ACTIONS OF ALCOHOL AND ISCHAEMIC BRAIN INFARCTION

HEIKKI NUMMINEN
Department of Neurology, University of Oulu and Department of Neurology, University of Helsinki

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HEIKKI NUMMINEN

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To Liisa, Anna and Emma
Abstract

Alcohol drinking may exercise both beneficial and untoward effects on the haemostatic and fibrinolytic systems. It may also predispose individuals to arterial thrombosis and trigger embolism in the brain. The aim here is to examine these problems.

Methods used for evaluating platelet function were platelet aggregation and associated thromboxane B₂ release, shear-induced platelet aggregation, and measurement of urinary prostaglandins. Changes in fibrinolytic system were evaluated by measuring plasminogen activator inhibitor type 1. The combined effects of alcohol drinking, physical exercise, eating a meal and circadian rhythms in healthy volunteers were examined in three experimental studies. Case-control studies were used for assessing the mechanism and etiology of ischaemic brain infarction triggered during alcohol intoxication.

Alcohol drinking did not potentiate the effects of physical exercise on platelet function. Sleeping while under acute intoxication resulted in a significant activation of platelets, as shown by increased urinary excretion of a thromboxane metabolite. On the other hand, ingestion of a moderate dose of red wine seemed to attenuate platelet aggregation measured ex vivo, irrespective of whether the wine was consumed with a meal or alone. However, both red wine and a larger acute dose of alcohol in fruit juice inhibited fibrinolytic activity.

In a case-control study, platelet count and function were evaluated in 426 consecutive patients hospitalized on account of acute brain infarction. Compared with the hospital-based controls, a higher than normal platelet count was observed immediately after admission. Heavy drinkers showed both higher and lower than normal platelet counts more often than the other patients with brain infarction. The changes in platelet function among the heavy drinkers reflected their recent drinking habits.

Another case-control study indicated that recent heavy drinking of alcohol was an independent risk factor for cardiogenic embolism to the brain. Recent heavy drinking also seemed to predispose subjects to some other types of ischaemic brain infarction such as artery to artery embolism due to large-artery atherosclerosis and cryptogenic stroke, but these observations need to be confirmed in larger studies.

In conclusion, the results show some untoward effects of acute heavy drinking of alcohol, which could contribute to the onset of brain infarction either as triggering or as predisposing factors. On the other hand, drinking of a moderate dose of red wine did not have any clear untoward effect on healthy human volunteers.

Keywords: platelets, thromboxanes, fibrinolysis, cardioembolic stroke
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Lappeenranta, July 2000

Heikki Numminen
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>AA</td>
<td>arachidonic acid</td>
</tr>
<tr>
<td>βTG</td>
<td>beeta thromboglobulin</td>
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<tr>
<td>cAMP</td>
<td>cyclic adenosine monophosphate</td>
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<tr>
<td>CI</td>
<td>confidence interval</td>
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<tr>
<td>COX</td>
<td>cyclo-oxygenase</td>
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<tr>
<td>ELISA</td>
<td>enzyme-linked immunosorbent assay</td>
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<tr>
<td>ET-1</td>
<td>endothelin 1</td>
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<tr>
<td>FVIII</td>
<td>clotting factor VIII</td>
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<tr>
<td>GP</td>
<td>glycoprotein</td>
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<tr>
<td>G-protein</td>
<td>guanine-nucleotide binding regulatory protein</td>
</tr>
<tr>
<td>PAI</td>
<td>plasminogen activator inhibitor</td>
</tr>
<tr>
<td>PF4</td>
<td>platelet factor 4</td>
</tr>
<tr>
<td>PGF$_{1α}$</td>
<td>prostaglandin F 1 alfa</td>
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<tr>
<td>PGI$_2$</td>
<td>prostaglandin I$_2$ i.e. prostacyclin</td>
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<tr>
<td>PPP</td>
<td>platelet poor plasma</td>
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<tr>
<td>PRP</td>
<td>platelet rich plasma</td>
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<tr>
<td>SIPA</td>
<td>shear-induced platelet aggregation</td>
</tr>
<tr>
<td>tPA</td>
<td>tissue plasminogen activator</td>
</tr>
<tr>
<td>TxA$_2$</td>
<td>thromboxane A$_2$</td>
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<tr>
<td>TxB$_2$</td>
<td>thromboxane B$_2$</td>
</tr>
<tr>
<td>vWF</td>
<td>von Willebrand factor</td>
</tr>
<tr>
<td>vWF:Ag</td>
<td>von Willebrand factor antigen</td>
</tr>
</tbody>
</table>
List of original articles


2.7.2. *Ex vivo* effects on platelet function ........................................... 34
2.7.3. *Ex vivo* effects on fibrinolytic activity ........................................ 36
2.7.4. *Ex vivo* effects on endothelial cell function .................................... 37
2.7.5. Plausible mechanisms for the effects of alcohol on haemostasis ........... 37

3. Aims of the present research ............................................................... 40

4. Subjects and methods ........................................................................... 41
   4.1. Subjects and study designs .............................................................. 41
   4.2. Laboratory methods ........................................................................ 43
   4.3. Statistical analyses ......................................................................... 44

5. Results .................................................................................................... 45
   5.1. Study I .......................................................................................... 45
   5.2. Study II .......................................................................................... 47
   5.3. Study III ....................................................................................... 49
   5.4. Study IV ....................................................................................... 52
   5.5. Study V ....................................................................................... 53

6. Discussion ............................................................................................... 55
   6.1. Methodological considerations ........................................................ 55
   6.2. Physical exercise and alcohol ingestion ............................................ 56
   6.3. Circadian rhythms and ingestion of alcohol ....................................... 57
   6.4. Postprandial effects of ingestion of alcohol ......................................... 58
   6.5. Platelets and ischemic brain infarction ............................................. 59
   6.6. Pathophysiological mechanisms of brain infarction in heavy drinkers .. 60

7. Conclusions ............................................................................................. 62

8. References .............................................................................................. 64
1. Introduction

The effects of ethyl alcohol on the cardiovascular system have aroused interest among clinicians and investigators for over a century now, but no physiological, clinical or epidemiological evidence of any beneficial or untoward effects can yet be integrated into any definite general concepts. The disparity in relations of alcohol drinking to various cardiovascular conditions has become increasingly clear in the past years (Klatsky 1998). Although it now seems likely that alcohol drinking provides some protection against coronary heart disease, there is no consensus about the relation between alcohol drinking and various types of cerebrovascular disease. In addition, the biological actions of alcohol which account for the reduction in coronary heart disease are unclear. Only about a half of the effect is mediated through an increase in high density lipoprotein (Criqui 1998). Thus what drinking habits may protect subjects against various cerebrovascular diseases or promote such diseases, and what are the possible biological mechanisms behind these effects, are still open questions.

The present work concentrates on the effects of short-term alcohol drinking. The pattern of drinking is important, as heavy binge drinking may be hazardous to the cerebral circulation (Palomäki & Kaste 1993a). Only a few previous experimental studies have analyzed the effect of possible co-factors of alcohol drinking. The aim of the present work can be presented in the form of two questions: how is alcohol drinking associated with the different aetiologies of brain infarction, and how does acute heavy drinking influence the haemostatic and fibrinolytic systems in conjunction with other factors. Answers to the first question were sought in two clinical case-control studies, while the other problems were investigated experimentally in healthy, non-alcoholic volunteers, who consumed an acute dose of alcohol. Special interest was concentrated on the combined effects of alcohol drinking and physiological factors, such as physical exercise, eating a meal and circadian rhythms. These co-factors were chosen because they often associate with drinking and they may modify the effects of alcohol itself.

The review of the literature starts with a short examination of recent epidemiological studies and discusses possible biases in the conclusions made. In addition to haemostatic and fibrinolytic factors, other possible aetiological mediators are discussed briefly. After an overview of methodological principles, attention is focused on previous findings in short-term human alcohol experiments. Recent results of animal studies are included briefly in the last section of the review.
2. Review of the literature

2.1. Alcohol drinking as a risk factor for stroke

In epidemiological studies the association of alcohol drinking with stroke mortality or morbidity is most often estimated on the base of weekly alcohol consumption. In case-control studies the results more precisely take account of the recent drinking habits, whereas in prospective studies the results are based more on the estimated average alcohol consumption during the follow-up. The amount of alcohol consumed can be reported as drinks, units or grams and the drinking habits are usually classified into light, moderate and heavy drinking in addition to abstainers which can be as well teetotallers as ex-drinkers if not otherwise stated. One drink or unit corresponds 8-12 grams of pure ethanol, but the limits of drinking categories varies so that in the recent review the exact amounts are reported if possible.

Two reviews have summarized the epidemiological data. In the first one, Camargo identified 62 epidemiological studies that examined the relation between moderate alcohol consumption and the risk of stroke (Camargo 1989). A J-shaped association with ischaemic stroke was found in predominantly white populations, but moderate drinking seemed to increase the risk of both intracerebral and subarachnoid haemorrhages in diverse populations. The evidence was insufficient to conclude whether recent alcohol consumption affects the risk of either ischaemic or haemorrhagic stroke.

A more recent review by the same author summarized the findings presented in 26 key articles (Camargo 1996). Twelve retrospective case-control studies from the USA, Europe and Australia suggested either no relation between moderate drinking and stroke, or possibly a protective association with both ischaemic and haemorrhagic events. Prospective cohort studies showed that moderate alcohol consumption reduced the risk of ischaemic strokes but increased the risk of haemorrhagic strokes.

The following epidemiologic survey concentrates on studies published after above-mentioned reviews.
2.1.1. Alcohol drinking and stroke mortality

Some new prospective studies concerning stroke mortality and the consumption of alcohol in Western countries have been published. A 13-year follow-up among British male doctors showed a progressive increase in adjusted death rates from stroke with the amount of alcohol consumed, although there was a non-significant reduction among light drinkers (1-14 units of alcohol per week) compared with non-drinkers (Doll et al. 1994). The death rate among those consuming more than 42 units per week was more than double that for light drinkers.

A Swedish ten-year follow-up study showed a reduction in the risk of death from ischaemic brain infarction in women who consumed less than 6 g of alcohol daily as compared with life-long abstainers (Hansagi et al. 1995). No benefit due to alcohol drinking was found among men, but binge drinking to the point of intoxication increased the risk of dying from ischaemic stroke.

The Busselton study in Australia found a reduction in the risk of stroke mortality among women who consumed an average of 11 g alcohol daily (Knuiman & Vu 1996). Men had a non-significant reduction.

The Cancer Prevention Study II in the USA, reporting multivariate-adjusted death rates for various diseases among almost half a million people after a follow-up of nine years (Thun et al. 1997), showed a significant reduction in the risk of stroke death in men regardless of the amount of alcohol consumed daily and in women at lower levels of alcohol consumption (up to one drink per day) as compared with life-long abstainers. However, the results of men did not show any dose-response effect, i.e. the risk reduction was exactly the same irrespective of whether the amount consumed daily was less than one drink or more than three drinks.

The most recent mortality results come from Scotland, where almost 6000 employed men were followed for 21 years (Hart et al. 1999). There was a slight non-significant reduction in stroke mortality among those who consumed 1-7 units of alcohol per week, but at higher levels, the risk exceeded that of non-drinkers even after adjustment for other risk factors. The risk for those who consumed more than 34 units per week was double that among the non-drinkers. Raised blood pressure seemed to account for much of the risk, but not all.

In addition to the Swedish study, an effect of binge drinking on cardiovascular mortality was found in Moscow, where a significant increase took place in deaths caused by alcohol poisoning, accidents and cardiovascular diseases on Saturdays, Sundays and Mondays (Chenet et al. 1998). Also in Finland, a binge effect of beer drinking was found to be a significant risk factor for deaths both from external causes and from fatal myocardial infarcts (Kauhanen et al. 1997). There is also one report on the effects of different alcohol drinks on cardiovascular mortality (Grønbæk et al. 1995). This ten-year follow-up carried out in Copenhagen showed a decreased risk in wine drinkers regardless of the amount consumed (up to 3-5 drinks per day) and an excess risk in those who consumed more than two drinks of spirits daily.
2.1.2. Alcohol drinking and stroke morbidity

As early as 1978 a retrospective study among Finnish patients suggested that acute alcohol intake may precipitate the onset of ischaemic brain infarction in young adults (Hillbom & Kaste 1978). The findings stimulated much further work in this area, and a later Finnish case-control study reported that the risk of ischaemic brain infarction was lower in men consuming less than 150 g of alcohol a week regardless of their drinking pattern (Palomäki & Kaste 1993a). Irregular consumption of more than 150 g weekly increased the risk relative to non-drinkers, although non-significantly. Heavy drinkers, i.e. those consuming more than 300 g weekly, had a four-fold risk. Still later a new case-control study confirmed that recent heavy drinking of alcohol is liable to increase the risk of brain infarction in young adults in Finland (Hillbom et al. 1995). The adjusted risk increased in both sexes when alcohol consumption exceeded 40 g during the 24 hours preceding the onset of stroke. However, an Australian case-control study using community-based control subjects instead of hospitalized patients did not find recent heavy drinking (more than 59 g of alcohol within the preceding 24 hours) to be a risk factor for ischaemic brain infarction among subjects aged from 15 to 55 years (You et al. 1997). Instead, long-term heavy drinking (more than 59 g of alcohol daily) increased the risk ten-fold relative to total abstinence from alcohol even when adjusted for age, cigarette smoking, hypertension, cholesterol, heart disease, diabetes and physical exercise.

The Northern Manhattan Stroke Study, investigating a multiethnic population aged over 39 years, found a decreased risk of ischaemic brain infarction in moderate drinkers (more than one drink per year and less than 2 drinks per day) irrespective of sex, age or ethnicity (Sacco et al. 1999). In intermediate drinkers (3-4 drinks per day) the adjusted risk was still lower, but in heavy drinkers (more than 7 drinks per day) it was significantly higher than in abstainers.

The British Regional Heart Study recruited over 7000 men aged 40 to 59 years in the late 1970s and made another survey among them five years later (Wannamethee & Shaper 1996). After 8.5 years of follow-up, neither a significant benefit nor any harm could be assigned to the different alcohol drinking categories by comparison with occasional drinkers, i.e. those who had 1-2 drinks per month or drank on special occasions. After various adjustments, only those who had 1-2 drinks at weekends had a lower risk of stroke, and all the other groups, including ex-drinkers and life-long abstainers separately, had a higher risk, although all these ratios were non-significant. The conclusions were hampered by the small numbers of cases in the different categories and by the fact that no information was available on the type of stroke. The authors concluded that if the increased risk among life-long abstainers is real, the finding that occasional drinkers, regular non-heavy drinkers and ex-drinkers do not differ significantly in their risk of stroke, i.e. there is no dose-response effect, makes it unlikely that the reason for life-long abstainers having an apparently increased risk of stroke lies in their abstinence from alcohol.

