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Effects of mental and physical stress on platelet function in patients with stable angina pectoris and healthy controls

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The effects of mental and physical stress on platelet function in patients with stable angina pectoris and healthy controls were investigated. Platelet function was studied at rest, and during mental stress (colour word test), or after exercise (bicycle ergometry), in 113 angina patients (21 on aspirin) and 50 matched controls. Platelet function was assessed by filtragometry ex vivo (reflecting platelet aggregability), by measuring platelet secretion (β-thromboglobulin and platelet factor 4 levels in plasma), and by Born aggregometry in vitro.

At rest, platelet function did not differ between patients and controls. Exercise increased platelet aggregability and secretion similarly in both groups. Aspirin did not attenuate the platelet activating effect of exercise despite inhibition at rest. Mental stress increased heart rate, blood pressure and plasma catecholamines, but platelet responses were highly variable. However, mental stress tended to shorten filtragometry readings in patients but not in controls ($P<0.05$ between the groups); plasma β-thromboglobulin showed a similar difference between patients and controls ($P<0.05$ between the groups; aspirin-treated patients included).

Physical exercise activates platelets in patients with stable angina pectoris and healthy controls. Aspirin is not an effective inhibitor of exercise-induced platelet aggregation. Platelet responses to mental stress are variable, but more pronounced in angina patients.
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Key Words: Platelet aggregation, platelet secretion, physical exercise, mental stress, ischaemic heart disease.

Introduction

Platelet activation plays a prominent role in arterial thrombosis, a phenomenon closely linked to acute myocardial infarction and sudden cardiac death[1]. Acute physical exertion increases the risk of having an acute myocardial infarction, especially in subjects with a sedentary life style[2,3], and there is mounting support that mental stress precipitates acute coronary syndromes[4]. The diurnal pattern of myocardial infarction and sudden cardiac death coincides with increased sympathoadrenal activity and increased platelet aggregability[5].

It may be hypothesized that stress-induced platelet activation with an increased risk of thrombus formation may be a link between stress and acute ischaemic coronary events. However, to what extent physical exertion or mental stress really cause platelet activation is still a matter of dispute[6–8]. Prothrombotic stimuli during stress may include 'stress hormones', such as catecholamines. We have previously shown that both adrenaline[9], and noradrenaline[10], have significant platelet activating effects in vivo in humans at physiologically and pathophysiologically relevant plasma concentrations. Interestingly, catecholamine-mediated platelet activation is only partially attenuated by aspirin treatment[10,11].

To further study the impact of stress on platelet function, we exposed patients with stable angina pectoris and healthy controls to a mental stress test, and a physical exercise test. Platelet function in vivo was evaluated by assessments of platelet secretion (β-thromboglobulin [βTG] and platelet factor 4 [PF4] levels in venous plasma), and platelet aggregability (filtragometry ex vivo). In addition, the effect of ADP, and the sensitizing effect of adrenaline on ADP-induced platelet aggregation, were measured in platelet-rich plasma from both patients and controls.

Methods

Subjects

The studies were approved by the Ethics Committee of the Karolinska Institute. All subjects gave their

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informed consent before participating. The present investigation was performed as a substudy of a prospective study of stable angina pectoris (the APSIS study) comprising 809 patients; the design and main treatment results have recently been described[12].

Patients with a typical history of angina pectoris (effort-induced, angina at rest or mixed type) below 70 years of age were included. Patients treated with β-blockers or calcium antagonists were switched to treatment with minimal doses of one of the study drugs, 25–50 mg . day^{-1} metoprolol or 40–80 mg . day^{-1} verapamil, for a run-in period of 2 weeks before the present baseline examination was performed. Aspirin-free patients were instructed to avoid platelet-active drugs during the run-in period before the experiments.

The healthy controls, matched with respect to age and sex to the entire population of the APSIS study, were recruited via the population registry of Stockholm county. The subjects underwent a thorough medical examination before inclusion, and were all free of signs of disease. Fifty controls participated in the exercise test; 21 of them also participated in mental stress experiments.

Protocol

The investigations were performed between 0900h and 1200h. Filtragometry measurements and blood sampling were performed after 30 min of rest in the supine position, and were repeated immediately the exercise test ended, or during the colour word test (after 15 min). A symptom-limited exercise test was carried out on a computer-assisted bicycle ergometer (Siemens AB, Solna, Sweden) starting at a workload of 30 W with increments of 10 W . min^{-1}. Perceived levels of chest pain and exertion during exercise were assessed by 10 and 20 graded category–ratio scales. Healthy controls exercised until exhaustion (Borg scale ≥ 17). Mental stress was induced by a modified video-taped version of Stroop's colour word conflict test[13]. Blood pressure, heart rate and ECG were measured repeatedly during the tests.

Filtragometry ex vivo

Platelet aggregability was assessed by filtragometry ex vivo, which measures platelet aggregates in blood continuously drawn from an antecubital vein[9,14]. Each reading requires a new venepuncture by a 19 G Butterfly needle. Heparin (final concentration 5 IU . ml^{-1}) is infused into the siliconized tubing system leading the blood to the apparatus. The time (tA; aggregation time) taken to occlude 25% of the pore area of a nickel filter (pore size 20 μm) is measured, and is inversely related to platelet aggregability. Due to a transient shortage of filters for filtragometry, five healthy controls (exercise experiments) had to be investigated using filters with a smaller surface area (diameter 2·0 mm instead of 2·3 mm). Filtragometry readings at rest tended to be shorter, but reactivity to exercise was identical with these filters. Therefore, these five controls were included in comparison of responses to exercise, but not of resting values.

