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Acute and subacute effects of strenuous exercise on platelet aggregation, coagulation and fibrinolysis in patients with stable coronary artery disease

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ABSTRACT

Introduction: Strenuous exercise may occasionally cause coronary thrombosis with myocardial infarction and sudden cardiac death.

Materials and methods: Patients with stable coronary artery disease (CAD) ($n = 164$) and healthy individuals ($n = 25$) performed strenuous exercise on a bicycle ergometer. Blood was drawn at baseline, immediately after exercise and 2 h later. Platelet aggregation was measured with Multiplate® Analyzer. Thrombin generation was determined using a thrombogram and by measuring prothrombin fragment 1 + 2 (F1 + 2). A clot lysis assay was used to investigate fibrinolysis.

Results: From baseline to immediately after exercise, thrombin receptor activating peptide (TRAP)-induced platelet aggregation increased in CAD patients (Δ77 AU × min, 95 % confidence interval (CI): 46;107) and in healthy individuals (Δ153 AU × min, 95%CI: 75;232). Endogenous thrombin potential (ETP) was unaffected by exercise, whilst F1 + 2 increased (Δ17%, 95%CI: 11;24) in CAD patients. Fibrin clot lysis time increased by 9 % (95%CI: 1–17) in CAD patients and by 26 % (95%CI: 8;45) in healthy individuals. When comparing baseline to 2 h post-exercise, TRAP-induced platelet aggregation remained slightly elevated in both CAD patients (Δ53 AU × min, 95%CI: 22;84) and healthy individuals (Δ140 AU × min, 95%CI: 62;219). In contrast, ETP and F1 + 2 decreased in CAD patients (Δ-6 %, 95%CI: −10;-1 and Δ-8 %, 95%CI: -14;-2). Moreover, clot lysis time decreased (Δ-19 %, 95%CI: −27;-11) in patients with CAD and returned to baseline in healthy individuals. All p-values were <0.05.

Conclusions: Platelet aggregation and F1 + 2 were substantially elevated immediately after exercise in CAD patients, indicating a pro-thrombotic state. After 2 h of recovery, they exhibited a markedly increase in fibrinolysis. Similar results were observed in healthy individuals.

1. Introduction

Coronary artery disease (CAD) is the leading cause of death worldwide [1]. Regular exercise training reduces cardiovascular mortality in patients with CAD [2,3], however, acute strenuous exercise has been reported to paradoxically increase the risk of coronary thrombosis and sudden cardiac death [2]. Although this serious adverse event is rare, it is more common in sedentary patients and those with advanced CAD [2,4]. While structural heart disorders and genetics account for the majority of sudden cardiac arrest cases among athletes under the age of 35 [5], atherosclerotic CAD is the leading cause of exercise-related mortality in the middle-aged and older populations [4,6]. In these atherosclerotic patients, cardiac arrhythmia and plaque rupture leading to an acute myocardial infarction are the most common causes of exercise-related death [7]. Immediately upon plaque rupture, platelets and coagulation are activated and a thrombus may be formed eventually

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inducing acute myocardial infarction [8]. Previous studies have shown that both primary [9–12] and secondary haemostasis [13–15] may be activated following acute strenuous exercise in patients with CAD, but only a few studies have investigated the duration of this response, and whether it applies to patients with CAD and also healthy individuals [13].

Therefore, it is reasonable to hypothesise that the greater mortality risk associated with exercise, may be explained by activated coagulation following acute strenuous exercise, which is our primary hypothesis. In the present study, we aimed to investigate the impact of a single bout of maximal cycling exercise on platelets, coagulation and fibrinolysis in patients with CAD and in healthy individuals. In addition to the immediate response of acute strenuous exercise, we also evaluated the response 2 h post-exercise.

