 2 PM, after which it increased again. A comparison between the values obtained during the control night and the control day also showed significant heterogeneity in collagen-induced aggregation (P<0.05) but not in AA-induced aggregation (P>0.05). The differences in the curves for the alcohol and control periods were not statistically significant, although drinking of alcohol tended to inhibit the daytime decrease in platelet aggregability.

PAI-1 activity was significantly increased by the intake of alcohol (Figure 3). An increase was observed irrespective of whether the alcohol was ingested in the morning (P<0.05) or in the evening (P<0.01). The alcohol-induced increase was transient and peaked at about 3 hours after the discontinuation of drinking. Thereafter, PAI-1 started to decrease, and it decreased rapidly until the end of the session. Unfortunately, we have no data to show the levels shortly after the end of each session. We did not follow the PAI-1 value after alcohol had been eliminated from the blood. PAI-1 showed circadian

---

**Urinary Excretion of Prostaglandin Metabolites, Urine Volume, and Creatinine Concentration in Each Session**

<table>
<thead>
<tr>
<th></th>
<th>Day</th>
<th>Night</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Ethanol</td>
</tr>
<tr>
<td>2,3-dinor TXB2, ng/12 h</td>
<td>140.9 (97.2–184.6)</td>
<td>153.0 (123.5–182.4)</td>
</tr>
<tr>
<td>2,3-dinor PGF1a, ng/12 h</td>
<td>161.5 (104.2–218.8)</td>
<td>123.6 (95.4–151.8)</td>
</tr>
<tr>
<td>Volume of urine, mL/12 h</td>
<td>1180 (890–1480)</td>
<td>2100 (1870–2330)</td>
</tr>
<tr>
<td>Creatinine concentration, mg/mL</td>
<td>0.84 (0.55–1.12)</td>
<td>0.37 (0.29–0.46)</td>
</tr>
</tbody>
</table>

Values are presented as mean, with 95% CIs in parentheses.

---

**Figure 2.** Circadian variation of mean platelet aggregation (mean±SEM) during the alcohol (△) and control (●) sessions. Aggregations were induced by AA (1 mmol/L) and collagen (5 μg/mL).

**Figure 3.** Effect of acute ingestion of a large dose of ethanol (1.5 g/kg in fruit juice) on PAI-1 activity. *P<0.05 compared with the respective baseline value (7 PM or 8 AM, by paired t test).
variation during the control session, as was expected. The PAI-1 levels were lowest in the evening and remained higher from 1 AM until 5 PM.

Discussion

The main finding of our experiment was the significant increase in urinary excretion of 2,3-dinor-TXB$_2$ in the absence of a simultaneous change in urinary excretion of 2,3-dinor-PGF$_{1\alpha}$ after acute ingestion of a large dose of alcohol. Another interesting finding was a significant alcohol-induced increase in plasma PAI-1 activity.

The increased urinary excretion of 2,3-dinor-TXB$_2$ reflects increased platelet activation in vivo. Platelets are activated in the arterial circulation, particularly at sites that are stenosed or ulcerated by atherosclerosis, but also at sites of bifurcation and sharp bends in the absence of atherosclerosis. The extent of activation could be influenced by hemodynamic factors, such as the velocity of blood flow and vasospasm.

The absence of a simultaneous change in urinary excretion of 2,3-dinor-PGF$_{1\alpha}$ suggests that factors capable of influencing endothelial prostacyclin were not significantly stimulated in subjects who were kept in a supine position. Unfortunately, we did not measure markers of endothelial activation, such as von Willebrand’s factor or thrombomodulin. However, other investigators have found these 2 markers to be uninfluenced or negatively associated with alcohol intake. The subjects were kept supine during the day and were asleep during the night. In fact, it has been shown that the endothelial formation of prostacyclin is low at rest but increases during physical activity.

Acute drinking of a large dose of alcohol produces tachycardia and increases blood flow. The effect is transient and dose dependent, and may lead to platelet activation because of increased shear. The increased urinary excretion of 2,3-dinor-TXB$_2$ in our experiment was possibly due to acute platelet activation caused by a change in turbulence and shear associated with an alcohol-induced rapid increase in blood flow.

Alcohol drinking increases urinary excretion of 2,3-dinor-TXB$_2$ dose dependently. In this study, a rather large dose of alcohol was ingested. We have recently shown that acute drinking of a moderate dose of alcohol does not increase urinary excretion of 2,3-dinor-TXB$_2$. Our findings seem to be reliable because the levels of prostanoids did not markedly deviate from those reported in physiological conditions.