β-thromboglobulin and PF4 in plasma

Sampling for measurements of βTG and PF4 in plasma was performed according to a validated procedure[15]. In brief, an antecubital vein was punctured with a Wasserman 18 G needle. After discarding the first 2 ml, 8 ml of blood was allowed to drip into ice-cooled sampling tubes containing 0·8 ml of a platelet-stabilizing solution (final concentrations: 9 mmol . l^{-1} EDTA, 1 mmol . l^{-1} theophylline and 1·4 μmol . l^{-1} prostaglandin E_1). All samples were immediately centrifuged at 15 000 × *g* (4 °C, 30 min). Plasma was carefully removed and stored at −80 °C. βTG immunoreactivity was analysed as described previously[16]. PF4 was analysed using an EIA-kit (Diagnostica Stago, Asnières, France).

Platelet aggregation in vitro

Born aggregometry was performed at rest only, as platelet activation in vivo (for instance during physical exercise or mental stress) may be difficult to interpret with in vitro tests[17]. Platelet-rich and platelet-poor plasma were prepared from venous blood anticoagulated with sodium citrate (final concentration 0·38% weight/volume) by centrifugation at 190 × *g* and 1400 × *g* for 10 min, respectively. Platelet aggregation was studied by using a four-channel platelet aggregation profiler (PAP-4, Bio/Data Corp., Hatboro, PA). ADP and L-adrenaline (Sigma Chemical Co., St Louis, MO) were diluted in TRIS-buffer and physiological saline with ascorbic acid (9·4 μg . ml^{-1} final concentration), respectively. Platelet aggregation was evaluated as previously described[11]. The EC_{50} for ADP-induced final aggregation (i.e. extent of aggregation after 4 min) and for primary aggregation (extent of aggregation during first minute), were determined; the rate of aggregation (i.e. slope) during the initial phase was also assessed, as was the enhancing effect of adrenaline (at 10 and 50 nmol . l^{-1}).

Other biochemical assays and blood cell counts

Catecholamines in venous plasma were determined as previously described[18]. Platelet counts and median platelet volume in whole blood (anticoagulated with EDTA, final concentration 10 mmol . l^{-1}) were measured 2–3 h after sampling using a cell counter (Cellanalyzer 460, Medonic AB, Solna, Sweden). Plasma fibrinogen was analysed by a polymerization test[19].

Table 1 Characteristics of angina patients and healthy controls

	Healthy controls n=50	Angina patients (aspirin free) n=92	Angina patients (on aspirin) n=21
Age (mean and range)	61 (41–73)	59 (43–69)	60 (39–69)
Sex (males/females; %)	70/30	79/21	90/10
Current smokers (%)	26	23	19
Fibrinogen (mean ± SD; $g \cdot l^{-1}$)	3·2 ± 0·6	3·6 ± 1·0	4·2 ± 1·1
Duration of angina (months; median and interquartiles)	—	12 (6–36)	12 (3·5–90)
Previous myocardial infarction (%)	—	8	24

Statistics

Due to asymmetrical distribution of data, filtragometry readings and platelet secretion data were logarithmically transformed prior to statistical evaluation. Unless otherwise stated, these data are presented as mean log values ± SD; the antilogarithm of the corresponding mean log value is also shown. Platelet reactivity to the interventions were calculated as the differences between log values for interventions and resting measurements (when values were ≤ 1, the log transformation procedure for this variable was performed using log[1+x]). A ratio was obtained by antilogarithmic transformation of the difference[20]. Born aggregometry data are presented as medians and interquartiles. Statistical evaluation was performed by an unpaired t-test or by Mann–Whitney's U-test, and by a paired t-test or by Wilcoxon's signed rank test.

Results

One-hundred and thirteen patients and 50 healthy volunteers were included. Their characteristics are shown in Table 1. Ninety-two patients were free from aspirin. Fifty-four of these patients (59%) were on minimal metoprolol treatment, six (7%) on minimal verapamil treatment and 37 (40%) on long-acting nitrates. Five patients were on ACE inhibitors, two on digoxin and seven on diuretics. Twenty-one patients were on aspirin. Fourteen of them (67%) received minimal metoprolol, and two minimal verapamil treatment (10%); 13 (62%) were on long acting nitrates. In addition, one patient received digoxin and three were on diuretics.

Plasma catecholamines, cardiovascular variables, and platelet counts and median platelet volume in whole blood, are shown in Table 2. As can be seen, the median platelet volume increased significantly in response to exercise in the patients (from 9·3 ± 0·7 fl at rest to 9·5 ± 0·8 fl after exercise; $P<0·01$), but remained unchanged in the controls. Platelet counts in platelet-rich plasma were 396 ± 73 and 394 ± 78 × $10^{-9} \cdot l^{-1}$ in angina patients and controls, respectively.

Platelet function at rest

Platelet function in vivo did not differ between angina patients and healthy volunteers at rest. Filtragometry readings at rest were 209 s in the patients (n=92) and 170 s in the controls (n=44; log values 2·32 ± 0 34 and 2·23 ± 0 29, respectively; $P=0·12$). Plasma βTG levels at rest were 25 and 23 $ng \cdot ml^{-1}$ in patients (n=54) and controls (n=28), respectively (log values: 1·39 ± 0·15 and 1·36 ± 0·15; $P=0·41$); corresponding levels of PF4 were 1·9 $U \cdot ml^{-1}$ (0·27 ± 0·24) in the patients and 1·6 $U \cdot ml^{-1}$ (0·21 ± 0·22) in controls ($P=0·10$). Filtragometry readings were significantly longer in aspirin-treated patients (309 s [2·49 ± 0·35]; n=20) than in patients not on aspirin ($P<0·05$; unpaired t-test); but platelet secretion variables did not differ.

Effects of physical exercise

Platelet aggregability (Figs 1 and 2) and platelet secretion (Fig. 1) were significantly enhanced by exercise. In angina patients performing exercise the filtragometry readings were reduced from 186 s (2·27 ± 0·32) to 110 s (2·04 ± 0·27) immediately after exercise (n=48, $P<0·001$). Levels of βTG in plasma increased from 22 $ng \cdot ml^{-1}$ (1·35 ± 0·14) to 26 $ng \cdot ml^{-1}$ (1·42 ± 0·15; n=20; $P<0·01$), and levels of PF4 in plasma increased from 1·7 $U \cdot ml^{-1}$ (0·24 ± 0·14) at rest to 2·3 $U \cdot ml^{-1}$ (0·37 ± 0·21) after exercise (n=20; $P<0·05$).