In the recent Physicians’ Health Study ischaemic and haemorrhagic strokes could be separated from each other. It reported, after a 12-year follow-up, that light-to-moderate alcohol consumption (from one to four drinks per week) reduces both the overall risk of
stroke and the risk of ischaemic stroke in men as compared with participants who had less than one drink per week (Berger et al. 1999).

Some recent case-control studies agree with the opinion that light-to-moderate alcohol consumption results in a reduced risk of ischaemic stroke (Caicoya et al. 1999) and intracerebral haemorrhage (Thrift et al. 1999) as well as of total stroke (Caicoya et al. 1999). Heavy drinking, however, results in an increased risk of ischaemic (Caicoya et al. 1999, Leppälä et al. 1999) and haemorrhagic strokes (Thrift et al. 1999).

In addition to the total amount of alcohol consumed, interest in different types of alcoholic drinks and the subsequent risk of cardiovascular disease has expanded since the publication of the French paradox for coronary heart disease (Renaud & de Lorgeril 1992). Based on mortality statistics arising from the World Health Organization’s MONICA project and figures for dairy fat and wine consumption in certain countries, the authors concluded that in France the untoward effects of saturated fats are counteracted by the intake of wine. This conclusion has been disputed on various grounds, however, e.g. by pointing to differences in diagnosing coronary heart disease (Murray & Lopez 1997).

A review of moderate alcohol consumption and the risk of coronary heart disease concluded that a substantial portion of the benefit comes from the alcohol rather than the other components of each drink (Rimm et al. 1996). A similar conclusion was drawn by Klatsky et al. (1997). Finally, one report assess the risk of stroke and the consumption of alcohol per week according to the type of drink (Truelsen et al. 1998). After a 16-year follow-up of over 13000 persons in Copenhagen, no significant benefit could be shown in any drinking category, but those who drank heavily (over 41 units per week) or less than one unit per week had moderately increased risk of ischaemic stroke compared with those who drank 1-7 units per week. When analyzed according to the type of alcohol, a significantly lower risk was seen among monthly, weekly and daily wine drinkers, but after adjustments including lifestyle factors, education and physical activity the significant effect could be seen only in weekly consumers. The drinking of beer or spirits did not have any effects.

2.1.3. Confounding factors and conclusions

Andréasson (1998) has recently summarized reasons given for the differences in the results of epidemiological studies. Prominent among these are 1) a large set of potential confounders, 2) the measurement of alcohol consumption and patterns of consumption, 3) cause-of-death patterns in different age groups and among men and women, and 4) the characteristics of abstainers.

Potential confounding factors include at least smoking, diet, physical activity and social support. Control of the smoking variable when assessing the impact of alcohol can be misleading if there is a relationship between smoking and drinking, and a similar association may exist between hypertension and drinking. When controlling for hypertension, the effect of heavy alcohol drinking as a risk factor for stroke is usually attenuated, because alcohol drinking itself can be a partial reason for hypertension. Differences in diet are not usually taken into account when assessing the effect of alcohol,
but in the Seven Countries Study a significant inverse relation between the intake of saturated fatty acids and alcohol undermined the beneficial effect of alcohol on the mortality from coronary heart disease found in univariate analysis (Kromhaut et al. 1996).

A well-known beneficial factor for cardiovascular diseases is physical activity, which is taken into account in some studies. Social support is a more complex factor, however, and has similarly been shown to explain mortality in Finland (Kaplan et al. 1988). In Sweden the social support that exist among abstainers has found to be different from that affecting moderate drinkers (Leifman et al. 1995). U-shaped curves were produced when indicators of poor sociability were depicted in relation to the level of alcohol consumption.

The problem of using abstainers as a control population generates other difficulties as well as those arising from differences in social support. Abstainers are a heterogeneous group, and in some studies also include former heavy drinkers. It is difficult to generalize the magnitude and direction of the latter effect on risk calculations among abstainers. One study showed that former drinkers had a similar cardiovascular mortality risk to abstainers (Klatsky et al. 1990), while another found a similar risk of stroke among previous drinkers as among moderate drinkers (Rodgers et al. 1993). Abstainers can also include small groups of persons who refuse to drink alcohol for various reasons. The risk of cardiovascular disease in a study among Norwegian Seventh-Day Adventists, who abstain from alcohol for religious reasons, was lower than in the general population (Fønnebø 1992). Thus abstaining can sometimes be associated with other factors, the effects of which are difficult to predict.

For these reasons, some authors strongly argue against using abstainers as a control group when assessing the health effects of alcohol. Occasional drinkers should be used as the baseline instead (Shaper & Wannamethee 1998). When this is done and a dose-response effect of alcohol is lacking, as in the British Regional Heart Study and the Cancer Prevention Study II, one can ask whether alcohol drinking has any beneficial effects at all on stroke morbidity and mortality. The conclusion may still be, as stated by Camargo (1996), that the available data do not provide any evidence that moderate drinking has an adverse effect on the risk of stroke.

### 2.2. Pathophysiological mechanisms of brain infarction

The causes of brain infarctions triggered during alcoholic intoxication are unknown. Alcohol drinking may have both beneficial and untoward effects on cerebral circulation, and these different effects may depend both on the pattern and amount of drinking.

#### 2.2.1. Cardiac diseases and arrhythmias

Alcoholic cardiomyopathy is linked to prolonged heavy drinking, although there is considerable variation in patients’ susceptibility to the disease. It may be associated with an alcohol consumption of 80 g daily and a duration of continuous exposure in excess of ten years or more, or a cumulative ingestion of 250 kg (Richardson et al. 1998). The
histological features are common to dilated cardiomyopathies in general and are indistinguishable from those of the other types of dilated cardiomyopathy. The hypokinetic left atrium and ventricle predispose to brain embolism. The prevalence of alcoholic cardiomyopathy may be higher than was thought, since many cases are subclinical and asymptomatic. Almost one third of a series of Spanish chronic alcoholics had findings suggestive of cardiomyopathy (Urbano-Marquez et al. 1989).

There is no convincing evidence of alcohol-induced cardiac arrhythmias in non-alcoholic persons (Kupari & Koskinen 1998). Studies in alcoholic populations during withdrawal and abstinence, have also shown little or no differences in the frequency of cardiac arrhythmias between alcoholics who were free of heart disease and the general population (Abbasakoor et al. 1976, Buckingham et al. 1985). Recently no arrhythmogenic cardiac disease could be demonstrated in small number of subjects prone to alcohol-induced atrial fibrillation (Mäki et al. 1998), although an exaggerated sympathetic reaction during moderate alcohol intoxication was observed.

Hospital population of idiopathic new-onset atrial fibrillation cases was shown to include a higher number of persons who had consumed over 30 g of ethanol daily during the preceding week than a control population admitted to the same hospital for other reasons (Koskinen et al. 1987), whereas patients admitted for supraventricular tachyarrhythmias other than atrial fibrillation did not differ from the control population in their alcohol consumption. In a prospective study of people participating in a health screening examination, the incidence of supraventricular tachyarrhythmias was significantly higher among persons with an intake of over five drinks per day than in people consuming less than one drink per day (Cohen et al. 1988).

Heavy drinking is thus associated with supraventricular tachyarrhythmias, mainly atrial fibrillation, which can predispose subjects to cardiac embolism. Heavy drinkers do not necessarily have any overt heart disease, however, although subclinical structural changes in the heart muscle are common (Kupari & Koskinen 1998).

### 2.2.2. Hypertension

Regular moderate-to-heavy alcohol intake (30-60 g/day) can be viewed as the second most important risk factor for hypertension, close behind the well-established risk factor of being overweight (Keil et al. 1998). Alcohol intake accounts for at least 10% of hypertension in the Western male population (Friedman et al. 1982, MacMahon et al. 1984, Keil et al. 1989). A crude rule can be derived from many observational and intervention studies: that above a daily alcohol intake of 30 g an increment of 10 g alcohol per day increases the average systolic blood pressure by 1-2 mmHg and diastolic blood pressure by 1 mmHg (Keil et al. 1998).

The effect of binge drinking on blood pressure is less clear, however. Experimental studies have often reported increased variability in blood pressure after short-term alcohol consumption (Howes et al. 1990, O’Callaghan et al. 1995, Maiorano et al. 1995). In a very recent study, ambulatory blood pressures were compared between social drinkers on a sober Saturday night, and on another Saturday night when they consumed alcohol (2.2 g/kg of their body weight) over a period of six hours (Seppä & Sillanaukee 1999). No
significant changes in blood pressure were evident when the drinking periods as a whole were compared, but both diastolic and systolic blood pressures were 5 mmHg higher and the pulse 18 beats per min faster during the state of intoxication than on the control night. By contrast, immediately after drinking, when blood alcohol levels were decreasing, ambulatory blood pressures were lower than during the control night, but the pulse was still higher. During the hangover no differences between blood pressures were found, but the pulse rate was still higher compared to the control weekend.

Regular moderate-to-heavy drinking increases thus blood pressure, but binge drinking results in both high and low blood pressure recordings in conjunction with high pulse rates. This may lead to increased shear stress in an artery through increasing flow rates (Kroll et al. 1996) and enhance the formation of a platelet thrombus, especially in already stenosed arteries, as has been shown both experimentally and in vivo (Ruggeri 1995, Holme et al. 1997).

2.2.3. Lipids and atherosclerosis

Evidence from both observational and experimental studies suggests that the beneficial effects of alcohol on plasma lipoproteins reduce the risk of coronary heart disease. Approximately 50% of this risk reduction can be explained by changes in total or high-density lipoprotein (HDL) cholesterol (Criqui et al. 1987, Langer et al. 1992, Suh et al. 1992, Gaziano et al. 1993).

As for the risk of brain infarction, no data are available on this. The effect of alcohol on the risk factors for brain infarction has been assessed in short-term experimental studies by reporting changes in plasma lipoproteins and by measuring carotid atherosclerosis by ultrasonography.

Recent prospective results on carotid atherosclerosis from the Bruneck Study after five years of follow-up (Kiechl et al. 1998) show significantly reduced progression of atherosclerosis in regular light drinkers (both men and women) after adjustment for the other risk factors relative to abstainers or heavy drinkers. The protection offered by an alcohol consumption of less than 50 g daily appeared to be due to inhibition of the injurious action of high levels of low-density lipoprotein cholesterol. Heavy drinkers, drinking 100 g or more alcohol daily, had an excess risk of atherosclerosis.

Two earlier case-control studies have shown similar results. Alcohol consumption was inversely associated with the severity of internal carotid artery stenosis as assessed by ultrasonography (Bogousslavsky et al. 1990) and regular light drinking was shown to reduce the risk of cervical atherosclerosis as detected by aortic arch angiograms (Palomäki et al. 1993).

Experimental trials over periods lasting from one weekend (Taskinen et al. 1985) to five weeks (Pikaar et al. 1987) and involving alcohol consumptions varying from 30 g per day (Välimäki et al. 1988) to greater amounts (Taskinen et al. 1985) of either wine (Pikaar et al. 1987, Seigneur et al. 1990), beer (Rakic et al. 1998) or diluted pure ethanol (Taskinen et al. 1985, Välimäki et al. 1988) have shown rather consistent effects on serum cholesterol. The concentration of total cholesterol has either increased (Seigneur et al. 1990) or remained unchanged after drinking wine (Pikaar et al. 1987) and decreased
after switching to a lower daily alcohol consumption from a higher one (Rakic et al. 1998). The HDL cholesterol concentration has followed a similar increasing (Pikaar et al. 1987, Seigneur et al. 1990) or decreasing (Rakic et al. 1998) tendency in these experiments, while the changes in the subfractions of HDL cholesterol seem to be both time and dose-dependent (Välimäki et al. 1988). Concentrations of low density lipoprotein cholesterol have shown minor changes (Taskinen et al. 1985, Seigneur et al. 1990, Rakic et al. 1998).

To sum up, light-to-moderate regular consumption of alcohol but not heavy drinking seems to have a beneficial effect on both the plasma lipoproteins and the course of carotid artery atherosclerosis regardless both of the type of alcoholic drink consumed and the drinking pattern (Rakic et al. 1998).

2.2.4. Sleep apnoea, vasospasm and trauma

There are many other alcohol-related potential pathophysiological mechanisms which may be either beneficial or unfavourable but lack yet enough evidence for firm conclusions to be drawn.

In addition to effects on plasma lipoproteins, alcohol, and particularly red wine, may exert further valuable effects on haemostatic parameters. Red wine may exert beneficial effects on plasma lipid peroxidation mediated by its antioxidant phenol content, which white wine does not have (Fuhrman et al. 1995). Increases in the proportions of polyunsaturated fatty acids in platelet phospholipids after the consumption of red wine, but not diluted alcohol, suggest an antioxidant effect of constituents of red wine other than ethanol (Pellegrini et al. 1996).

A considerable proportion of brain infarctions occur during sleep or during the first hours after awakening (Palomäki et al. 1989, Haapaniemi et al. 1992). Since snoring has been shown to be an independent risk factor for ischaemic stroke (Partinen & Palomäki 1985, Palomäki et al. 1989, Smirne et al. 1993) and alcohol ingestion may cause oxygen desaturation during sleep (Block et al. 1986), which associates with enhanced platelet activation (Palomäki & Kaste 1993b), a tempting hypothesis would be that alcohol-related ischaemic strokes are precipitated by alcohol-induced breathing disturbances during sleep, causing platelet activation. There are many other potential mediators, however, such as cardiac arrhythmias and decreased cerebral blood flow (Palomäki et al. 1992).

Alcohol-induced spasms of the cerebral arteries were observed in experimental studies on brain ischaemia some years ago (Altura et al. 1983). Alcohol consumption is known to be the most notorious cause of depletion of body magnesium (Altura & Altura 1994), and magnesium deficient rats are prone to hypoxic-lethal stroke insults induced by alcohol administration (Altura et al. 1998). Ischaemic and haemorrhagic stroke patients have demonstrated a significant elevation in the ratio of ionized calcium to magnesium in serum, which has been considered a sign of increased vascular tone and cerebral vasospasm (Altura et al. 1997). The experimental observations of Altura and coworkers support their hypothesis that alcohol induces stroke by causing either spasms or rupture of
the cerebral blood vessels because of a rise in the concentrations of intracellular calcium-ions.

Finally, alcohol intoxication predisposes subjects to head trauma, which may cause tears in the vessel intima and dissections. The relationship between traumatic dissection and alcohol consumption is difficult to establish, because the onset of trauma may have occurred long before the onset of stroke. There is one case report that suggests such a mechanism, however (Hess et al. 1990).

2.3. Haemostatic and fibrinolytic mechanisms

2.3.1. Platelet activation

Arterial thrombosis characteristically results from a primary vascular abnormality. A thrombus usually develops in an area of disturbed blood flow, most commonly caused by an occlusive atherosclerotic plaque, where intimal injury leads to the adhesion and subsequent recruitment of circulating platelets.