The rapid increase in PAI-1 activity after an acute ingestion of a large dose of alcohol suggests decreased fibrinolytic activity. Unfortunately, we did not measure plasminogen activator (tPA) activity, which is the most important parameter reflecting fibrinolytic activity. However, Hendriks et al. have shown that an acute intake of even a moderate dose of beer, spirits, or wine, if ingested with an evening meal, causes a significant decrease of tPA activity. Our observations indicate that the alcohol-induced increase of PAI-1 activity occurs to the same extent during the night and the day, and that an acute intake of a relatively large dose of alcohol disturbs the normal circadian pattern of PAI-1 activity. Accordingly, it is likely that the decrease of fibrinolytic activity observed by Hendriks et al. also occurs irrespective of whether alcohol is ingested in the evening or in the morning.

A high shear and a sudden increase in shear could cause the activation of circulating platelets. This may not be a harmful effect if the vessels are nonatheromatous. However, a rapid increase in shear-induced platelet activation may aggravate the imbalance between thromboxane and prostacyclin at sites of severe atherosclerosis, because it has been shown that prostacyclin synthesis is deficient in atherosclerotic carotid plaques. Such an imbalance could rapidly develop during heavy alcoholic intoxication and lead to local thrombus formation if not simultaneously compensated for by enhanced prostacyclin formation. Because fairly heavy physical activity is needed to stimulate prostacyclin formation, bed rest and severe alcoholic intoxication could be a harmful combination for individuals with atherosclerotic arteries. In addition, sleep apneas are aggravated by alcohol and may precipitate atrial fibrillation. Acute intake of large doses of alcohol by those who snore and experience sleep apnea may therefore be detrimental. Drinking of alcohol may promote thrombogenesis, and atrial fibrillation may detach emboli from intracardiac thrombi. Palomäki and Kaste have already observed increased morning production of platelet thromboxane B$_2$ in subjects suffering from the obstructive sleep apnea syndrome.

The sharp increase of PAI-1 activity caused by acute alcohol intake could result in a transient decrease of fibrinolysis. PAI-1 is released into the vasculature by platelets and endothelial cells. It has recently been shown that the thrombolysis resistance of platelet-rich thrombi is largely due to the presence of PAI-1. Accordingly, the increase of PAI-1 activity in vivo by moderate-to-heavy acute alcohol intake suggests that alcohol ingestion could transiently enhance thrombolysis resistance and thereby retard lysis of the already-formed thrombi. However, the effect seems to be rapidly compensated for, and regular alcohol intake results in enhanced fibrinolytic activity. In vitro experiments indicate enhancement of fibrinolysis by alcohol, but the significance of such findings with regard to alcohol intake in humans is unclear.

The present study is the first to show effects of acute heavy alcohol ingestion on urinary excretion of prostanoids. The increase in the urinary excretion of 2,3-dinor-TXB$_2$ by alcohol intake, a parameter of platelet function in vivo, was modest compared with the effects observed in clinical cardiovascular disease causing signs and symptoms. None of our healthy volunteers showed signs or symptoms of any cardiovascular disease, nor did they report any adverse events after drinking the relatively large dose of alcohol. Heavy drinkers who suffer a stroke after binge drinking certainly have other factors that predispose to stroke, such as underlying atherosclerotic lesions and cardiac abnormalities. Our findings could be among those which contribute, but alcohol drinking may cause also other effects that are still more important and need to be identified.

Taken together, the present results indicate that acute ingestion of a relatively large but tolerable dose of alcohol results in a marked elevation of plasma PAI-1 activity and a modest increase of urinary excretion of 2,3-dinor-TXB$_2$. The effects seem to be independent of the circadian variation of the hemostatic system and may favor thrombogenesis. The circadian variation in hemostatic factors that maintains resis-
tance to thrombosis in healthy men during sleep seems to be more hazardous than heavy drinking during the daytime.

Acknowledgments

This study was supported by the Oulu Medical Research Foundation (Drs Numminen and Hillbom). We wish to thank Mrs Saija Eirola for technical assistance.

References


The Effect of Acute Ingestion of a Large Dose of Alcohol on the Hemostatic System and Its Circadian Variation
Heikki Numminen, Martti Syrjälä, Günther Benthin, Markku Kaste and Matti Hillbom

Stroke. 2000;31:1269-1273
doi: 10.1161/01.STR.31.6.1269

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/31/6/1269

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke 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 Stroke is online at:
http://stroke.ahajournals.org//subscriptions/