In aspirin-treated patients, filtragometry measurements were 513 s (2·72 ± 0·27) at rest and 178 s (2·25 ± 0·36) immediately after exercise ($P<0·01$, n=8; Fig. 2). Sampling for βTG and PF4 was only possible in four subjects, but all four subjects showed increased platelet secretion following exercise; βTG increased from 25 to 45 $ng \cdot ml^{-1}$, and PF4 from 1·6 $U \cdot ml^{-1}$ to 6·8 $U \cdot ml^{-1}$. When all patients were analysed together, βTG levels increased from 23 to 29 $ng \cdot ml^{-1}$ ($P<0·01$; n=24), and PF4 levels increased from 1·7 to 2·8 $U \cdot ml^{-1}$ ($P<0·01$; n=24).

Among controls, filtragometry readings were 174 s (2·24 ± 0·30) at rest and 91 s (1·96 ± 0·26; n=41, $P<0·001$) after exercise. βTG rose from 22 $ng \cdot ml^{-1}$

Table 2 Effects of mental stress and physical exercise on cardiovascular variables, levels of catecholamines in venous plasma and whole blood platelet counts, in angina patients and healthy subjects

	Mental stress		Physical exercise	
	Healthy subjects	Angina patients	Healthy subjects	Angina patients
Work capacity (Watts)			153 ± 45§	123 ± 33
Heart rate (beats . min^{-1})				
Baseline	63 ± 11	64 ± 10	64 ± 10	61 ± 9
5 min	86 ± 17	81 ± 15		
End	81 ± 14†	80 ± 16†	157 ± 15†	134 ± 22†
Systolic blood pressure (mmHg)				
Baseline	122 ± 12	124 ± 19	134 ± 19	135 ± 20
5 min	154 ± 14	152 ± 25		
End	151 ± 15†	151 ± 26†	194 ± 27†	182 ± 32†
Noradrenaline (nmol . l^{-1})				
Baseline	2·65 ± 0·82	2·74 ± 1·06	2·12 ± 0·67	2·37 ± 0·93
End	3·02 ± 0·93*	3·30 ± 1·07†	15·09 ± 5·44†	12·38 ± 6·30†
Adrenaline (nmol . l^{-1})				
Baseline	0·21 ± 0·10	0·25 ± 0·17	0·18 ± 0·16	0·17 ± 0·11
End	0·35 ± 0·17†	0·44 ± 0·32†	0·95 ± 0·79†	0·58 ± 0·41†
Platelet count (× 10^{-9} . l^{-1})				
Baseline	227 ± 42	227 ± 37	232 ± 46	227 ± 42
End	232 ± 47	233 ± 39†	264 ± 56†	258 ± 52†
Median platelet volume (MPV;fl)				
Baseline	9·4 ± 0·6	9·4 ± 0·7	9·3 ± 0·9	9·3 ± 0·7
End	9·3 ± 0·7	9·3 ± 0·7	9·3 ± 0·8‡	9·5 ± 0·8*

Data are presented as mean ± SD.
Statistical calculations within groups were performed by one-way ANOVAs (repeated measures design; heart rate and blood pressure) or paired t-tests (*P<0·01, †P<0·001).
For differences between groups unpaired t-tests were used (‡P<0·05, §P<0·01).

(1·34 ± 0·11) to 28 ng . ml^{-1} (1·45 ± 0·18) after exercise (n=14; P<0·01), and PF4 increased from 1·4 U . ml^{-1} (0·16 ± 0·15) to 2·5 U . ml^{-1} (0·39 ± 0·27) after exercise (n=14; P<0·01).

Effects of mental stress

Relative platelet responses to mental stress are shown in Fig. 3. In the angina patients, filtragometry readings were reduced from 240 s (2·38 ± 0·35) at rest to 200 s (2·30 ± 0·31) during the colour word test (n=43, P=0·16). βTG in plasma increased from 25 to 28 ng . ml^{-1} (1·40 ± 0·15 to 1·45 ± 0·26; n=32, P=0·16), and PF4 increased from 1·9 U . ml^{-1} (0·28 ± 0·28) at rest to 2·1 U . ml^{-1} (0·33 ± 0·41) during mental stress (n=32, P=0·32). Filtragometry data were not greatly affected by inclusion of aspirin-treated patients, whereas plasma βTG increased from 23 to 28 ng . ml^{-1} (1·37 ± 0·15 to 1·45 ± 0·26; n=45, P<0·05), and PF4 from 1·7 to 2·2 U . ml^{-1} (0·24 ± 0·25 to 0·35 ± 0·45; P=0·06) during the colour word test.

Among healthy volunteers the filtragometry readings tended to be prolonged from 135 s (2·13 ± 0·31) at rest to 186 s (2·27 ± 0·38) during the colour word test (n=21, P=0·12). Plasma βTG did not change in response to mental stress; levels were 21 ng . ml^{-1} (1·33 ± 0·12) at rest and 19 ng . ml^{-1} (1·28 ± 0·10; n=14; P=0·21) during stress. PF4 levels were slightly reduced, from 1·6 U . ml^{-1} (0·20 ± 0·15) at rest to 1·3 U . ml^{-1} (0·10 ± 0·07; P<0·05) during stress.

The reactivity to mental stress differed between groups with respect to filtragometry readings and βTG levels in plasma; PF4 levels also tended to differ (see Fig. 3).