After loosened contacts between adjacent endothelial cells blood flow at high shear rates can mechanically tear away the endothelium, exposing adhesive molecules in the subendothelial matrix. The highest wall shear rates in normal circulation occur in small arterioles (Slack et al. 1993). In such areas of the vasculature the haemostatic role of platelets is of paramount importance.

Platelet adhesion to the endothelium is mediated by von Willebrand Factor (vWF) A1 domain, which binds to the glycoprotein (GP) Ib receptor of the platelet membrane (Kroll et al. 1991, Lefkovits et al. 1995, Savage et al. 1996). Platelets tethered to the vessel wall in this manner move constantly in the direction of flow and become activated. This activation leads to irreversible adhesion between the platelet receptor GP IIb-IIIa and the C1 domain of vWF (Savage et al. 1996, Ruggeri 1997). Subendothelial components such as collagen (van Zanten et al. 1994) and soluble platelet agonists such as adenosine diphosphate (ADP) (Ikeda et al. 1991) and epinephrine (Goto et al. 1992, Mustonen & Lassila 1996) can greatly enhance this response by contributing synergistically to GP IIb-IIIa activation. Thrombus formation follows when platelets become aggregated. Fibrinogen and vWF are the principal macromolecules linking the platelets together, doing so by binding to activated GP IIb-IIIa receptors on adjacent platelets (Lefkovits et al. 1995). vWF is the main mediator of platelet aggregation induced in the cone-and-plate viscometer, where high shear rates are achieved (Ikeda et al. 1991).

By contrast, platelets can adhere to immobilized fibrinogen at low shear rates through the GP IIb-IIIa receptor independent of their activation (Savage et al. 1996). In platelet aggregometers, where shear rates in platelet-rich plasma (PRP) are usually low, platelet aggregation is mainly mediated by the binding of soluble fibrinogen to GP IIb-IIIa receptors (Ruggeri 1993). This requires, however, that the platelets should first be activated by adding an aggregating agent to PRP (Ruggeri 1993).

Many aggregating agents, e.g. thrombin and ADP, act largely via guanine-nucleotide binding regulatory protein (G-protein) -coupled receptors to stimulate phospholipase C, with a resultant stimulation of protein kinase C by diacylglycerol and mobilization of
intracellular calcium-ions by inositol triphosphate (Siess 1991). Such aggregating agents also cause an influx of extracellular calcium-ions (Hourani & Hall 1994). Shear stress-induced platelet protein kinase C activation differs from agonist-induced activation, since it occurs independently of any measurable change in diacylglycerol or hydrolysis of phosphatidylinositol diphosphate (Kroll et al. 1996).

Cytoplasmic calcium elevation and protein kinase C activation are responsible for activating phospholipase A, which catalyses the cleavage of arachidonic acid (AA) from membrane phospholipids. AA is converted to prostaglandin G2 and this in turn is further converted to prostaglandin H2 by cyclo-oxygenase (COX). Thromboxane A2 synthase then converts the prostaglandin H2 to thromboxane A2 (TxA2), which is the active end product of this cascade (Wu 1996). TxA2 is an aggregator of platelets and an extremely potent constrictor of bovine cerebral vessels (Ellis et al. 1977) and human pial arteries (Uski et al. 1984). Since TxA2 can diffuse across the platelet plasma membrane, it can serve as a second messenger between platelets as well as within platelets, interacting with receptors on the cell surface (Brass et al. 1993). TxA2 has a very short half-life in plasma and is converted by non-enzymatic hydrolysis to the inactive but more stable thromboxane B2 (TxB2), which is further converted to 2,3-dinor-TxB2 and 11-dehydro-TxB2 and excreted into the urine (Lawson et al. 1985, Catella et al. 1986).

2.3.2. Platelet aggregation and bleeding time

The standard method to investigate platelet aggregation uses an optical aggregometer and is based on the turbidometric method described by Born in 1962 (Born 1962). Thereafter other methods have partially superseded Born’s method, which is based on platelets’ sensitivity to agonist. These methods are platelet aggregation in whole blood, platelet perfusion studies and flow cytometry for ascertaining the activity status of platelets.

When platelets aggregate in PRP, the turbidity of the PRP falls and light transmission through it increases proportionally. Two phases of aggregation can be distinguished, a primary and a secondary wave, although these responses often become incorporated into each other. Primary aggregation is reversible, since the platelets have not yet released their granule content and can separate from each other, whereas secondary aggregation is irreversible. The results are usually expressed as either the speed of early aggregation, the maximal change in light transmission or the concentration of agonist needed for irreversible aggregation.

A reliable determination of platelet aggregation by the method of Born nevertheless suffers from the instability of PRP (O’Brien 1964, Harrison et al. 1967). Once the platelet concentration in PRP and the test time after blood sampling have been standardized, the measurements of platelet aggregation and the associated TxB2 release can be fairly well reproduced (Siess et al. 1981). Simultaneous measurements of both parameters is feasible for platelet studies (Siess et al. 1981), even though the measurement of TxB2-formation in PRP does not reflect the actual production rate in vivo.

Shear-induced platelet aggregation (SIPA) is measured in a cone and plate viscometer in which shear stress can be varied. The viscometer generates mechanical forces in vitro similar to haemodynamic forces in vivo (Kroll et al. 1996). In a tubular blood vessel,
maximal blood velocity and minimal shear rate occur at the centre of the blood flow stream, and minimal velocity and maximal shear rate at the vessel wall. The difference in velocity between the laminae of the flowing blood generates shearing forces. Shear stress, expressed in dynes/cm², is the product of the shear rate and blood viscosity. A laser light is used to measure SIPA. Changes in the intensity of the transmitted laser light are proportional to the occurrence of platelet-to-platelet interactions leading to the formation of aggregates in PRP (Kroll et al. 1996).

The use of ADP as an aggregating agent in PRP is controversial, because aggregation induced by ADP at physiological levels of extracellular calcium-ions is reversible, and only if the extracellular calcium-ions are reduced by an anticoagulant such as citrate does ADP cause a secondary, irreversible phase of aggregation and TxB₂-release (Mustard et al. 1975, Macfarlane et al. 1975). On the other hand, ADP is an important contributor to platelet aggregation in whole blood (Saniabadi et al. 1984, Carter & Heptinstall 1985), and both ADP and collagen-induced aggregation correlate inversely with brachial artery shear stress measured in vivo (Mazeaud et al. 1994). In addition, SIPA measured at physiological calcium concentrations is dependent on endogenous ADP (Moake et al. 1981, Oda et al. 1995).

The length of bleeding time is a measure of primary, i.e. platelet-dependent haemostasis in vivo. Acquired conditions, such as aspirin treatment, prolong the bleeding time and reduce the amount of TxB₂ released into bleeding time blood (Thorngren et al. 1983, Kyrle et al. 1987, Gerrard et al. 1989). A significant negative correlation has been shown between bleeding time and platelet aggregation (Gerrard et al. 1989). The differences between the two standard bleeding time techniques, the Ivy method and the Simplate II method, have proved to be minimal as far as sensitivity and specificity are concerned (Srámek et al. 1992).

2.3.3. Platelet release products

Beta thromboglobulin (βTG) and platelet factor 4 (PF4) are specific platelet proteins released from alpha granules of activated platelets (Kaplan & Owen 1981). PF4 binds to the endothelial surface heparin-like molecules, and hence has a short half-life, whereas βTG is not bound by the vascular endothelium and is cleared from the circulating blood via renal excretion (Wu 1996). The interpretation of PF4 or βTG measurements is often confronted by methodological problems, however, due to in vitro activation of platelets during blood sampling and plasma processing (Wu 1996).

The same limitations concern the measuring of plasma TxB₂, as the amount of TxB₂ in plasma samples exceeds the estimated physiological production rate several times over (Patrono et al. 1986, Catella et al. 1986b). Measurement of the stable metabolites of TxB₂ avoids these problems. TxB₂ has two major pathways of metabolism in humans: β-oxidation, resulting in the formation of 2,3-dinor-TxB₂, and dehydrogenation of the hemiacetal alcohol group at C-11, resulting in the formation of a series of metabolites with a Δ-lactone ring structure (Roberts et al. 1981). Taking advantage of the linear relation between the rate of exogenous TxB₂ infusion and the urinary excretion of both 2,3-dinor-TxB₂ and 11-dehydro-TxB₂, the rate of entry of endogenous TxB₂ into the
human circulation can be estimated to be 0.11-0.12 ng/kg/min (Patrono et al. 1986, Ciabattoni et al. 1989). Roughly 80% of what is excreted in urine appears to derive from the platelets under physiological circumstances (Catella & FitzGerald 1987). The plasma half-lives of 2,3-dinor-TxB₂ and 11-dehydro-TxB₂ are 15 and 45 min respectively (Lawson et al. 1986).

In addition to measuring TxB₂ formation in PRP, the capacity of the blood to form TxB₂ can be studied by allowing whole blood to clot at 37° C. In one hour, 1 ml of blood can generate approximately 300 ng of TxB₂ (Patrono et al. 1986). The amount of TxB₂ generated in aggregated PRP varies greatly according to the agonist used and its concentration (Siess et al. 1981).

2.3.4. Fibrinolytic activity

The blood fibrinolytic system contains a proenzyme, plasminogen, that can be converted to the active enzyme plasmin, which degrades fibrin into soluble products. Two physiological plasminogen activators (PA) have been identified: tissue-type PA (tPA) and urokinase-type PA. tPA-mediated plasminogen activation is mainly involved in the dissolution of fibrin in the circulation. tPA is produced by the vascular endothelial cells, and various stimuli can bring about a rapid increase in its level in the blood. Inhibition of the fibrinolytic system may occur either at the level of PA, through the specific plasminogen activator inhibitor type 1 (PAI-1), or at the level of plasmin, mainly by α₂-antiplasmin (Lijnen & Collen 1995).

PAI-1 is produced in endothelial cells and plays an important role in the regulatory mechanisms of the fibrinolytic system (Wiman 1995). Regulation of PAI-1 production occurs mainly at the transcriptional level of protein synthesis (Wiman 1995). PAI-1 mRNA has been demonstrated in a large variety of tissues (Loskutoff 1991) and PAI-1 is rapidly secreted after its synthesis (Lijnen & Collen 1995). One exception concerns the platelets which store PAI-1 in their a-granules (Lijnen & Collen 1995). The functional half-life of PAI-1 is around 4 hours (Wiman 1995).

2.3.5. Endothelial cell function

Endothelial cells possess antithrombotic properties under normal conditions. They form a metabolically active interface between blood and tissue, and their luminal surface is antithrombotic and anticoagulant, owing to a variety of surface molecules. The pattern of endothelial cell responses depends on the initiating stimulus. They can produce several haemostatically important factors, e.g. prostacyclin, nitric oxide, vWF, thrombomodulin and endothelin (Pearson 1993).

The synthesis of prostacyclin (PGI₂), a potent vasodilator and inhibitor of platelet aggregation (Moncada & Vane 1979), is localized in the endothelial cells (Weksler et al. 1977). Systemic levels of circulating PGI₂ are too low to affect platelet function (Blair et al. 1982). Its synthesis and secretion are rapidly and transiently stimulated locally by a variety of agonists, including thrombin and ADP, which is secreted from activated
platelets (Pearson 1993). This results in the liberation of AA from membrane phospholipids via the activation of phosphoinositidase C, the release of inositol triphosphate and the liberation of calcium from internal stores, which activates the rate-limiting step, phospholipase A₂, to yield AA (Pearson 1993). AA in endothelial cells is converted to PGI₂ by COX and PGI₂ synthase. Another substrate for PGI₂ production can be the platelet-derived cyclic endoperoxide intermediate prostaglandin H₂, which is synthesized in platelets during their activation and transferred to the adjacent endothelial cells (Chesterman et al. 1986, Fitzgerald et al. 1983). PGI₂ is unstable, with a half-life of 2-3 minutes (Moncada 1979), and breaks down into several urinary metabolites, of which one of the most abundant is 2,3-dinor-6-keto-PGF₁α (Brash et al. 1983).

vWF is a glycoprotein which, after its synthesis in endothelial cells or megakaryocytes, forms multimers that are stored in specific organelles of endothelial cells known as Weibel-Palade bodies, or in α-granules of mature platelets (Ruggeri 1997). From the endothelium, low-molecular-weight forms are secreted into the blood, where vWF acts as the carrier for Clotting Factor VIII (FVIII) and as a cofactor mediating platelet adhesion to the endothelium or to subendothelial matrix components such as collagen (Pearson 1993). Multimerized forms, which are efficient in platelet adhesion, are secreted both from endothelial cells in response to a limited range of agonists, i.e. thrombin, adrenaline or fibrin, and from platelets during their activation (Pearson 1993, Ruggeri 1997).

Endothelins are produced in a variety of tissues, where they act as modulators of vasomotor tone, cell proliferation and hormone production. Endothelin-1 (ET-1) is the only one that is also produced in endothelial cells (Levin 1995). After stimulation, e.g. by hypoxia or shear stress, ET-1 is synthesized and secreted by the endothelial cells within minutes (Levin 1995), mostly towards the vascular smooth-muscle side of the cells (Yoshimoto et al. 1991). The plasma half-life is a few minutes and it is cleared mostly by the lungs during the first passage (de Nucci et al. 1988). ET-1 acts both through endothelin-A receptors, expressed on vascular smooth-muscle cells, and through endothelin-B receptors, expressed on endothelial cells (Sakurai et al. 1990, Levin 1995). Both receptors mediate the vasoconstrictor action of ET-1 by leading to increases in intracellular calcium concentration (Simonson & Dunn 1990), although in some endothelial cells the B-type receptors are linked to inhibitory G-proteins (Aramori & Nakanishi 1992). The vasodilators nitric oxide and PGI₂ both inhibit the production of ET-1 and the vasoconstriction induced by it (Levin 1995).

2.4. Physical exercise

2.4.1. Effect on platelet function

Strenuous exercise has consistently been reported to result in an increase in the platelet count (Warlow & Ogston 1974, Mehta & Mehta 1982, Ohri et al. 1983, Piret et al. 1990, Winther et al. 1992, Chicharro et al. 1994). This increase has been attributed to the release of platelets from the vascular beds of the spleen and the bone marrow, and also from an intravascular pool found in the pulmonary circulation and in the lungs (El-Sayed
It occurs at least partly through the action of adrenaline on vascular α2 receptors (Anfossi & Trovati 1996). The increase seems to be short-lived, i.e. from 30 to 60 min, and it depends on the degree of the exercise (Chicharro et al. 1994, Piret et al. 1990).