2. Methods

2.1. Study population

The present cohort was part of a randomised controlled trial, of which some of the results already are published [16,17]. We included 169 patients above 18 years of age with angiographically verified CAD or previous myocardial infarction referred to the Department of Medicine, National Hospital of the Faroe Islands. Exclusion criteria were acute myocardial infarction and/or revascularisation within the previous 12 months, inability to perform strenuous exercise, severe heart failure (ejection fraction <30 % or New York Heart Association >2), severe valvular heart disease, hospitalisation with serious arrhythmia within six months, treatment with anticoagulants, implantable cardioverter-defibrillator or cardiac resynchronisation therapy and chronic obstructive pulmonary disease GOLD IV. In addition, 25 healthy individuals were studied. The inclusion criteria for healthy individuals were age ≥ 18 years, no chronic illness, and no regular medication usage. The exclusion criterion was inability to perform strenuous exercise. Moreover, in this study, we also excluded four aspirin-naïve patients in the cohort. The research ethics council of the Faroe Islands approved the project, and all participants signed a written informed consent form. The study was conducted according to the ethical principles of the Helsinki declaration.

2.2. Acute strenuous exercise

All participants performed acute strenuous exercise on an electronically braked bicycle ergometer (Excalibur Sport, Lode, Groningen, Netherlands). The exercise protocol was slightly different for patients with CAD and healthy individuals. For patients with CAD, it started with 6 min warm-up. The first 3 min, the bicycle had a load of 50 W for men and 30 W for women, increasing to 70 W and 50 W the last 3 min of the warm-up. Afterwards, the load was increased every min by 20 W for men and 15 W for women until exhaustion. An electrocardiogram was obtained before and constantly throughout the test in CAD patients. Any severe ST-segment deviations, chest pain, or desire to stop the test resulted in termination of exercise. In healthy individuals, the warm-up lasted for 10 min; the first 5 min at 50 W for men and 40 W for women and the last 5 min at 100 W and 80 W. The load was increased every min with 25 W for men and 20 W for women until the point of exhaustion. Maximal peak power is the highest load that the study participants were able to complete on the bicycle ergometer. The formula for calculating the maximum power output is described in a previous paper from our group [16].

2.3. Blood samples and laboratory methods

Venous blood was drawn from an antecubital vein using standard procedures. Prior to blood sampling, all participants were asked to refrain from pain killers containing acetylsalicylic acid for 1 week, alcohol for 24 h, caffeine for 12 h, food and liquids (except water) for 1.5 h. The first blood sample was obtained after 30 min of rest (baseline). The second sample was taken within 5 min after stopping strenuous exercise and the last sample was obtained 2 h after strenuous exercise was terminated.

2.4. Platelet parameters

Blood was anticoagulated with hirudin and analysed with the Multiplate® Analyzer (Roche, Basel, Switzerland) using the following agonists: 6.5 μM adenosine diphosphate (ADP), 0.5 mM arachidonic acid (ASPI) and 32.3 μM thrombin receptor activating peptide (TRAP). The blood rested between 30 min and 2 h before analysis. Platelet count and mean platelet volume (MPV) were measured using the Sysmex XN-1000 (Norderstedt, Germany).

2.5. Thrombin generation and prothrombin fragment 1 + 2

Ex vivo thrombin generation and *in vivo* prothrombin fragment 1 + 2 (F1 + 2) were measured in citrated plasma. Plasma for thrombin generation and F1 + 2 was centrifuged for 25 min at 3100*g*. Plasma for thrombin generation was centrifuged twice, secondly for 15 min at 2500*g*. Plasma was frozen at −80 °C until analysis. The analyses were run in duplicates on 96-well microtiter plates using 4 μM phospholipid and 5 pM tissue factor. Parameters for thrombin generation were calculated using the calibrated automated thrombogram (Thrombinoscope BV, Maastricht, the Netherlands): a) lag time: time from start of analysis until first amount of thrombin is produced, b) peak thrombin: the maximal concentration of thrombin generated at a given time point, c) time to peak (tt-peak): length of time needed to reach the maximal thrombin concentration and d) endogenous thrombin potential (ETP): the total amount of thrombin generated during the whole analysis. Plasma for F1 + 2 was measured in duplicates using a commercial enzyme-linked immunosorbent assay (Enzygnost® F1 + 2 Mono, Siemens Healthcare GmbH).