Effects of ADP and adrenaline on platelet aggregation in vitro

The sensitivity to ADP in vitro (EC_{50} for final aggregation; median and interquartiles), was 1·1 μmol . l^{-1} (0·8–1·6) in patients and 1·0 μmol . l^{-1} (0·8–1·6) in controls; corresponding EC_{50} values for primary aggregation were 1·1 μmol . l^{-1} (0·9–1·3) and 1·0 μmol . l^{-1} (0·8–1·3), respectively (n=75 and n=48; P=0·44 and P=0·88 for group comparisons of final and primary aggregation, respectively). ADP sensitivity was profoundly reduced in the aspirin-treated patients (EC_{50} for final aggregation was 2·8 μmol . l^{-1} [1·6–3·8]; n=20; P<0·001 compared to patients not on aspirin). The enhancing effect of 10 nmol . l^{-1} adrenaline on ADP-induced aggregation was slightly lower among patients compared to controls; the slope of aggregation was enhanced by 2 (0·5–4) units in patients and 4 (2–6) units

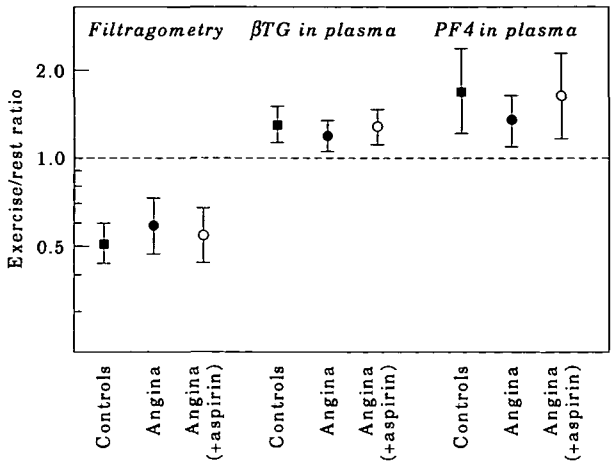

Figure 1 **Effects of physical exercise on platelet aggregability (filtragometry readings) and platelet secretion (β-thromboglobulin [βTG] and platelet factor 4 [PF4] levels in plasma). Data are presented as means and 95% confidence intervals for intervention/rest ratios. Filtragometry readings were shortened by 49% in controls (■), 41% in aspirin-free angina patients (●) and 46% in all patients (○). Corresponding alterations in βTG levels were +29%, +18% and +27%, and for PF4 +68%, +33% and +62%. There were no statistically significant differences in relative effects of exercise between patients and controls.**

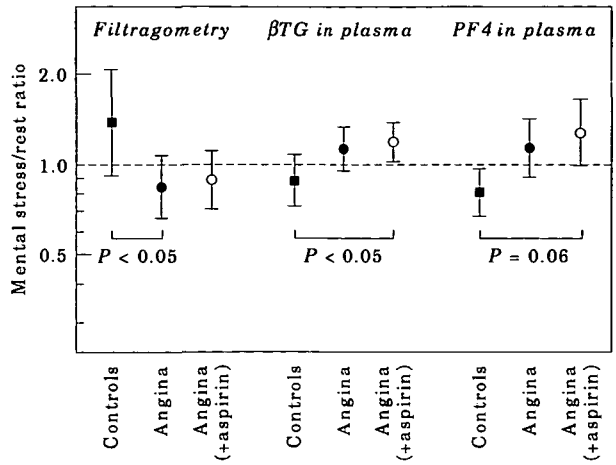

Figure 3 **Effects of mental stress on platelet aggregability (filtragometry readings) and platelet secretion (β-thromboglobulin [βTG] and platelet factor 4 levels [PF4] in plasma). Data are presented as means and 95% confidence intervals for intervention/rest ratios. Alterations in filtragometry readings were +38% in controls (■), −16% in aspirin-free patients (●) and −11% in all angina patients (○). Corresponding alterations in βTG levels were −11%, +13% and +19%, and for PF4 −19%, +14% and +28%. The relative effects of mental stress were significantly different with respect to filtragometry readings (aspirin free patients vs controls), and βTG levels (all patients vs controls; $P<0.05$ for both); PF4 levels also tended to differ ($P=0.06$; unpaired t-test).**

in controls, and the extent of aggregation was enhanced by 16 (12–28) units in patients, and 25 (15–39) units in controls ($P<0.05$ for both). Effects of 50 nmol . l^{-1} adrenaline did not differ between groups (data not shown).

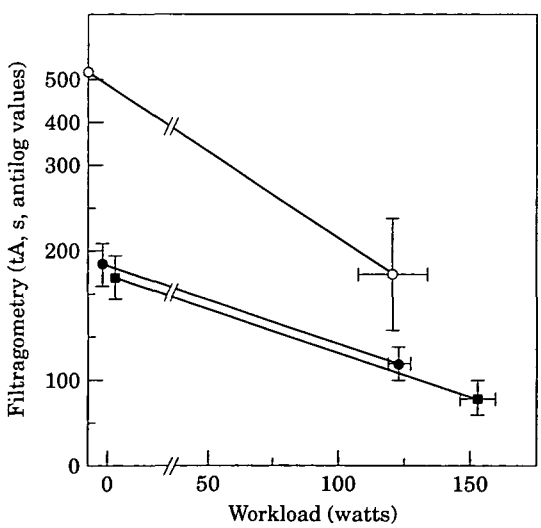

Figure 2 **Platelet aggregability (filtragometry readings) in relation to workload in aspirin-free angina patients (●, n=48), angina patients on aspirin treatment (125–500 mg daily; ○, n=8) and healthy controls (■, n=41). Data are shown as mean ± SEM.**

Platelet function in relation to gender and smoking

Healthy men and women did not differ in the basal state. Healthy men tended to respond more strongly to exercise but also had higher work loads (170 vs 110 Watts; $P<0.001$); exercise/rest ratios for filtragometry readings were 0·47 in men (n=32) and 0·65 in women (n=14; $P=0.06$). There were no gender differences with respect to platelet responses to mental stress among either controls or patients.

In the in vitro measurements, platelets from women with angina were more sensitive to ADP, but less responsive to high dose adrenaline than were platelets from male patients; EC_{50} values for ADP-induced primary aggregation were 0·8 (0·7–1·0) μmol . l^{-1} in females (n=17) and 1·1 (0·9–1·3) μmol . l^{-1} in males (n=58; $P<0.01$), whereas the potentiating effect of 50 nmol . l^{-1} adrenaline on the slope of ADP-induced aggregation (at the EC_{50} for ADP) was 5 (3–6) units in females and 8 (5–11) units in males ($P<0.01$).