ADP-induced maximal platelet aggregation has usually remained unchanged in healthy volunteers immediately after submaximal exercise (Siess et al. 1982, Taniguchi et al. 1984, Lassila & Laustiola 1988, Winther et al. 1992, Chicharro et al. 1994, Wang et al. 1994), whereas maximal exercise has resulted in a transient increase of aggregation lasting for not more than 30 min (Chicharro et al. 1994, Wang et al. 1994). Three of the studies (Siess et al. 1982, Taniguchi et al. 1984, Winther et al. 1992) report high simultaneous plasma catecholamine concentrations, and one recent work reports both an enhanced SIPA and increased plasma catecholamines after a submaximal treadmill exercise, although no correlation was observed between SIPA and noradrenaline or adrenaline levels (Tokuue et al. 1996).

As already mentioned, studies on platelet release products from plasma are hampered by the in vitro activation of platelets during blood sampling and plasma processing. Both unchanged (Mehta et al. 1983, Taniguchi et al. 1984, Todd et al. 1994) and increased (Todd et al. 1992) levels of plasma TxB2 have been reported after submaximal exercise. A repeated sequence of exercise until exhaustion produced an increase in plasma TxB2 but not in serum TxB2 (Laustiola et al. 1984), nor were there any changes in serum TxB2 after submaximal exercise (Lassila & Laustiola 1988) nor in aggregation-associated TxB2 formation (Siess et al. 1982). The results concerning βTG have also been variable (Mehta & Mehta 1982, Mant et al. 1984, Piret et al. 1990, Todd et al. 1994). Wang et al. measured both βTG and PF-4 at different levels of exercise (Wang et al. 1994) and found both indices of platelet activation to be increased after maximal exercise but not after submaximal exercise. Their ratio did not change, however, suggesting that the increase was artificial and that platelet secretion in vivo was not activated.

Few studies have looked at the urinary excretion of thromboxane metabolites. Neither submaximal (Wennmalm & FitzGerald 1988) nor maximal exercise, i.e. a marathon run (Ronni-Sivula et al. 1993), increased the excretion of 2,3-dinor-TxB2.

In conclusion, strenuous exercise yields a short-lived increase in platelet counts, but no constant changes in platelet sensitiveness to agonist are observed. Platelet release products have neither shown any signs of platelet activation.
2.4.2. Effects on blood coagulation and fibrinolytic activity

Blood is hypercoagulable immediately after strenuous exercise. This has been attributed to an increase in FVIII coagulant activity (El-Sayed 1996).

Overall fibrinolytic activity, measured as a shortened euglobulin lysis time, is increased after exercise (Hawkey et al. 1975, Knudsen et al. 1982, Ferguson et al. 1987), and determination of tPA and PAI-1 can be expected to give a more exact view of the changes which occur in the individual components of this system. A variety of exercise protocols have been shown to increase tPA significantly (Hansen et al. 1990, Winther et al. 1992, Handa et al. 1992, Molz et al. 1993, Szymanski & Pate 1994, Rankinen et al. 1995, El-Sayed et al. 1995, Mustonen et al. 1998), its increase evidently being dependent on exercise intensity (Handa et al. 1992, Molz et al. 1993, Szymanski & Pate 1994, Rankinen et al. 1995). A cycle ergometer test performed at a maximal or anaerobic threshold level produced a greater response in tPA than did a test at an aerobic threshold level (Rankinen et al. 1995). On the other hand, the mere assuming of an upright position in the morning after sleep has been shown to elevate tPA significantly (Winther et al. 1992). Increased tPA appears to be accompanied by a reduction in PAI-1 activity (Szymanski et al. 1994, Rankinen et al. 1995, van den Burg et al. 1995, El-Sayed et al. 1995), although no change was reported by Chandler et al. (1992). The decrease in PAI-1 activity seems to correlate positively with the intensity of exercise (Rankinen et al. 1995). Simultaneous measurements of coagulation and fibrinolytic variables during submaximal exercise have shown a moderate increase in coagulant activity, which is balanced by an enhanced fibrinolytic potential (van der Burg et al. 1995). During the recovery period (up to 25 min), however, the balance shifts towards hypercoagulation due to a persistent increase in FVIII (van der Burg et al. 1995).

Although exercise-induced hyperfibrinolysis seems to be a constant finding, the mechanisms responsible for this are not entirely understood (El-Sayed 1996). Approximately a half of the increase in tPA can be explained by an exercise-induced rise in adrenaline level, based on the finding that an infusion of adrenaline resulted in an increase in tPA level, although this was smaller than during an exercise test (Chandler et al. 1992).

2.4.3. Effect on endothelial cell function

By contrast to the lack of increase in urinary 2,3-dinor-TxB2 excretion, levels of urinary 2,3-dinor-6-keto-PGF1α excretion have been found to increase after both submaximal (Vesterqvist et al. 1984, Wennmalm & FitzGerald 1988) and maximal exercise (Piret et al. 1990, Ronni-Sivula et al. 1993). Both unchanged (Taniguchi et al. 1984, Todd et al. 1992) and increased plasma levels of 6-keto-PGF1α (Mehta et al. 1983, Laustiola et al. 1984) have been reported.

An increase in vWF in conjunction with a rise in the FVIII level has been observed after various exercise protocols (Brown et al. 1979, Andrew et al. 1986, Hansen et al. 1990, van den Burg et al. 1995). This increase is mediated through β2-adrenergic stimulation, which also leads to increases in FVIII (Small et al. 1984).
An increase in ET-1 has recently been demonstrated after strenuous but not after moderate exercise (Röcker et al. 1996). Earlier studies had suggested similar findings (Appenzeller & Wood 1992, Maeda et al. 1994).

2.5. Circadian variability

2.5.1. Effect on platelet function

Several investigations have demonstrated a circadian variation of platelet aggregability. The threshold concentration of ADP needed for biphasic aggregation has been shown to be lower in the morning (Tofler et al. 1987, McCall et al. 1991, Jafri et al. 1992). Towards the afternoon, platelet sensitiveness has decreased (Jafri et al. 1992, Knöfler et al. 1995), but not in all studies (Jovicic & Mandic 1991, Malyszko et al. 1994). In agreement with most of these findings is morning increases in urinary βTG excretion (Musumeci et al. 1986).

This increased platelet responsiveness in the morning has been shown to be associated with the assumption of an upright posture (Brezinski et al. 1988, Winther et al. 1992). A return to the supine posture for 2 hours was sufficient to permit a return of platelet aggregability to the level observed after the overnight supine period, and reassumption of the upright posture produced an increase in aggregability indistinguishable from that observed initially (Winther et al. 1992). Simultaneous increases in plasma adrenaline and noradrenaline levels are associated with increased platelet aggregability (Tofler et al. 1987, Brezinski et al. 1988, McCall et al. 1991, Winther et al. 1992) as are increases in plasma renin activity and angiotensin II concentration (Brezinski et al. 1988). In addition, 24 hours of bedrest has been found to abolish the circadian variation in both platelet aggregation and plasma catecholamines (Rosenfeld et al. 1994).

2.5.2. Effects on fibrinolytic activity and endothelial cell function

The first findings of circadian fluctuation in fibrinolytic activity go back to the 1950s (Andreotti & Kluft 1991). Later, Rosing et al. confirmed the presence of a diurnal increase in fibrinolytic activity between 8:00 a.m. and 3:00 p.m. by a euglobulin fibrin plate lysis area method (Rosing et al. 1970). The reason for this variation was found to lie in a decrease in PAI-1 activity during the daytime, resulting in a net rise in tPA activity (Kluft et al. 1988). This observation has been confirmed by others (Andreotti et al. 1988, Angleton et al. 1989, Akiyama et al. 1990).

The mechanisms regulating plasma PAI-1 are not fully understood (Andreotti & Kluft 1991). Circadian variation in PAI-1 activity occurs despite a bedrest of 24 hours (Rosenfeld et al. 1994) and fibrinolytic activity is not related to posture (Kofod et al. 1994). This variation is not correlated with plasma insulin, cortisol or catecholamines (Chandler et al. 1990). Circadian changes in endothelial function do not appear to be
responsible for it either, since the plasma concentration of vWF does not vary over the 24 h period (Akiyama et al. 1990).

Other markers of endothelial function also remain stable day and night. Urinary excretion of 2,3-dinor-6-keto-PGF$_{\alpha}$ did not show any significant variation during consecutive 3-hour collection periods over 24 hours (Wennmalm et al. 1992). On the other hand, a slight difference, 24% higher day values than night ones, has been reported despite the fact that the subjects were resting during the collections (Nadler & Yamamoto 1986). Plasma ET-1 has been found both to show minimal circadian variation in 24 hours (Kanai et al. 1996) and to vary with in a period of eight hours (Herold et al. 1998).

### 2.6. Postprandial state

#### 2.6.1. Effect on platelet function

Observations of postprandial platelet function are contradictory and difficult to compare because of the different methods and parameters used. It has been claimed that chylomicrons isolated from postprandial plasma suppress platelet function (Lippel et al. 1981). Patients with marked hypertriglyceridaemia show decreased platelet response to ADP and collagen (Aviram et al. 1985), and this platelet response and the capacity of platelets to produce TxB$_2$ during aggregation are associated inversely with the amount of saturated fat in the diet (Mutanen et al. 1995). However, platelet activity has been shown variably to increase (Nordoy et al. 1984, Fuhrman et al. 1986, Belch et al. 1987), decrease (Johnston et al. 1982, Nimpf et al. 1989, Kozima et al. 1993, Orth et al. 1995, Tholstrup et al. 1996) or remain unchanged (Jakubowski et al. 1985, Aznar et al. 1987) after consuming a fatty meal. Eating of a fatty meal did not influence the TxB$_2$ released during collagen-induced platelet aggregation (Nimpf et al. 1989) nor did the postprandial increase in triglycerides result in any change in platelet aggregation (Fuhrman et al. 1986, Nimpf et al. 1989).

Two recent studies have compared the circadian variation in serum remnant-like lipoproteins with platelet aggregation. A close positive correlation between ADP-induced platelet aggregation and remnant lipoprotein cholesterol was found by Knöfler et al. (1995), whereas a negative association between collagen-induced aggregation and remnant lipoprotein cholesterol was reported by Rydzewski et al. (1998). Postprandial platelet responses are thus variable and the reasons for these variations remains yet to be defined.
2.6.2. Effects on fibrinolytic activity and endothelial cell function

Some case-control studies have suggested that hypertriglyceridaemia may be associated with a predisposition to thrombosis, because of low fibrinolytic activity (Hamsten et al. 1985, Mehta et al. 1987). In addition, it has been shown experimentally that very low density lipoproteins stimulate the secretion of PAI-1 from human umbilical vein endothelial cells (Stiko-Rahm et al. 1990).

The results of one short-term dietary programme partly support these findings. A three-week diet containing plenty of carbohydrates and a low fat content reduced both tPA and PAI levels in conjunction with a decrease in triglycerides. Triglycerides correlated significantly with tPA and PAI both before and after the programme (Mehrebiam et al. 1990). In another experiment, however, a two-week diet low in fat and high in fibre, produced no significant change in either tPA or PAI-1 (Marckmann et al. 1994). This was also the case with three single meals rich in either saturated fatty acids, polyunsaturated fatty acids or carbohydrates, which failed to have any significant influence on tPA or PAI-1, or vWF:antigen (vWF:Ag) (Salomaa et al. 1993). A two-day programme of a high-fat diet and a single high-fat meal had no significant effect on tPA or PAI-1 levels (Marckmann et al. 1993, Kozima et al. 1993) despite a significant correlation of triglycerides with PAI-1 (Kozima et al. 1993). In conclusion, human studies suggest no changes in fibrinolytic activity or endothelial cell function after different kind of diets.

2.7. Alcohol

2.7.1. In vitro effects on platelet function

The addition of ethanol to PRP usually results in a decrease in platelet aggregation, although the response depends on the concentration of ethanol and on the agonist used. Ethanol alone does not cause any platelet aggregation or any secretion of granular contents at concentrations up to as high as 500 mmol/L (23‰) (Rubin et al. 1988) and at concentrations lower than 100 mmol/L (4.6‰) it has little effect on ADP-induced primary (i.e. reversible) aggregation (Haut & Cowan 1974, Fenn & Littleton 1982, Rand et al. 1988). By contrast, secondary (irreversible) platelet aggregation was already inhibited by ethanol concentrations of 20-40 mmol/L (Quintana et al. 1980, Rand et al. 1988), although there are two studies that did not confirm such an effect (Davis & Phillips 1970, Duarte et al. 1995). One report points to a decrease in the maximal aggregation percentage for adrenaline and collagen at ethanol concentrations higher than 43 mmol/L and a decrease in aggregation-associated TxB₂ release even at an ethanol concentration of 22 mmol/L (Mikhailidis et al. 1983). Ethanol seems to inhibit the formation of TxB₂ during spontaneous clotting of whole blood at concentrations higher than 88 mmol/L (Toivanen et al. 1983).

The effects of ethanol on collagen-induced aggregation are fairly uniform. It has no effect on aggregation at low concentrations, but at higher concentrations (50-85 mmol/L) the aggregation induced by low concentrations of collagen is inhibited (Haut & Cowan...
1974, Fenn & Littleton 1982, Rand et al. 1988, Rubin 1989, Duarte et al. 1995), a finding which is consistent with the significant role of ADP in low collagen-induced aggregation. High collagen concentrations can overcome the inhibition, however (Fenn & Littleton 1982, Rubin et al. 1988, Jakubowski et al. 1988). In conjunction with the suppression effect on aggregation, ethanol causes a dose-dependent decrease in aggregation-associated TxB2 release (Hwang 1981, Jakubowski et al. 1988) and also lowers the quantities of some secretion products found in PRP (Fenn et al. 1982, Rubin et al. 1988). The in vitro effect of ethanol on SIPA has not yet been studied.

### 2.7.2. Ex vivo effects on platelet function

The pattern of alcohol consumption used in human experimental studies has either been acute, i.e. a single fixed dose, or habitual, i.e. a fixed dose repeatedly for several days, but these protocols have seldom reached ethanol concentrations comparable to the levels used in vitro. Platelet function in alcoholics has been studied both during prolonged heavy drinking and shortly after its discontinuation. Despite the uncertainty surrounding alcohol consumption during a drinking bout, the results of these observational studies are fairly consistent (Renaud & Ruf 1996).

There is only one cohort study, which compares platelet aggregation with the amount of alcohol consumed in a general population (Renaud et al. 1992). This pointed to a negative association of ADP and collagen-induced platelet aggregation with the amount of alcohol consumed, but the aggregation results induced by thrombin showed a controversial tendency.