Healthy smokers did not differ from non-smokers, either at rest or during interventions. Smoking angina patients had higher plasma βTG levels at rest compared to non-smokers (31 vs 24 ng . ml^{-1}; n=13 and n=42; $P<0.05$), but other platelet parameters did not differ.

Correlations

Among healthy controls, increments in platelet aggregability (filtragometry) during exercise were related to increments in heart rate (Fig. 4) and to workload (r=0·56, $P<0·001$, n=46); among angina patients there was a similar but weaker relationship between aggregability and heart rate (Fig. 4). Correlations between filtragometry and catecholamine responses to exercise were considerably weaker; among controls, filtragometry responses tended to correlate with increases in noradrenaline (r=0·30, $P=0·073$) and adrenaline (r=0 25, $P=0·096$).

Discussion

The present study shows that physical and mental stress influences platelet function in vivo. Physical exercise clearly increased platelet aggregability and platelet secretion in both angina patients and healthy controls. Platelet responses to mental stress were variable, but angina patients were clearly more prone to respond with platelet activation than controls.

In the angina patients, plasma βTG increased significantly during mental stress, whereas effects on PF4 levels and platelet aggregability were less clear-cut. Among healthy controls, platelet function, if anything, was attenuated by mental stress. Gross haemodynamic and catecholamine responses to the stress test were similar in the two groups, even though platelet activation was more pronounced among angina patients. However, catecholamines are unlikely to mediate platelet activation by mental stress, as neither adrenaline nor noradrenaline reach plasma concentrations high enough to activate platelets in vivo[6,10]. The mechanism(s) behind the different platelet response patterns of angina patients and healthy controls are unclear and cannot be explained by the present study.

Previously, we have shown that mental stress evoked by the present colour word test, increases platelet aggregability in young healthy male volunteers[6]; this did not occur in the present elderly healthy individuals, despite clearcut haemodynamic and plasma catecholamine responses. The reason behind this discrepancy is unclear, but the findings are nevertheless interesting. The colour word test evokes pronounced vasodilatation in the forearm in younger individuals[21]; we do not know if this occurs to the same degree in elderly individuals. Flow-mediated shear stress activates platelets[22], and stimulates the release of nitric oxide from the normal vascular endothelium[23]. Perhaps healthy elderly individuals have a different balance between platelet activating and inhibiting systems during vasodilatation compared to young individuals. This, however, remains to be examined.

We found clear-cut platelet aggregability and secretory responses to exercise. Previous studies on this issue have yielded variable results[7]. Elevations of βTG in plasma following dynamic exercise have been reported in healthy individuals[24–30], as well as in patients

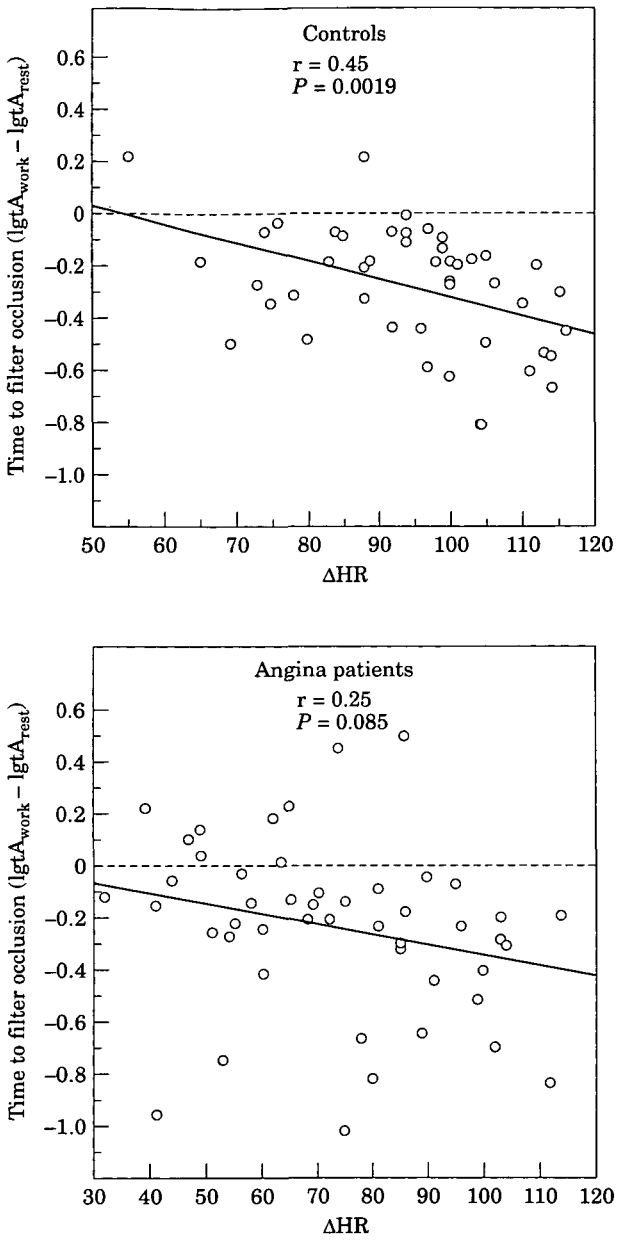

Figure 4 **Relationships between maximal increments in heart rate (HR) and alterations in platelet aggregability (filtragometry readings) in response to physical exercise in controls (upper panel) and angina patients (lower panel); if patients on verapamil and metoprolol were excluded, the relationship improved (r=0·53, $P<0·05$, n=16).**