In an experimental study (Dunn et al. 1981), volunteers consumed commercial liquor ad libitum for four hours. Their blood alcohol levels varied from 15 to 63 mmol/L, and there was no evidence of any change in platelet aggregation induced by various agonists. In accordance with this, a small dose of alcohol (50 g ethanol), resulting in a blood concentration of 11 mmol/L, did not significantly influence the bleeding time (Deykin et al. 1982), although it did potentiate aspirin-induced prolongation of the bleeding time. Mikhailidis studied the effect of a fixed dose (1 ml ethanol per kg body weight) consumed within 15 min (Mikhailidis et al. 1983) and found the blood ethanol concentrations of the volunteers to rise to 15.6 and 18.7 mmol/L 30 and 60 min after the end of ethanol ingestion, respectively. No change in platelet aggregation induced by a low concentration of collagen or ADP was seen, but the amount of TxB2 produced during collagen-induced aggregation decreased significantly. The conclusions concerning the effect of ethanol could have been biased, however, since the observation period was restricted to the early state of intoxication and the ethanol had been partly diluted in grapefruit juice, which can itself have an effect on platelet function (Demrow et al. 1995).

Ingestion of a large dose of whisky containing 1.6 g ethanol per kg body weight within 15 min produced a significant decrease in platelet aggregation and a prolongation of bleeding time. These effects were observed one and two hours after ingestion (Elmér et al. 1984). The blood ethanol concentrations rose only to 19.3 and 15.5 mmol/L, respectively, approximately a half of what they would have been if the dose of alcohol had been ingested more slowly. The low blood ethanol concentrations may have been due
to attenuated gastric emptying and nausea. By contrast, the results of two previous studies by our own group demonstrated a slight increase in ADP-induced platelet aggregation 4-8 hours after starting to drink an equally large dose of ethanol (Kangasaho et al. 1982, Hillbom et al. 1985a). Accordingly, variable effects of an acute intake of alcohol on platelet aggregation have been observed. Although the studies may have been hampered by methodological problems, the general consensus seems to be that alcohol reduces rather than increases platelet aggregation if measured *ex vivo* (Renaud & Ruf, 1996).

Two reports exist on the effects of alcohol consumed together with a meal on platelet function. When a meal high in saturated fatty acids was eaten and a bottle of white wine was consumed with it, both ADP and collagen-induced platelet aggregation decreased, i.e. the concentration of inducer needed for the aggregation response was increased (Fenn & Littleton 1984). Blood ethanol levels reached an average of 24 mmol/L during these experiments. The second study compared a meal rich in protein with one rich in fat when consumed together with an aperitif and two glasses of red wine (Veenstra et al. 1990). The meal did not influence the effects of the alcohol on platelet function, but the alcohol tended to increase platelet aggregation one hour postprandially despite the fact that its concentration was very low, 3.8 mmol/L. No previous studies have explored the combined effects of either alcohol and physical exercise or alcohol and the circadian rhythms.

Habitual alcohol intake for several days has usually attenuated platelet aggregation (Renaud & Ruf, 1996). When heavy drinkers, after withdrawal from prolonged alcohol consumption, were served increasing daily doses of whisky (up to 990 ml per day) for four weeks, their platelet aggregation had already decreased after two weeks of consumption (Haut & Cowan 1974). Blood ethanol levels varied in the range 60-115 mmol/L. In other studies a much smaller dose of alcohol ingested in the form of red wine (from 120 to 480 ml daily for at least four weeks) produced a decrease in platelet aggregation induced by collagen (Pikaar et al. 1987, Pellegrini et al. 1996). ADP-induced aggregation was either not influenced by the consumption of wine (red or white) (Pikaar et al. 1987, Pellegrini et al. 1996) or decreased (Seigneur et al. 1990, Pace-Asciak et al. 1996). No significant changes were observed either in bleeding times (Pikaar et al. 1987, Pellegrini et al. 1990) or in various platelet release products (Haut & Cowan 1974, Pikaar et al. 1987).

Alcoholics frequently show thrombocytopenia during prolonged drinking (Cowan & Hines 1971), and one third of them may develop transient rebound thrombocytosis shortly after discontinuation of drinking (Lindenbaum & Hargrove 1968). The reason for the thrombocytopenia is the inhibition of thrombopoiesis at the level of megakaryocyte maturation by ethanol (Haut & Cowan 1974). Maximum thrombocytosis occurs on average from 10 to 14 days after stopping drinking (Cowan 1980), and this rebound thrombocytosis has been thought to predispose subjects to thromboembolic disease (Haselager & Vreeken 1977). During regular drinking, the platelets of alcoholics usually show impaired aggregability compared with those of control subjects (Hutton et al. 1981, Hillbom et al. 1985b, Mikhailidis et al. 1986, Desai et al. 1986). This hypoaggregability of platelets reverses to hyperaggregability within two weeks after withdrawal from alcohol (Hutton et al. 1981, Fink & Hutton 1983, Hillbom et al. 1985b, Mikhailidis et al. 1986). Aggregation of the hyperactive platelets is associated with increased TxB₂ release during ADP induced aggregation, and with a shortened bleeding time (Hillbom et al. 1985b).
Studies reporting urinary excretion of TxB$_2$ metabolites are few in number. Both increased during the early withdrawal period (Neiman et al. 1994), and normal excretion of urinary 2,3-dinor-TxB$_2$ has been observed (Förstermann & Feuerstein 1987). The decreased reactivity in platelets of alcoholics and their recovery upon withdrawal is probably due to an inherent platelet defect rather than alterations in plasma factors, as the time course of recovery generally corresponds to the period of platelet turnover (Rubin & Rand 1994).

2.7.3. Ex vivo effects on fibrinolytic activity

Both occasional and habitual alcohol consumption seem to influence fibrinolytic activity. Although the first published study did not find any alcohol-induced effects on FVIII, coagulation time, euglobulin clot lysis time, plasminogen or fibrinogen, there was a distinct decrease in platelet aggregation after ingestion of a large dose of whisky (Elmér et al. 1984). In a comprehensive study performed later on, ingestion of a small dose of wine, beer or spirits (40 g ethanol) together with an evening dinner produced a sharp rise in PAI-1 which lasted for five hours (Hendriks et al. 1994). The effect coincided with a moderate drop in tPA activity. PAI-1 activity returned to control levels during the night following the evening of alcohol intake, but tPA antigen levels remained elevated, resulting in increased circulating tPA activity in the early morning. In experiments comparing two evening dinners, one rich in protein and the other rich in fat, similar responses to alcohol were observed in the fibrinolytic parameters (Veenstra et al. 1990).

A more prolonged consumption of moderate doses of red wine (from 320 to 480 ml daily for at least four weeks) reduced tPA activity in one study (Pikaar et al. 1987), but had no effect on it in another (Pellegrini et al. 1996). A small daily dose of beer (330 ml) for one month did not influence PAI-1 activity in patients with coronary artery disease (Gorinstein et al. 1997). In cohort studies, the consumption of alcohol has been found to be associated positively both with tPA (Ridker et al. 1994) and PAI-1 activity (Marques-Vidal et al. 1995).

The association between alcohol consumption and blood fibrinogen levels is a controversial matter (Belleisen et al. 1985, Folsom et al. 1991, Krobot et al. 1992, Marques-Vidal et al. 1995). An early prospective study demonstrated a negative association between alcohol consumption and fibrinogen concentration and showed that a 10-g increase in daily alcohol intake resulted in a decrease in fibrinogen concentration (Meade et al. 1979). Taken together, these observations suggest that an acute alcohol ingestion appears to shift the fibrinolytic balance in an adverse direction, i.e. favouring resistance to fibrinolysis, but this is followed by a rebound increase in fibrinolytic activity, and fibrinolytic activity is usually increased in habitual drinkers.
2.7.4. Ex vivo effects on endothelial cell function

Few observations are available concerning the effect of alcohol on prostacyclin or other endothelial products. Two early studies failed to point to any significant change in plasma 6-keto-PGF$_{1\alpha}$ after an acute alcohol intake resulting in peak blood ethanol levels of nearly 30 mmol/L (Kontula et al. 1982, Kangasaho et al. 1982). Later (Landolfi & Steiner 1984), an increase in plasma 6-keto-PGF$_{1\alpha}$ levels after a single dose of alcohol producing smaller blood ethanol concentrations (from 14 to 21 mmol/L) was reported. The urinary metabolites of both thromboxane and prostacyclin during early withdrawal from alcohol have shown reduced excretion of both 2,3-dinor-6-keto-PGF$_{1\alpha}$ and 6-keto-PGF$_{1\alpha}$ (Neiman et al. 1987, Förstermann & Feuerstein 1987). The effect of regular drinking on the functioning of the endothelium is not known, but there is one report of no change in plasma vWF during a four-week period of daily ingestion of 320 ml of red wine (Pellegrini et al. 1996).

2.7.5. Plausible mechanisms for the effects of alcohol on haemostasis

Several mechanisms are thought to mediate the effects of alcohol on haemostasis. The role of platelets has been explored more thoroughly. Ethanol itself appears to exert a direct toxic effect on the maturing megakaryocyte compartment at blood alcohol concentrations usually encountered in vivo (Sullivan et al. 1977, Levine et al. 1986, Gewirtz & Hoffman 1986). This toxic effect causes reversible thrombocytopenia. The mechanisms mediating the effects of alcohol on platelet function are not as simple. A common feature of platelet activation is the binding of platelet agonists to the receptors of the plasma membrane, leading to the activation of specific enzymes, such as adenylate cyclase and phospholipases (Rubin 1990). The stimulation of these enzymes is generally mediated by the membrane associated G-proteins (Casey & Gilman 1988). Alcohol increases the formation of cyclic adenosine monophosphate (cAMP) in a variety of cell types, probably by acting at the level of G-proteins (Hoffman & Tabakoff 1990), but it seems not to exert its inhibitory actions on platelets by this mechanism, as no measurable increases in cAMP have been demonstrated in human platelets incubated with ethanol (Jakubowski et al. 1988). Alcohol can also have stimulatory effects on human platelet function. Two possible proofs of this are the increased phospholipase C (Rubin & Hoek 1988) and protein kinase C activities (Deitrich et al. 1996) caused by tissue incubation in a solution containing ethanol, although the physiological significance of these changes is unknown (Rubin & Rand 1994, Deitrich et al. 1996). The most probable mechanism by which alcohol influences platelet function is the inhibition of phospholipase A$_2$, and thereby inhibition of the liberation of AA from platelet membrane phospholipids (Rubin & Rand 1994). There is plenty of indirect and experimental evidence for this effect (Stubbs & Rubin 1992, Rubin & Rand 1994).

Alcohol consumption influences plasma lipids, but does the altered lipid status modify platelet function or the effect of alcohol on it? One in vitro investigation has explored this problem (Fenn & Littleton 1983). It appeared that platelets, with their membranes incorporated with unsaturated fats were less susceptible to inhibition of aggregation by
alcohol than those which were incorporated with saturated fats. In two later studies, rabbits were fed either a normal diet, a cholesterol-enriched diet or a cholesterol-enriched diet plus 6% alcohol in their drinking water (Latta et al. 1994a, Latta et al. 1994b). The overall fatty acid composition of the platelet phospholipids was not affected by either cholesterol feeding or alcohol intake. The primary ADP induced aggregation was not affected, but cholesterol feeding did enhance collagen-induced aggregation and the formation of TxB2. These effects were significantly reduced by the intake of alcohol, however. The authors suggest that the alterations in platelets as a result of alcohol intake may occur at the level of the megakaryocyte.

The compositions of two common phospholipids, phosphatidylinositol and phosphatidylcholine were compared up on simultaneous changes in platelet function in alcoholics shortly after withdrawal from alcohol (Neiman et al. 1987). Despite the increased aggregation response and TxB2 release in PRP, no corresponding changes could be observed in the fractions of the phospholipids. Thus these studies suggest that changes in platelet function can be induced by diet or heavy alcohol consumption but they are not a straightforward consequence of the phospholipid composition of the platelets.

Attention has recently been directed to the possibility that other compounds in alcoholic beverages than ethanol itself could influence haemostatic mechanisms. In addition to ethanol, wine contains glycerol and phenolic compounds such as tannins and flavonoids, which are powerful antioxidants (Frankel et al. 1993). These phenolic compounds inhibit platelet aggregation and depress TxB2 synthesis in vitro (Mower et al. 1984, Landolfi et al. 1984, Corvazier & MacLouf 1985, Pace-Asciak et al. 1995), and they are thought to modify cAMP (Landolfi et al. 1984), inhibit COX (Mower et al. 1984) or, bind selectively to platelet thrombi and, owing to their free radical scavenging properties, resuscitate the biosynthesis and action of endothelial prostacyclin and nitric oxide (Gryglewski et al. 1987).

In rats fed on red wine for two weeks, the rebound increase in platelet aggregation after deprivation of the alcoholic beverage was totally abolished. Instead of that, rats pair-fed with pure 6% alcohol showed a clear rebound increase in platelet aggregation (Ruf et al. 1995). The protective effect of red wine on platelets was reproduced by tannins extracted from grape seeds or red wine added to the 6% alcohol. The effect was probably due to an inhibition of increased lipid peroxidation. This was supported by the higher level of lipid peroxides and conjugated dienes in rats drinking 6% alcohol than in rats drinking water or red wine.

When the effects of red and white wine administered by gastric tube on cyclic flow reductions in mechanically stenosed dog arteries were investigated (Demrow et al. 1995), the red wine, contrary to the white wine, resulted in a reversible elimination of the cyclic flow reductions at an average blood alcohol concentration of 8 mmol/L. The same effect also occurred in dogs administered grape juice-saline solution intravenously. These findings demonstrate that red wine constituents other than ethanol could have a profound inhibiting effect on platelet function.

Alcohol in vitro enhances the secretion of plasminogen activator from various vascular endothelial cells (Laug 1983, Aikens et al. 1997). This effect was shown to depend on the stimulation of both tPA and uPA gene expression (Grenett et al. 1998). Alcohol has also been found to directly stimulate endothelin 1 and 2 release from cultured human umbilical vein endothelial cells, although the mechanism of the effect is unknown (Tsuji...
et al. 1992). On the other hand, exposing endothelial cells to alcohol (100 mmol/L) for four days enhanced the response of nitric oxide synthase to various agonists (Davda et al. 1993). Thus the effects of alcohol on the endothelium may depend on the time of exposure.
3. Aims of the present research

I want to find out whether an acute alcohol consumption could predispose to vascular thrombosis in various physiological situations, which may be combined with alcohol drinking, and whether patients, having an acute ischaemic brain infarction after a drinking bout, show signs of platelet mediated mechanisms as a cause of their stroke. For this purpose I investigated:

1. whether moderate exercise during acute alcoholic intoxication and the following hangover could predispose a subject to thrombosis by increasing platelet aggregability.
2. whether the physiological circadian rhythms of fibrinolytic activity and platelet aggregability are modified by an acute intake of a large dose of alcohol.
3. whether ingestion of a moderate dose of red wine with or without an evening meal influences the haemostatic and fibrinolytic systems.
4. whether alcohol-induced alterations in the platelet count and platelet activation are frequent findings in heavy drinkers stricken by an acute brain infarction.
5. the mechanisms of brain infarctions that are triggered during alcoholic intoxication.
4. Subjects and methods

4.1. Subjects and study designs

Studies I, II and III investigated the actions of alcohol in male volunteers who were healthy, non-obese, non-smoking and aged from 20 to 59 years. They were all light-to-moderate, infrequent drinkers of alcohol, and had abstained from alcoholic beverages and drugs for at least one week before the trials.