with coronary artery disease[29,30], hypertension[31] or diabetes[32]. However, others have failed to find such effects in healthy individuals[33–38] or in patients with coronary artery disease[35,36,38,39]. Measurements of platelet secretory products in plasma, such as βTG, are associated with considerable methodological difficulties[15,40]. We have found that sampling through venous catheters or butterfly needles (as performed in several of the 'negative' studies reviewed above[35,37,38]), lack of adequate platelet stabilizing additives in the sampling

tubes and/or delays in the centrifugation of samples (also practiced in several 'negative' studies[35,36,39]) can easily lead to artifactual platelet release[15]. There are similar problems regarding PF4 responses to exercise. Elevations of plasma PF4 have been reported in healthy subjects[27,28,30,41] and in patients with coronary artery disease[28,30,42–44], whereas others fail to find such effects in healthy subjects[35,37,38,45] or in patients with coronary artery disease[35,38,39,45,46]. In the present study, data on PF4 levels in plasma were slightly more variable than βTG data, but the main findings were similar, with signs of increased platelet secretion from basal βTG levels around 20–25 ng . ml^{-1} and PF4 levels around 1–2 U . ml^{-1}. The 'positive' studies noted above tend to have lower resting βTG levels and better sampling procedures than the 'negative' ones, but the methods descriptions are frequently incomplete. Thus, our conclusion is that exercise indeed increases platelet secretion in vivo and that discrepancies in the literature may be explained by methodological differences.

Our data suggest that the magnitude of platelet activation is related to the degree of exertion, in accordance with other studies. Schernthaner *et al.*, who measured PF4 and βTG in plasma before and after a symptom-limited exercise test in healthy subjects and patients with coronary artery disease, found significant platelet secretion only in those who reached more than 75% of their calculated maximal exercise capacity[30]. Others have also found that exercise-induced platelet activation is time[28] and intensity[47] dependent.

Several mechanisms may contribute to the platelet-activating effect of physical exercise, including serotonin and catecholamine stimulation[48], as well as shear induced aggregation brought about by increased cardiac output and alterations in blood flow[22,49]. We found no clear-cut relationships between plasma catecholamine and platelet responses to exercise but this does not rule out that noradrenaline contributes to exercise-induced platelet activation.

Noradrenaline infusion elicits concentration dependent shortening of filtragometry readings and elevates plasma βTG at plasma concentrations similar to, or lower than, those found after exercise in the present study[10]. Plasma adrenaline levels during exercise were not sufficiently high enough to cause significant platelet activation in vivo[17]. The previously observed relationship between platelet secretion and adrenaline[28] may reflect that adrenaline is a marker of exertion[50]. Intensity and duration of exercise have been shown to influence the magnitude of thrombin generation[51,52], and it is tempting to speculate that thrombin may also be involved.

Aspirin treatment was associated with prolonged filtragometry readings at rest, as expected, but did not attenuate responses to exercise, in accordance with our observations regarding noradrenaline-induced platelet aggregation[10]. In addition, platelet secretory responses to exercise seem to be preserved. Thus, aspirin treatment does not seem to protect against exercise-induced platelet activation.

The absence of significant differences between angina patients and healthy controls with respect to platelet aggregability or platelet secretory responses in vivo to exercise should be interpreted with caution, as the platelet-activating effects of exercise seem to be intensity dependent and the healthy controls exercised more vigorously. A different exercise protocol, with equal exertion in both groups, might reveal enhanced platelet reactivity among patients also during exercise. Indeed, there was a greater heterogeneity with respect to platelet responses to exercise among angina patients (see Fig. 4), with some patients responding very vigorously. Thus, platelets may well be more responsive to a variety of stressors in stable angina pectoris.

Increased platelet volume has been related to poor outcome after acute myocardial infarction[53], and larger platelets seem to be more haemostatically active[54]. We found no obvious differences in platelet volume at rest between controls and patients. Median platelet volume increased significantly after exercise among patients, but not controls. Increased platelet volume following vigorous exercise may be related to adrenergically mediated release from the spleen[55]. However, it seems unlikely that platelet release from the spleen would be greater among patients as judged by blood pressure and catecholamine responses. Possibly, the exercise-induced increase in platelet volume might reflect an increased occurrence of platelet shape change (i.e. more pronounced in vivo activation) in the angina patients.

It should be emphasized that mental stress and physical exercise differ substantially with respect to physiological response patterns[56]; for example, the different forearm vascular responses — vasodilatation during mental stress and vasoconstriction during leg exercise — may be important in this context (sampling is performed in the forearm). Platelet-activating mechanisms during mental stress probably differ from those operating during physical exercise, and it is interesting that mental stress differentiates patients and controls more clearly than exercise.

Platelet function at rest did not differ between angina patients and matched healthy controls, in agreement with results from previous larger studies. The Northwick Park Heart Study found similar ADP induced aggregation in vitro in men with and without a history of ischaemic heart disease[57]. The Caerphilly study found no relationships between platelet aggregability in vitro and the presence or absence of angina pectoris, although aggregation was enhanced in individuals with previous myocardial infarction and/or electrocardiographic evidence of coronary ischaemia[58]. Thus, platelets from patients with stable angina pectoris do not seem to by hyper-reactive in the basal state. Surprisingly, the proaggregatory effects of adrenaline in vitro were less pronounced in our patients. However, aggregability in platelet rich-plasma may poorly reflect platelet function in vivo, due to loss of cells during sample preparation and the unphysiological environment used for in vitro studies[11,17].

There was a slight imbalance between the groups with respect to sex (21% females in the patient group vs 30% in the control group), as the controls were matched with respect to the entire APSIS population, and not to the platelet substudy population (which required good veins for methodological reasons). It is, however, unlikely that this greatly influenced the results, as the imbalance was slight and sex differences were mainly found for in vitro data (greater ADP sensitivity and reduced adrenaline sensitivity in females).

In conclusion, heavy exercise has a significant platelet activating effect which is evident in both patients with stable angina pectoris and matched healthy controls, and which does not seem to be attenuated by aspirin treatment. The effect of mental stress on platelet function, on the other hand, shows considerable inter-individual variability and more pronounced platelet responses among angina patients. Stress-induced platelet activation in vivo may contribute to atherothrombotic complications, and is also of interest when evaluating new antiplatelet regimens.