In study I the volunteers participated in two exercise sessions at least one week apart. Alcohol was given in one of the sessions and fruit juice in the other. A crossover design was used, and the subjects did not know beforehand whether they had to start with an alcohol or a control session. The alcoholic beverage was prepared by diluting pure ethanol in fruit juice, and 5 drinks of equal volume were served half an hour apart for two and half hours. The total amount of ethanol given was 1.5 g/kg of body weight, and the volumes of the drinks (alcohol and control) were kept the same during both the sessions. The exercise was performed three times during each session: first in the morning before the test drink was given, then one hour after the subjects had ceased drinking and for the last time in the evening, when eight and half hours had elapsed from the end of drinking. The exercise was a graded bicycle exercise with a work load increasing stepwise from 125 W (ten minutes) to 175 W (ten minutes). During the last ten minutes, the load was increased until a heart rate of at least 170 beats/min was reached. Blood samples were taken 30 min before and after each exercise, except for the samples for measuring plasma catecholamines, which were taken immediately after each exercise. Urine was collected on the day before the session and on the session day. Bleeding time was measured 15 min before and after each exercise.

In study II each volunteer participated randomly in three sessions, one of which was a control session and the other two alcohol sessions. The alcohol sessions started either at 7.00 p.m. or at 8.00 a.m. and lasted for 12 hours. If the session started in the morning, the subjects arrived at our research department the preceding evening and spent the night sleeping in beds. The control session started in the evening and lasted for 24 hours, including both a fruit juice night and a juice day. In the morning, the subjects were woken up at 7 a.m. and were then allowed to walk within the research department. From 7.30 till 10.00 p.m. or from 8.30 till 11.00 a.m., they received either alcohol or fruit juice as in
study I. They were given carbohydrate-rich snacks together with the fruit juice to balance their calory intake to the level of the alcohol session. Blood samples were taken five times at three-hour intervals starting either at 7 p.m. or at 8 a.m. Urine was collected for 12 hours after voiding the bladder at the beginning of each session.

In study III each volunteer participated in four 12-hour sessions according to a randomized cross-over design. Two sessions included alcohol, one with a meal and the other without. In the two control sessions, also with or without a meal, the alcoholic beverage was replaced by an equal volume of water. The sessions always began at 6 p.m. and the period allowed for eating and drinking lasted for three hours. The volunteers were kept supine and allowed to sleep the following night from 10 p.m. till 6 a.m. During the day they had breakfast and lunch. Alcohol was served in the form of red wine (60 g ethanol). During the water session an equal volume of mineral water was served. The meal always contained the same proportions of carbohydrates (55%), proteins (15%) and fats (30%). Blood samples were taken four times at four-hour intervals, starting at 6 p.m., and urine was collected from 10 a.m. till 6 p.m. and from 10 p.m. till 6 a.m.

In study IV a series of 426 consecutive patients aged from 16 to 59 years, who had been admitted within 24 hours after the onset of their first-ever brain infarction were examined together with controls comprising patients taken into the same hospital on an emergency basis because of some acute illness not considered to accumulate among either heavy drinkers or teetotallers. Data on alcohol consumption and platelet count were gathered for all the patients and controls. They were interviewed using a structured questionnaire within 48 hours of admission, and their recent alcohol consumption was recorded in terms of g of ethanol consumed during the preceding week. Problem drinking was assessed with the short CAGE questionnaire (Bush et al. 1987). This interview includes four question on abnormal drinking behaviour, and patients with two or more positive answers are considered CAGE-positive. The patients who were CAGE-positive or whose regular weekly alcohol intake had exceeded 300 g of ethanol, were included in the category of heavy drinkers. In addition to drinking behaviour, data were collected on other risk factors for stroke: previously diagnosed hypertension, diabetes, hyperlipaemia, smoking and cardiac disease, which included current atrial fibrillation, previous myocardial infarction and congestive heart failure. Laboratory procedures included measurement of the platelet count within three days of admission and measurements of erythrocyte mean corpuscular volume (MCV) and g-glutamyl transferase (GGT). Serial blood and 24-hour urine samples for analyses of platelet function were collected from 13 of the patients who had not taken any drugs known to affect platelet function.

In study V we compared 212 consecutive patients aged between 16 and 60 years who had been thoroughly evaluated for the aetiology of their brain infarction with 274 control subjects admitted to the emergency unit of the same hospital. The subjects were grouped according to their drinking and smoking behaviour in the same manner as in study IV. Former heavy drinkers were placed in a separate group from the heavy drinkers by virtue of reporting no alcohol intake during the week preceding the index brain infarction. The complete aetiological investigations allowed us to classify their brain infarctions into seven categories according to the TOAST criteria (Adams et al. 1993). The additional investigations included both duplex imaging of the carotid and vertebral arteries and/or an aortic arch angiogram and cardiac imaging by transaesophageal and/or transthoracic echocardiography. Besides these, special laboratory tests were devised, if needed, to
investigate haematological and other abnormalities in order to reveal the precise aetiology of the brain infarction.

4.2. Laboratory methods

Blood samples for analysing platelet aggregation and associated TxB₂ release were taken with minimal stasis via a plastic cannula into polyethylene tubes containing 1:10 volume of 3.8% sodium citrate. PRP and platelet poor plasma (PPP) were prepared by centrifuging the samples at 330 and 1500 x g for ten minutes, respectively, after which autologous PPP was used to adjust the platelet count in the PRP either to 200 x 10⁹/L (study I) or to 250 x 10⁹/L (study II and IV) and the tubes were capped until used. 450 ml of PRP was moved to an aggregation tube and aggregation was induced after 45 min (studies II and IV) or 60 min (study I) of blood sampling by adding 50 ml of the necessary inducer to the tube and stirring at a constant speed with a magnetic stirrer in a dual-channel Chorono-Log aggregometer (Coulter Electronics Ltd, UK). Aggregation was allowed to continue for five minutes, after which it was stopped by adding 1 mol/L HCl. The PRP was stored at -20° C for further analyses. Various concentrations of ADP were used to produce a secondary aggregation wave. Collagen and AA were also used as aggregation inducers (study II). Maximal aggregation, i.e. the maximum percentage change in light transmittance, was used as an expression of the aggregation response.

The blood sampling technique and the preparation of PRP were the same in study III as elsewhere. SIPA was measured by a turbidometric technique that used a cone-plate streaming chamber through which the laser light beam passed, measuring platelet aggregation due to shear stress caused by the rotation of the cone (Fukuyama et al. 1989). A high shear force (10⁸ dynes/cm²), corresponding values achieved in stenotic vessels, was applied for 5 min and SIPA was expressed as the maximum percentage change in light transmittance. SIPA was also measured after adding ethanol in PPP to a test tube containing PRP.

Aggregation-associated TxB₂ release and serum TxB₂ were measured either with a commercial radioimmunoassay (Amersham International, UK) or with a commercial enzyme-linked immunosorbent assay (ELISA) (Cayman Co, USA). After storing at -70° C, urine was analysed for the metabolites of TxB₂ and PGI₂ with a stable isotope dilution assay using a mass spectrometer coupled to a gas chromatograph (Falardeau et al. 1981, Lawson et al. 1985). 11-dehydro-TxB₂ was analysed with a commercial ELISA (Cayman Co, USA), except in study III, where a radioimmunoassay after solid-phase extraction was used for the analyses of 2,3-dinor-6-keto-PGF₁α, 11-dehydro-TxB₂ and 2,3-dinor-TxB₂ (Riutta et al. 1992, Riutta et al. 1994). For the determination of plasma-free arachidonic acid (p-AA) free fatty acids were isolated with thin-layer chromatography and analysed with gas chromatography.

The blood samples taken for the measurement of PAI-1 activity were immediately put into ice and centrifuged at 4° C and 1500 g. The activity was measured with a colorimetric assay (Diagnostica Stago, France) in the kinetic mode using an automatic coagulometer (Behnk Elektronic GMBH, Germany). FVIII-related antigen was measured by rocket immunoelectrophoresis (Laurell 1966) and vWF:Ag by the ELISA technique,
using antibodies to rabbit anti-human vWF and to peroxidase-conjugated rabbit anti-
human vWF (Dako A/S, Denmark) and expressed in units. The standards (FVIII and
vWF) came from the WHO (International Institute for Biological Standards and Controls,
UK). Plasma samples for the assessment of vWF multimers were diluted in modified Tris
buffer and run in SDS (sodium dodecyl sulphate) - agarose, after which the proteins were
transferred to nitrocellulose paper and the vWF multimers were visualized with rabbit
immunoglobulins to human vWF and alkaline phosphatase-conjugated swine anti-rabbit
immunoglobulins (Dako A/S, Denmark). The results were expressed as the number of
multimers.

Plasma endothelin was analysed by enzyme-linked immunoassay (Cayman Chemical
Company, USA) after purification in cartridges (C18 Sep-Pak, Millipore Corporation,
USA).

4.3. Statistical analyses

The data were transformed as necessary on account of skewed distributions. In study I,
the pre-exercise and post-exercise results were originally compared using Student’s t-test
and the results were reanalyzed for the purposes of this dissertation by using repeated
measures ANOVA. In studies IV and V the BioMedical Data Package statistical
programme (BMDP Statistical Software Inc, version 1993, USA) were used to compare
categorial and continuous variables by means of the appropriate tests and to calculate
odds ratios and confidence intervals by logistic regression. In studies II and III the
comparisons between the sessions were calculated using Student’s t-test or Wilcoxon
signed-rank test according to the distribution of the data. For time trend analyses
repeated-measures ANOVA was used, either as a one-way analysis for a single session or
as a two-way analysis between sessions. Results are presented as means and 95%
confidence intervals (CI) unless otherwise stated. All analyses except those in studies IV
and V, were calculated using Statview and SuparAnova (Abacus Concepts, USA).
5. Results

5.1. Study I

Ten male volunteers aged 20-24 years participated in the trial. Their heart rate and blood pressure and the effort used in exercise remained at the same level during each of the three exercises. Blood ethanol reached 28 mmol/L (95% CI 26-30 mmol/L) before the second exercise and decreased to 6 mmol/L (95% CI 4-9 mmol/L) before the third exercise in the evening.

ADP-induced platelet aggregation (ADP concentration 8 µmol/L) did not differ between the alcohol and control sessions when the changes from pre-exercise and post-exercise values were compared (F1,7=0.07, p=0.798) (Fig. 1). On the other hand, there was significant heterogeneity in the aggregation responses during the control session (F5,7=4.24, p=0.004), but not during the ethanol session (F5,7=1.75, p=0.148) (Fig. 2).

Changes in TxB2 released during ADP-induced aggregation reflected the changes in aggregation and did not differ between the sessions (Fig. 1). The changes observed during the control session (F5,7=3.2, p=0.018) were inhibited by the drinking of alcohol, however (F5,6=1.82, p=0.139) (Fig. 2).

Fig. 1. Absolute change (mean ± 95% CI) between pre-exercise and post-exercise values for platelet aggregation (left) and associated TxB2 release (right) in three exercises (I, II, III). Negative deflection means decreased post-exercise values and vice versa. Mean percent changes were used in the corresponding figure 3 of the original paper.
Excretion of 2,3-dinor-6-keto-PGF$_{1\alpha}$, a urinary metabolite of PGI$_2$, increased significantly upon exercise, without any differences between the session days. The mean percentage increases were 60% (95% CI 17-104%) and 55% (95% CI 3-107%) during the control and alcohol session, respectively. Exercise or alcohol ingestion did not cause any significant changes in serum TxB$_2$ formation, which reflects the capacity of platelets to form TxB$_2$ (Fig. 3). Levels of plasma arachidonic acid decreased between the first and second exercise irrespective of whether fruit juice or alcohol was consumed (Fig. 3). The changes were significant (control: $F_{5,9}=3.21, p=0.015$ and alcohol: $F_{5,8}=5.76, p<0.001$). FVIIIR:Ag increased significantly during the session day, the effect being similar in magnitude during the control 76% (95% CI 58-95%) and alcohol sessions 116% (95% CI 82-150%). Skin bleeding time was not significantly influenced by either exercise or alcohol drinking.

Levels of plasma catecholamines increased significantly during each exercise. The highest absolute levels of adrenaline (1.8 nmol/L) were achieved after the second exercise. Changes in plasma noradrenaline were comparable and the highest absolute levels were 23.4 nmol/L (95% CI 15.8-31.0) and 24.1 nmol/L (95% CI 15.2-33.0) for the control and alcohol sessions, respectively.
5.2. Study II

Twelve men aged 20-39 years volunteered for this trial, which included both alcohol and control sessions. They ingested a large acute dose of alcohol, resulting in relatively high blood alcohol concentrations (30 mmol/L). Nine hours later the ethanol concentrations were around 10 mmol/L.

Platelet aggregations induced by collagen (5 µg/mL) and measured from night samples ex vivo showed no significant changes either between the sessions or during either session, but they showed significant heterogeneity ($F_{4,8}=6.64$, $p<0.001$), which was observed during the daytime control session (Fig. 4). Platelet aggregability decreased from 8 a.m. to 2 p.m. Drinking of alcohol abolished the variation ($F_{4,8}=0.96$, $p=0.442$), but the difference between the curves was not significant ($F_{1,8}=0.00$, $p=0.98$) and no corresponding changes in aggregation-associated TxB$_2$ release were seen. A slight morning increase in platelet aggregation (mean change +2%, 95% CI -8-11) was observed during the control session between 7 a.m. and 8 a.m.
Despite the lack of significant changes in platelet function measured ex vivo, the urinary excretion of 2,3-dinor-TxB$_2$ increased after alcohol ingestion (Fig. 5). The mean increases between the alcohol and control sessions were 13.0 pg/mmol creatinine (95% CI 6.2-19.8) during the night and 4.5 pg/mmol creatinine (95% CI -0.9-9.8) during the day. Urinary excretion of 2,3-dinor-TxB$_2$ was significantly lower during the control night than during the control day (mean difference 3.3 pg/mmol creatinine, 95% CI 1.0-5.7). In contrast, the urinary excretion of 2,3-dinor-6-keto-PGF$_{1\alpha}$ (Fig. 5), did not differ significantly between the control day and the control night. Ingestion of alcohol did not significantly influence the urinary excretion of this metabolite.
PAI-1 activity was significantly increased by acute ingestion of a large dose of alcohol (Fig. 6). An increase compared with the control session was observed both during the day ($F_{1,7}=8.3$, $p=0.028$) and during the night ($F_{1,6}=23.6$, $p=0.003$). PAI-1 activity remained fairly constant during the control session, but was slightly decreased in the afternoon and evening (Fig. 6).

Variations in plasma endothelin were negligible, and neither circadian nor alcohol induced changes were observed. The mean values were 0.94 pg/ml (95% CI 0.86-1.02) and 0.97 pg/ml (95% CI 0.89-1.06) for the control and alcohol sessions, respectively.

5.3. Study III

Twelve men aged 33-59 years participated in the trial. After the ingestion of a moderate dose of red wine with or without an evening meal, their blood alcohol concentrations reached 18.1 mmol/L (95% CI 16.4-19.8) and 12.9 mmol/L (95% CI 11.4-14.3), respectively.

The wine intake resulted in a transient decrease in SIPA (Fig. 7). The effect was small, but similar irrespective of whether the wine was drunk with a meal ($F_{3,11}=4.67$, $p=0.008$) or not ($F_{3,10}=3.34$, $p=0.032$). The differences between the wine and water sessions were not statistically significant, however. A meal accompanied by mineral water produced a similar but insignificant decrease in SIPA (Fig. 7).
When pure ethanol mixed in PPP was added to the test tube containing PRP, SIPA decreased slightly but insignificantly at an ethanol concentration of 4 mmol/L (Fig. 8), but increased significantly at high ethanol concentrations of ≥33 mmol/L ($F_{6,5}=29.8$, $p<0.001$).

Urinary excretion of the TxB$_2$ metabolites 11-dehydro-TxB$_2$ and 2,3-dinor-TxB$_2$ and the PGI$_2$ metabolite 2,3-dinor-6-keto-PGF$_{1\alpha}$ were not influenced by the drinking of a moderate dose of red wine and the eating of a meal.

PAI-1 activity increased significantly upon the intake of wine (Fig. 9), irrespective of whether the wine was ingested with ($F_{3,11}=28.0$, $p<0.001$) or without a meal ($F_{3,10}=10.7$, $p<0.001$). The increase compared with the corresponding control session session was significant when wine was drunk with a meal ($F_{1,10}=19.7$, $p=0.001$), but did not reach statistical significance when it was drunk without a meal ($F_{1,10}=2.5$, $p=0.144$). There was a significant increase in PAI-1 activity at night ($F_{3,10}=12.8$, $p<0.001$).
Serum triglycerides increased on the ingestion of wine (Fig. 10), the effect reaching its peak five hours after the end of wine drinking, irrespective of whether the wine was ingested with (F$_{3,11}$=22.2, p<0.001) or without a meal (F$_{3,10}$=10.7, p<0.001), whereas eating a meal with mineral water caused a weaker and earlier increase (F$_{3,10}$=12.9, p<0.001). There was a significant difference in serum triglycerides between the two meal sessions (F$_{1,10}$=9.4, p=0.012). Serum triglyceride values correlated with those for PAI-1 both after consuming a meal (slope 5.6, 95% CI 2.9-8.4, p<0.001, $r^2=0.16$) and after wine (slope 4.9, 95% CI 2.1-7.7, p<0.001, $r^2=0.12$), but not without.

Neither the drinking of wine nor the eating of a meal influenced plasma vWF:Ag or fibrinogen significantly. By contrast, the number of vWF multimers increased after eating a meal (F$_{3,11}$=6.5, p=0.001), but the effect was reversed by the simultaneous drinking of wine (F$_{3,11}$=3.5, p=0.025) (Fig. 11). The difference between the curves was significant (F$_{1,11}$=43.0, p<0.001). SIPA showed no significant correlation with vWF multimers, triglycerides or fibrinogen, but it did correlate with vWF:Ag (slope 1.96, 95% CI 1.23-2.69, p<0.001, $r^2=0.24$) (Fig. 11).
5.4. Study IV

This case-control study focused on 426 consecutive brain infarction patients and 157 hospitalized control subjects. The classic risk factors for brain infarction, such as hypertension and smoking, were more prevalent among the patients than among the controls. In addition, both heavy drinking, i.e. regular consumption of more than 300 g of alcohol per week or being CAGE-positive, and recent heavy drinking, i.e. consumption of more than 300 g of alcohol during the week preceding the brain infarction, were significantly more frequent (odds ratio, OR, 2.16, 95% CI 1.38-3.38 and 5.77, 95% CI 2.28-14.6, respectively) among the patients than among the controls. Recent heavy drinking was further emphasized as a risk factor for brain infarction in the multivariate analysis. After adjustment for classical risk factors and heavy drinking, the OR was 4.92 (95% CI 1.64-14.72).

The platelet count on admission was higher among the patients with brain infarction than among the control subjects, 244 x 10^9/L (95% CI 236-252) and 227 x 10^9/L (95% CI 216-238), respectively. A high platelet count remained an independent risk factor in the multivariate analysis after adjustment for other risk factors, including recent alcohol consumption and a history of heavy drinking (OR 1.05 per 1 x 10^9/L, 95% CI 1.02-1.09).

The 426 patients included 144 who were heavy drinkers. Their risk of having a higher or lower than normal platelet count (150-300 x 10^9/L) on admission was non-significant if their alcohol consumption during the preceding week before the brain infarction was not taken into account, but the heavy drinkers who had recently abstained from alcohol (n = 20) had platelet counts over 300 x 10^9/L more often than the non-drinkers and the light-to-moderate drinkers (OR 2.30, 95% CI 0.82-6.44), whereas the current drinkers (n = 124) more frequently (OR 3.20, 95% CI 1.19-8.59) showed a low platelet count (<151 x 10^9/L). Accordingly, the heavy drinkers with brain infarction showed both high and low
platelet counts more often than the other patients with brain infarction. Thrombocytopenia was associated with current heavy drinking, whereas thrombocytosis was associated with recent withdrawal from heavy drinking.

As many patients had been receiving medications known to influence platelet function, the release of TxB₂ during ADP-induced platelet aggregation was analyzed only from the plasma samples of 13 patients who had not received any medication, all of these patients had had normal platelet counts on admission. In the eight of them, who had consumed less than 300 g of alcohol during the week preceding the brain infarction, aggregation-associated TxB₂ release decreased during the first week after admission (median decrease -70 fmol/10⁷ platelets in PRP, 25th and 75th percentiles -359 and +67), whereas in the five who had consumed more than 300 g of alcohol, TxB₂ release increased (median increase +688 fmol/10⁷ platelets in PRP, 25th and 75th percentiles +136 and +1157). The difference between the changes was significant (p < 0.05). Urinary 11-dehydro-TxB₂ values did not show any significant correlation with the corresponding platelet counts, aggregation percentages, or aggregation-associated TxB₂ release.

5.5. Study V

This case-control study included 212 patients who had been completely evaluated to reveal the aetiology of their brain infarction (and 274 controls). They were divided into the following categories: 76 cases with cardioembolism (38 with a high-risk source), 68 with a negative evaluation (cryptogenic stroke), 34 with large-artery atherosclerosis, 24 with cervicocerebral arterial dissection and 10 with small-artery occlusion (lacunar stroke).

Multiple stepwise logistic regression showed that hypertension (adjusted RR 2.20, 95% CI 1.32-3.68), current smoking (adjusted RR 1.79, 95% CI 1.16-2.77) and heavy drinking (adjusted RR 1.82, 95% CI 1.08-3.05) were significant independent risk factors. Omission of hypertension from the model did not change the association between heavy drinking and ischaemic brain infarction, but after the exclusion of recent heavy drinkers, i.e. those who had consumed more than 300 g of alcohol during the preceding week, the risk for former heavy drinkers was not significant. Both moderate (150-300 g) and heavy alcohol intake during the week preceding the stroke appeared to be significant risk factors. The adjusted RRs were 3.61 (95% CI 1.67-7.79) and 3.74 (95% CI 1.61-8.72), respectively.

Risk factor analysis according to the aetiology of brain infarction is shown in Table 1. In addition to heavy drinking both recent moderate and recent heavy drinking (adjusted RR 3.67, 95% CI 1.19-11.3 and 3.43, 1.04-11.3, respectively) were significant risk factors for cardiogenic brain embolism. The cases with small-artery occlusion were too few for any meaningful analysis to be performed.
Table 1. Multivariate RRs and 95% confidence intervals in parentheses of ischaemic brain infarctions (BI) grouped according to their etiology.

<table>
<thead>
<tr>
<th></th>
<th>Large-artery atherosclerotic BI</th>
<th>Cardiogenic BI</th>
<th>Cryptogenic BI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertension</td>
<td>2.68 (0.81-8.87)</td>
<td>2.06 (0.98-4.34)</td>
<td>2.31* (1.07-4.99)</td>
</tr>
<tr>
<td>Current smoking</td>
<td>3.34* (1.08-10.3)</td>
<td>1.78 (0.93-3.40)</td>
<td>1.75 (0.95-3.21)</td>
</tr>
<tr>
<td>Heavy drinking</td>
<td>2.01 (0.66-6.07)</td>
<td>2.44* (1.16-5.11)</td>
<td>1.74 (0.84-3.60)</td>
</tr>
<tr>
<td>Hyperlipemia</td>
<td>2.01 (0.55-7.39)</td>
<td>1.68 (0.69-4.13)</td>
<td>1.20 (0.43-3.35)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>2.09 (0.47-9.31)</td>
<td>1.22 (0.42-3.57)</td>
<td>1.76 (0.62-4.95)</td>
</tr>
<tr>
<td>Migraine</td>
<td>0.27 (0.03-2.67)</td>
<td>1.49 (0.66-3.37)</td>
<td>2.09* (1.03-4.23)</td>
</tr>
</tbody>
</table>

RRs represent comparisons with patients without a risk factor and have been adjusted for age, sex, body mass index, and the other variables listed in the table.

* p<0.05.

If the time for recent drinking was limited to the 24 hours preceding the brain infarction instead of one week, the effect of drinking alcohol was enhanced. Drinking more than 40 g of alcohol appeared to be a risk factor not only for cardioembolism (adjusted RR 3.49, 95% CI 1.47-8.28) and cryptogenic brain infarction (adjusted RR 3.84, 95% CI 1.69-8.71), but also for large-artery atherosclerosis (adjusted RR 7.68, 95% CI 1.82-32.30) and cervicocerebral arterial dissection (adjusted RR 3.67, 95% CI 0.97-13.8).

Gender-specific analyses yielded higher RRs for men and non-significant RRs for women, due at least partially to the low number of heavy drinkers among the women.
6. Discussion

The main purpose of the present experimental studies was to find out whether acute drinking of alcohol, could cause either beneficial or untoward effects on the fibrinolytic or haemostatic system in various physiological situations, which may be combined with alcohol drinking. The aims of the clinical surveys were to see whether altered platelet sensitivity to agonist plays a role in the first-ever ischemic brain infarction suffered by a heavy drinker, and to demonstrate the causes of brain infarctions triggered by alcohol. Both our epidemiological and earlier experimental studies had suggested that alcohol can behave as a triggering factor for brain infarction. Firstly, alcoholic intoxication seemed to be a precipitating factor for brain infarctions among young adults (Hillbom & Kaste 1978, Hillbom & Kaste 1981), and secondly, alcohol ingestion seemed to sensitize platelets to aggregate (Kangasaho et al. 1982, Hillbom et al. 1985). Recent epidemiological (Renaud et al. 1992) and methodological (Uchiyama et al. 1994) observations drew our attention to the possible beneficial effect of red wine and also to utilize a wider range of methods when assessing platelet aggregation.

6.1. Methodological considerations

Platelet function can be examined either by taking blood samples for in vitro and ex vivo analyses or by collecting urine for in vivo analyses of metabolites formed during platelet activation and excreted into the urine via the blood. The methodological artefacts of ex vivo measurement of platelet function can be circumvented by analysing the metabolites of platelet activation products in urine. This avoids the possibility of the platelets being activated by blood sampling or centrifuging and desensitized or sensitized by subsequent handling of the PRP sample. This requires, however, that the platelets should be activated and release their granular content or other activation agents such as TxB2. The question of timing and the methods of measurement are also important for evaluation of the fibrinolytic system and endothelial function, but probably not as crucial as for the evaluation of platelet function.

One can ask whether ex vivo measurement of platelet function has any biological relevance. Increased platelet function has been observed immediately after ischaemic

The choice of young, healthy, normal weight, non-smoking men who were at most moderate consumers of alcohol as volunteers for the experiments in order to avoid biases in measurements may display a different pattern of responses as compared with responses in patients who already have had some thrombotic complication. There are no other ethically acceptable possibilities, however, for choosing volunteers for experiments of this kind.

It may also be that patients who have had a brain infarction triggered by acute alcoholic intoxication may have individual and unknown predisposing factors for such events. The results of study IV did not support the view that heavy drinkers with brain infarction form a uniform group as far as platelet function is concerned. Thus, a common platelet related mechanism appeared to be unlikely as an aetiopathological factor for brain infarctions in heavy drinkers.

### 6.2. Physical exercise and alcohol ingestion

In study I both platelet aggregation and the associated TxB2 release measured ex vivo decreased after the first exercise in the morning. The pre-exercise values were nevertheless at a higher level than at other times during the sessions. As the changes after the second and third exercise were negligible, there was no pronounced effect of submaximal physical exercise on platelet aggregation and TxB2 release. This agrees with the previously published reports of other investigators using platelet aggregation method (Siess et al. 1982, Taniguchi et al. 1984, Lassila & Laustiola 1988, Winther et al. 1992, Chicharro et al. 1994, Wang et al. 1994) as well as one report using a whole blood flow cytometry to study platelet activation state and reactivity (Kestin et al. 1993).

The effect of exercise in reducing platelet function in the morning was probably modified by the well-known circadian rhythm of platelet aggregation. The high starting level of both platelet aggregation and TxB2 release favours this possibility. The lack of such a response later during the sessions could also have been influenced by the drinking of fruit juice and alcohol, as both juice and alcohol can counteract the increase in serum free fatty acids, including p-AA, that is exaggerated by exercise (Pruett 1970, Heikkonen 1989) and as platelets are able to utilize free, exogenous AA to form thromboxane.


(Sautebin et al. 1983), so that the responses could have been attenuated by a decrease in the substrate pool.

We cannot exclude the possibility that PGI₂, although having a very short half-life, had an effect on platelet function as a consequence of the exercise alone, as reported previously (Vesterqvist et al. 1984, Wennmalm & FitzGerald 1988, Piret et al. 1990, Ronni-Sivula et al. 1993). Since urinary metabolites of TxB₂ were not measured, the significance of the increase of PGI₂ for the haemostatic balance remained uncertain. Plasma TxB₂ levels have remained unchanged after exercise in the majority of reports (Mehta et al. 1983, Taniguchi et al. 1984, Todd et al. 1992, Todd et al. 1994). If this is true, our observations together with these favour the view that submaximal exercise has an anti-thrombotic effect.

Plasma catecholamine levels were increased by exercise irrespective of whether alcohol was ingested or not. We did not observe any significant activation of platelet function in relation to these, but as the samples were taken immediately after the exercise, the results should be interpreted as showing that the increases in plasma catecholamines had no long-lasting effect on platelet function. This is in accordance with some of the previously reported results (Siess et al. 1982, Taniguchi et al. 1984, Winther at al 1992).