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References

[1] Fuster V, Badimon L, Badimon JJ, Chesebro JH. The pathogenesis of coronary artery disease and the acute coronary syndromes. N Engl J Med 1992; 326: 242–50, 310–18.

[2] Willich SN, Lewis M, Löwel H, Arntz H-R, Schubert F, Schröder R. Physical exertion as a trigger of acute myocardial infarction. N Engl J Med 1993; 329: 1684–90.

[3] Mittleman MA, Maclure M, Tofler GH, Sherwood JB, Goldberg RJ, Muller JE. Triggering of acute myocardial infarction by heavy physical exertion. N Engl J Med 1993; 329: 1677–83.

[4] Willich SN, Maclure M, Mittleman M, Arntz H-R, Muller JE. Sudden cardiac death. Support for a role of triggering in causation. Circulation 1993; 87: 1442–50.

[5] Tofler GH, Brezinski D, Schafer AI *et al.* Concurrent increase in platelet aggregability and the risk of myocardial infarction and sudden cardiac death. N Engl J Med 1987; 316: 1514–18.

[6] Hjemdahl P, Larsson PT, Wallén NH. Effects of stress and β-blockade on platelet function. Circulation 1991; 84 (Suppl VI): VI-44–VI-61.

[7] Kestin AS, Ellis PA, Barnard MR, Errichetti A, Rosner BA, Michelson AD. Effect of strenuous exercise on platelet activation state and reactivity. Circulation 1993; 88 (part 1): 1502–1511.

[8] Hjemdahl P. Editorial: Platelet reactivity, exercise and stable coronary heart disease. Eur Heart J 1995; 16: 1017–19.

[9] Larsson PT, Hjemdahl P, Olsson G, Egberg N, Hornstra G. Altered platelet function during mental stress and adrenaline infusion in humans: evidence for an increased aggregability in vivo as measured by filtragometry. Clin Sci 1989; 76: 369–76.

[10] Larsson PT, Wallén NH, Hjemdahl P. Norepinephrine induced human platelet activation in vivo is only partly counteracted by aspirin. Circulation 1994; 89: 1951–7.

[11] Wallén NH, Held C, Rehnqvist N, Hjemdahl P. Impact of treatment with acetylsalicylic acid on the proaggregatory effects of adrenaline in vitro in patients with stable angina pectoris: influence of the anticoagulant. Clin Sci 1993; 85: 577–83.

[12] Rehnqvist N, Hjemdahl P, Billing E *et al.* Effects of metoprolol versus verapamil in patients with stable angina pectoris. Eur Heart J 1996; 17: 76–81.

[13] Frankenheuser M, Mellis I, Rissler A, Björkvall C, Patkai P. Catecholamine excretion as related to cognitive and emotional reaction patterns. Psychosom Med 1968; 30: 109–20.

[14] Hornstra G, ten Hoor F. The filtragometer: A new device for measuring platelet aggregation in venous blood of man. Thromb Diath Haemorrh 1975; 34: 531–44.

[15] Beck O, Wallén NH, Bröijersén A, Larsson PT, Hjemdahl P. On the accurate determination of serotonin in human plasma. Biochem Biophys Res Comm 1993; 196: 260–6.

[16] Hjemdahl P, Perneby C, Theodorsson E, Egberg N, Larsson PT. A new assay for β-thromboglobulin in urine. Thromb Res 1991; 64: 33–43.

[17] Larsson PT, Hjemdahl P, Olsson G, Angelin B, Hornstra G. Platelet aggregability in humans: contrasting *in vivo* and *in vitro* findings during sympatho-adrenal activation and relationship to serum lipids. Eur J Clin Invest 1990; 20: 398–405.

[18] Hjemdahl P. Catecholamine measurements in plasma by high-performance liquid chromatography with electrochemical detection. Methods Enzymol 1987; 142: 521–34.

[19] Vermylen C, de Vreker R, Verstraete M. A rapid enzymatic method for assay of fibrinogen fibrin polymerization time (FPT test). Clin Chim Acta 1963; 8: 418–24.

[20] Fleiss JL. In: Design and analysis of clinical experiments. John Wiley & Sons, New York, 1986: 67.

[21] Lindqvist M, Kahan T, Melcher A, Hjemdahl P. Cardiovascular and sympatho-adrnal responses to mental stress in primary hypertension. Clin Sci 1993; 85: 401–9.

[22] O'Brien JR. Shear induced platelet aggregation. Lancet 1990; 335: 711–13.

[23] Busse R, Mülsch A, Fleming I, Hecker M. Mechanisms of nitric oxide release from the vascular endothelium. Circulation 1993; 87 (Suppl V): V-18–V-25.

[24] Naesh O, Hindberg I, Trap-Jensen J, Lund JO. Post-exercise platelet activation–aggregation and release in relation to dynamic exercise. Clin Physiol 1990; 10: 221–30.

[25] Piret A, Niset G, Depiesse E *et al.* Increased platelet aggregability and prostacyclin biosynthesis induced by intense physical exercise. Thromb Res 1990; 57: 685–95.

[26] Vicari AM, Macagni A. Primary platelet activation in recent-onset type I diabetes mellitus. Scand J Clin Lab Invest 1990; 50: 429–32.

[27] Röcker L, Drygas WK, Heyduck B. Blood platelet activation and increase in thrombin activity following a marathon race. Eur J Appl Physiol 1986; 55: 374–80.

[28] Stratton JR, Malpass TW, Ritchie JL, Pfeifer MA, Harker LA. Studies of platelet factor 4 and Beta thromboglobulin release during exercise: lack of relationship to myocardial ischemia. Circulation 1982; 66: 33–43.

[29] Mehta J, Mehta P. Comparison of platelet function during exercise in normal subjects and coronary artery disease patients: potential role of platelet activation in myocardial ischemia. Am Heart J 1982; 103: 49–53.

[30] Schernthaner G, Mühlhauser I, Böhm H, Seebacher C, Laimer H. Exercise induces in vivo platelet activation in patients with coronary artery disease and in healthy individuals. Haemostasis 1983; 13: 351–7.

[31] Virgolini I, Fitscha P, Sinzinger H. Effects of bopindolol on platelet function in hypertension at rest and during exercise. Prostaglandins leukotrienes and essential fatty acids 1990; 40: 125–30.

[32] Trovati M, Anfossi G, De Facis R *et al.* Moderate exercise increases platelet function in type I diabetic patients without severe angiopathy and in good control. Diabetes care 1992; 15: 1742–6.
[33] Davis RB, Boyd DG, McKinney ME, Jones CC. Effects of exercise and exercise conditioning on blood platelet function. Med Sci Sports Exerc 1990; 22: 49–53.
[34] Small M, Tweddel AC, Rankin AC, Lowe GDO, Prentice CRM, Forbes CD. Blood coagulation and platelet function following maximal exercise: effects of beta-adrenoceptor blockade. Haemostatis 1984; 14: 262–8.
[35] Marcella JJ, Nichols AB, Johnson LL *et al.* Excercise-induced myocardial ischemia in patients with coronary artery disease: lack of evidence for platelet activation or fibrin formation in peripheral venous blood. J Am Coll Cardiol 1983; 1: 1185–93.
[36] Hughes ASB, Ilsley CD, Wilkinson L. Platelet activation during exercise-induced myocardial ischemia. Thromb Res 1982; 26: 425–30.
[37] Mant MJ, Kappagoda CT, Quinlan J. Lack of effect of exercise on platelet activation and platelet reactivity. J Appl Physiol 1984; 57: 1333–7.
[38] Strauss WE, Cella G, Parisi AF, Sasahara AA. Serial studies of platelet factor 4 and beta thromboglobulin during exercise in patients with coronary artery disease. Am Heart J 1985; 110; 293–9.
[39] Berglund U, Lassvik C, Wallentin L. Effects of the platelet inhibitor ticlopidine on exercise tolerance in stable angina pectoris. Eur Heart J 1987; 8: 25–30.
[40] Kaplan KL. Plasma levels of platelet secretory proteins. Crit Rev Oncol Hematol 1986; 5: 235–55.
[41] Bjerre Knudsen J, Brodthagen U, Gormsen J, Jordal R, Nørregard-Hansen K, Paulev PE. Platelet function and fibrinolytic activity following distance running. Scand J Haematol 1982; 29: 425–30.
[42] Green LH, Seroppian E, Handin RI. Platelet activation during exercise-induced myocardial ischemia. N Engl J Med 1980; 302: 193–7.
[43] Levine SP, Suarez AJ, Sorenson RR, Raymond NM, Knieriem LK. Platelet factor 4 release during exercise in patients with coronary artery disease. Am J Hematol 1984; 17: 117–27.
[44] Rotmensch HH, Vlasses PH, Carpenter KL, D'Amelio LF, Swanson BN, Ferguson RK. Plasma platelet products and exercise-induced myocardial ischemia. J Lab Clin Med 1983; 102: 63–9.
[45] Mathis PC, Wohl H, Wallach SR, Engler RL. Lack of release of platelet factor 4 during exercise-induced myocardial ischemia. N Engl J Med 1981; 304: 1275–8.
[46] Ek I, Falkenberg C, Bygdeman S, Thunell S. Platelet factor 4 plasma levels at rest and after exercise in patients with recent myocardial infarction. Act Med Scad 1982; 212: 43–6.
[47] Wang J, Jen CJ, Kung H, Lin L, Hsiue T, Chen H. Different effects of strenuous exercise and moderate exercise on platelet function in men. Circulation 1994; 90: 2877–85.
[48] Eidt JF, Ashton J, Golino P, McNatt J, Buja LM, Willerson JT. Treadmill exercise promotes cyclic alterations in coronary blood flow in dogs with coronary artery stenoses and endothelial injury. J Clin Invest 1989; 84: 517–27.
[49] Diodati JG, Cannon RC, Epstein SE, Quyyumi AA. Platelet hyperaggregability across the coronary bed in response to atrial pacing in patients with stable coronary artery disese. Circulation 1992; 86: 1186–93.
[50] Svedenhag J, Martinsson A, Ekblom B, Hjemdahl P. Altered cardiovascular responsiveness to adrenaline in endurance-trained subjects. Acta Phys Scand 1986; 126: 539–50.
[51] Herren T, Bärtsch P, Haeberli A, Werner Straub P. Increased thrombin–antithrombin III complexes after 1 h of physical exercise. J Appl Physiol 1992; 73: 2499–2504.
[52] Prisco D, Paniccia R, Guarnaccia V *et al.* Thrombin generation after physical exercise. Thromb Res 1993; 69: 159–64.
[53] Martin JF, Bath PMW, Burr ML. Influence of platelet size on outcome after myocardial infarction. Lancet 1991; 338: 1409–11.
[54] Kristensen SD, Martin JF. Platelet heterogeneity and coronary artery thrombosis. Platelets 1991; 2: 11–17.
[55] Chamberlain KG, Tong M, Penington DG. Properties of the exchangeable splenic platelets released into the circulation during exercise-induced thrombocytosis. Am J Hematol 1990; 34: 161–8.
[56] Hjemdahl P. Plasma catecholamines — analytical challenges and physiological limitations. Baillere's Clin Endocrinol Metab 1993; 7: 307–53.
[57] Meade TW, Vickers MV, Thompson SG, Stirling Y, Haines AP, Miller GJ. Epidemiological characteristics of platelet aggregability. Br Med J 1985; 290: 428–32.
[58] Elwood PC, Renaud S, Sharp DS, Beswick AD, O'Brien JR, Yarnell JWG. Ischemic heart disease and platelet aggregation. The Caerphilly Collaborative Heart Disease Study. Circulation 1991; 83: 38–44.