Since there were no significant differences in platelet function measured ex vivo between the control and alcohol sessions it must be concluded that acute heavy drinking of alcohol followed by submaximal exercise was unable to precipitate platelet hyperactivity.

6.3. Circadian rhythms and ingestion of alcohol

We observed a slight increase in ex vivo platelet aggregation after waking and getting up from bed, after which it decreased until the afternoon. This change was attenuated by alcohol ingestion. The slight morning increase is in accordance with previous reports (Toffler et al. 1987, McCall et al. 1991, Jafri et al. 1992) and the rather small change could reflect the gradual assumption of an upright posture in the case of our volunteers. The decrease in platelet aggregation towards the afternoon is not reported in all the previous studies (Jovicic & Mandić 1991, Jafri et al. 1992, Knöfler et al. 1995). This could be attributed to the gentle daytime activities of the volunteers, but such a hypothesis is not supported by previous observations (Rosenfeld et al. 1994). Another possible explanation is that the morning snack with fruit juice influenced platelet aggregation. The snack mainly included carbohydrates, which is a type of meal that has earlier resulted in conflicting effects on platelet aggregation (Johnston et al. 1982, Nordoy et al. 1984, Jakubowski et al. 1985, Fuhrman et al. 1986, Belch et al. 1987, Aznar et al. 1987, Nimpf et al. 1989, Kozima et al. 1993, Orth et al. 1995, Tholstrup et al. 1996). A slight decrease in aggregation after the evening snack, also accompanied by fruit juice, is in accordance with this assumption.

Our main finding was that urinary excretion of the TxB₂ metabolite 2,3-dinor-TxB₂ was significantly increased on the night of the alcohol session relative to the respective control sessions, and less so during the day. By contrast, urinary excretion of the PGI₂ metabolite 2,3-dinor-6-keto-PGF₁α remained at a constant level day and night,
irrespective of whether alcohol was consumed or not. These results could not be explained by changes in urinary volumes and reflect thus a real shift in the balance of urinary prostaglandins. The results are also in accordance with the observed effects on platelet aggregation.

The effect of alcohol ingestion in increasing the urinary excretion of 2,3-dinor-TxB2 has not been reported earlier. Similar effects were seen at night and during the day, although the observed increase reached statistical significance only after an evening intake of alcohol. One explanation for the effect could be enhanced shear stress induced by increased heart rate and variable blood pressures, which are consequences of intoxication (Seppä & Sillanaukee 1999). Since heart rate is a factor in volumetric flow rate, which is directly associated with shear stress in the vessel wall (Kroll et al. 1996), the conclusion may be that the increased urinary excretion of 2,3-dinor-TxB2 was a result of platelet activation caused by an increase in shear stress due to an alcohol-induced increase in blood flow. We believe that the finding reflects platelet activation in vivo and that it is of biological significance. For example, it may contribute to the consequences of the sleep apnoea syndrome (Palomäki & Kaste 1993b), as alcohol is known to provoke apnoeic periods during sleep.

Fibrinolytic activity usually shows diurnal variation (Kluft et al. 1988, Andreotti et al. 1988, Angleton et al. 1989, Akiyama et al. 1990). In our experiment, alcohol ingestion resulted in a rapid rise in PAI-1 activity irrespective of whether it was consumed in the morning or in the evening. This finding may depend on the ability of alcohol to stimulate tPA gene expression (Aikens et al. 1997, Grenett et al. 1998). Our findings suit to previous observations (Veenstra et al. 1990, Hendriks et al. 1994) and indicate that alcohol can disturb the normal circadian variation in fibrinolytic activity.

Although alcohol has been found to directly stimulate endothelin release from cultured human endothelial cells (Tsuji et al. 1992), we did not observe any marked variation in endothelin concentrations caused by alcohol ingestion.

In conclusion, our observations indicate that acute ingestion of a relatively large but tolerable dose of alcohol results in a marked elevation in plasma PAI-1 activity and a modest increase in urinary excretion of 2,3-dinor-TxB2. These effects seem to overcome the natural circadian variation in the haemostatic system and may favour thrombogenesis.

### 6.4. Postprandial effects of ingestion of alcohol

A moderate dose of red wine produced a decrease in SIPA irrespective of whether it was consumed with an evening meal or without. The effect was over after twelve hours. The effects of alcohol ingestion on SIPA have not been reported on earlier. There was a trend for a decrease in SIPA when eating an evening meal without alcohol, but the meal did not seem to have any additive effect on SIPA compared with that of the wine alone. Thus, the transient decrease in SIPA was not specific to alcohol.

In experimental studies using conventional methods to measure platelet function, platelet aggregation has been found to be attenuated by the ingestion of red wine (Ruf et al. 1995, Demrow et al. 1995). It has been claimed that this effect is not to due to ethanol but, to the phenolic compounds in red wine. This problem was not addressed in the design
of our study, but the addition of pure ethanol to a test tube containing PRP increased SIPA in a dose-dependent manner.

There were no changes in urinary excretion of either the thromboxane metabolites 11-dehydro-TxB₂ and 2,3-dinor-TxB₂ or the PGL₂ metabolite 2,3-dinor-6-keto-PGF₁α. The former finding indicates the absence of any platelet activation in vivo. Accordingly, ingestion of a moderate dose of red wine did not cause platelet activation (study III), whereas the intake of a larger dose of alcohol did (study II).

Eating a meal had no effect on PAI-1 activity, and the increase in PAI-1 after wine drinking was weaker than the corresponding increase caused by intake of a larger dose of alcohol in study II. Wine drinking also further enhanced the increasing effect of the meal in serum triglycerides. The PAI-1 values correlated with those of serum triglycerides in both the meal sessions and the wine sessions, but neither in the water sessions nor in the fast sessions. This suggests an induction of endothelial cells and is also in accordance with some previous reports (Mehrebiam et al. 1990, Salomaa et al. 1993, Kozima et al. 1993).

Ingestion of red wine inhibited the postprandial increase in vWF multimers, but had no effect on vWF:Ag values. We believe that this effect does not depend on decreased synthesis of vWF in endothelial cells, but merely on the effect of wine on vWF multimers that are already circulating. It is possible that the inhibition could contribute to the observed decrease in SIPA, although no correlation between vWF multimers and SIPA was observed, which is contrary to suggestions made in a previous study (Uchiyama et al. 1994). There are no previous reports on the effects of alcohol on vWF multimers except in alcoholic liver cirrhosis where an increased concentration of vWF multimers are though to be responsible for a hyperagglutination response with platelets (Beer et al. 1995).

In conclusion, the ingestion of a moderate dose of red wine induced a significant decrease in SIPA. This could be considered a beneficial effect on the haemostatic system, but the effect was not specific to alcohol. Instead of that, alcohol specifically but transiently attenuated fibrinolytic activity, which must be considered as a detrimental effect with regard to the risk of ischaemic stroke.

### 6.5. Platelets and ischemic brain infarction

Our main purpose was to show whether rebound thrombocytosis coincides with the time of onset of brain infarction among heavy drinkers of alcohol. This was not the case. Although a high platelet count and recent heavy drinking of more than 300 g of alcohol during the preceding week were both independent risk factors, the heavy drinkers had normal platelet counts at the onset of their stroke. Only when they had been abstaining from alcohol for the preceding week were their platelet counts higher than those of heavy drinkers who continued to consume alcohol. Only three out of the eight patients who had high platelet counts (> 450 x 10⁹/L) had a probable rebound thrombocytosis caused by withdrawal from alcohol. A higher than normal platelet count in itself was nevertheless found to be a common finding and a risk factor for ischaemic brain infarction.
Bias due to false-negative results could have been possible through the missing of those heavy drinkers who had stopped drinking alcohol earlier than one week before the onset of their stroke. By using the CAGE questions, however, we were able to include these patients in the group of heavy drinkers, so that bias is unlikely.

The platelet count at the onset of ischemic brain infarction has been investigated only in a few studies. Previous studies report lower platelet counts among stroke patients than among controls within 48 hours of the onset of stroke (D'Erasmo et al. 1990, O’Malley et al. 1995), whereas we found higher than normal platelet counts. The controls in these studies were different, which could explain the contradictory observations. Consumption of platelets due to thrombosis or haemodilution of platelets due to treatment procedures cannot be excluded as a confounding factor in the above-mentioned studies.

Changes in platelet TxB₂ formation after the onset of brain infarction seemed to be associated with alcohol consumption. Aggregation-associated TxB₂ release decreased in patients who had consumed less than 300 g of alcohol during the preceding week, whereas it increased during the first week in those who had consumed more. Indices of platelet release in plasma or urine have usually been higher in patients within the first few days after the onset of brain infarction than in controls (Mulley et al. 1983, Vicari et al. 1987, Joseph et al. 1989, Satoh et al. 1991, Koudstaal et al. 1993, D’Andrea et al. 1994, van Kooten et al. 1997), although the opposite findings have also been reported (Tohgi et al. 1991). This later study found an increase in platelet activity during the weeks following the brain infarction (Tohgi et al. 1991). Our finding of increased aggregation-associated TxB₂ release ex vivo in the acute phase of brain infarction probably reflects a metabolic change in the platelets and is in accordance with the majority of published reports. We did not find any support for the hypothesis that rebound thrombocytosis and associated platelet activation predispose heavy drinkers to ischaemic stroke. Accordingly, aetiologies other than platelet-dependent mechanisms should be looked for as risk factors for ischaemic stroke in these patients.

6.6. Pathophysiological mechanisms of brain infarction in heavy drinkers

Of the 212 patients classified by the TOAST criteria, 36% had a cardioembolic brain infarction. This is twice the frequency that the TOAST authors themselves had found in their hospital-based material of young patients (Adams et al. 1995), but the patients in our study were selected to be consecutive on the basis of complete investigations, which may explain the higher frequency of cardiac sources. The frequency of cryptogenic strokes was almost the same in both studies.

Previous studies investigating the risk entailed in acute alcohol intake for brain infarction have not all found an association. This can be explained by several pitfalls. Either the number of heavy drinkers has been too small to allow meaningful statistical analysis, or patients having atrial fibrillation have been excluded (You et al. 1997, Henrich & Horwitz 1989). One study which initially found a light weekly alcohol consumption to be a significant risk factor, was focused on middle-aged and elderly ischaemic brain infarction patients, among whom the habit of heavy drinking is rare. Not
surprisingly, the risk became insignificant after adjustment for cigarette smoking and hypertension (Gorelick et al. 1989).

Our main finding, that recent heavy drinking is associated with cardiogenic brain embolism in men, is not surprising, albeit a new one. The most important reason for cardioembolism in heavy drinkers is probably the high frequency of atrial fibrillation (Koskinen et al. 1987, Cohen et al. 1988). The provocation of atrial fibrillation by alcohol intake does not necessarily need any underlying cardiac disease (Koskinen et al. 1987). Subjects prone to alcohol-induced atrial fibrillation seem to develop an exaggerated sympathetic reaction during acute alcoholic intoxication (Mäki et al. 1998).

Drinking more than 40 g of alcohol during the 24 hours preceding the onset of the brain infarction also predisposed patients to cryptogenic and large-artery atherosclerosis-mediated infarctions. We assume that the group of cryptogenic infarctions included patients who actually had an embolic source which remained undiscovered. Patients with large-artery atherosclerosis probably suffered an artery to artery embolism in the brain, which may have been precipitated because of an intoxication-induced increase in blood flow. Changes in the haemostatic balance, either in fibrinolytic, endothelial or platelet-dependent mechanisms, probably have had some influence, especially in patients who already had large-artery atherosclerosis.

In conclusion, the main message of this study is that recent heavy drinking, including occasional and binge drinking, may trigger cardiogenic embolism to the brain.
7. Conclusions

The aim of the present research was to explore the mechanisms by which alcohol drinking could predispose subjects to an ischemic brain infarction or protect them against one. The experimental part focused on factors that are known to be important for the formation of an arterial thrombus, while the clinical studies were concentrated demonstrating the causes of an acute brain infarction triggered during alcoholic intoxication and ascertaining whether the alcohol-induced changes in platelet count and sensitivity to agonist contribute to the onset of stroke.

Acute heavy drinking of alcohol followed by a submaximal bicycle exercise was not able to precipitate platelet hyperactivity, despite marked increases in plasma catecholamines. The exercise, performed three times during the same day, caused some variation in platelet aggregation and associated TxB2 release, which were abolished rather than potentiated by the drinking of alcohol.

Acute heavy drinking of alcohol also abolished the circadian variation in fibrinolytic activity and platelet aggregability. Alcohol caused a 7 to 8-fold increase in PAI-1 activity, irrespective of whether it was ingested in the morning or in the evening. This finding is likely to associate with impaired fibrinolytic activity. Alcohol smoothed the diurnal variation in platelet aggregability to collagen, but had no significant effects on aggregation-associated thromboxane release. Neither did it alter the capacity of the blood to form thromboxane. Urinary excretion of 2,3-dinor-TxB2 increased significantly after alcohol ingestion, however, suggesting platelet activation in vivo. In contrast, urinary excretion of the prostacyclin metabolite 2,3-dinor-PGF1α was not influenced by alcohol drinking but was increased by a bicycle exercise.

Drinking of a moderate dose of red wine also led to a modest increase in PAI-1 activity, although the urinary excretion of thromboxane and prostacyclin metabolites was not influenced. The wine intake resulted in a slight transient decrease in shear-induced platelet aggregation, but the effect was not specific to alcohol, since eating a meal produced a similar effect. Ingestion of red wine inhibited the postprandial increase in vWF multimers, which may also be a beneficial effect.

A high platelet count and recent heavy drinking were both found to be independent risk factors for brain infarction. Heavy drinkers did not show any particular pattern in platelet counts or function at the onset of the stroke, although these parameters seemed to
be associated with the preceding pattern of alcohol consumption. The observations indicated that other aetiologies in addition to platelet-dependent mechanisms should be looked for as predisposing factors for brain infarction in heavy drinkers.

Recent heavy drinking was found to be associated with cardiogenic embolism to the brain in men. In addition, alcohol also seemed to predispose subjects to cryptogenic stroke and artery to artery embolism if they had a large-artery atherosclerotic carotid arterial disease. Accordingly, recent heavy drinking, including occasional and binge drinking, was found to be a significant triggering factor for several types of ischaemic brain infarction.

These observations demonstrate that heavy drinking of alcohol should be avoided because it predisposes to cardiogenic embolism in the brain. Alcohol abuse causes clear effects which influence the fibrinolytic and haemostatic systems. Some of the observed effects may be considered beneficial, whereas others are detrimental. This disparity may depend on the amount of alcohol consumed. Drinking of a moderate dose of red wine together with an evening meal showed a slight beneficial effect on the haemostatic system, but simultaneously a slight untoward effect on the fibrinolytic system.
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