2.6. Fibrinolytic parameters

Clot lysis, plasminogen activator inhibitor-1 (PAI-1), fibrinogen and D-dimer were all measured in citrated platelet-poor plasma. Plasma was centrifuged for 25 min at 3100*g* before being stored at −80 °C until analysis. Clot lysis time (50 % lysis time) evaluates the time it takes for half of the clot to resolve from peak clot formation. It was measured with an in-house dynamic turbidimetric assay [18]. Plasma was mixed with a solution including HEPES buffer, 4 μM phospholipid, 1:5000 tissue factor, 116 ng/mL tissue plasminogen activator and 26.7 mmol/L calcium. The analysis was performed on 96-well microplates which were read using a Victor X4 (Perkin Elmer, Turku, Finland). PAI-1 was measured in duplicates with an ELISA technique (TECHNOZYM PAI-1 Antigen ELISA Kit, Technoclone GmbH). Fibrinogen was measured by ACL TOP 500 CTS (Werfen, Cheshire, UK) and D-dimer was evaluated using CS2100i (Sysmex, Norderstedt, Germany).

2.7. Statistics

Data were analysed using IBM SPSS Statistics version 28.0.0.0. Graphs were generated in GraphPad Prism 9.3.1. (La Jolla, CA, USA). A p-value <0.05 was considered statistically significant. A paired samples t-test was completed for all parameters to identify possible within-group time-specific differences. To evaluate the model's assumptions, data were logarithmically transformed for peak thrombin, ETP, F1 + 2, 50 % lysis time, PAI-1 and D-dimer. Data are presented as means with standard deviation (SD) or 95 % confidence interval (CI) if normally distributed and as medians with 95 % CI if not. Categorical data are shown in percent and were compared with Pearson's chi squared test or Fisher's exact test. In the supplemental material, variations in

haemostatic parameter changes over time between the groups were evaluated with a linear mixed-model analysis for repeated measures. The following parameters were assigned as fixed effects in Table S1: group (CAD *vs.* healthy individuals), time (baseline *vs.* 5 min *vs.* 2 h post exercise) and group × time interaction. Data on F1 + 2 had several obvious outliers, and we used Tukey box-plot method to identify them [quartile (Q)1–1.5(Q3 - Q1), Q3 + 1.5(Q3 - Q1)] [19]. The power calculation was based on a mean 50 % clot lysis time on 521 s with SD 244 s in healthy individuals known from a previous study from our group [20]. Using a significance threshold (two-sided alpha) of 5 % and within-group changes in patients with CAD ($n = 164$) and healthy individuals ($n = 25$), our study had a 100 % power to identify differences in clot lysis time within each of these two groups.

3. Results

A total of 164 patients with CAD and 25 healthy individuals were included in the statistical analyses of the present study. Of the initial 169 patients with CAD, five were excluded. Four did not receive aspirin, and one patient was excluded due to inability to perform strenuous exercise. As shown in Table 1, patients with CAD were approximately 15 years older than healthy individuals, and the sex composition was different; in patients with CAD, the majority were men and in healthy individuals the majority were women. Both groups had body mass index higher than 25, however this was more pronounced among patients with CAD. All patients with CAD received mono antiplatelet therapy with aspirin 75 mg, whilst healthy individuals received no daily medication. Patients with CAD had lower VO_{2peak}, maximum heart rate and peak power than healthy individuals as illustrated in Table 2.

3.1. Platelet count and platelet aggregation

Results on platelet count and aggregation are presented in Fig. 1 and Tables S1-S7. Changes in mean or median values are given below. Platelet count increased immediately after strenuous exercise in CAD patients by 25 × 10^9/L (95%CI: 22;28) and in healthy individuals by 34 × 10^9/L (95%CI: 26;42, both $p < 0.001$). After 2 h, platelet count returned to baseline values in both groups.

Immediately after strenuous exercise, ADP-induced platelet aggregation increased by 107 AU × min (95%CI: 76;137) in patients with CAD and by 198 AU × min (95%CI: 120;277) in healthy individuals (both $p < 0.001$). After 2 h, it remained slightly elevated by 31 AU × min (95%CI: 0;62, $p = 0.049$) in patients with CAD and by 97 AU × min (95%CI: 19;176, $p = 0.01$) in healthy individuals. Likewise, ASPI-induced platelet aggregation increased by 90 AU × min (95%CI: 69;111) in patients with CAD and by 192 AU × min (95%CI: 142;242) in healthy individuals (both $p < 0.001$) immediately after strenuous exercise. However, 2 h after the test, ASPI-induced platelet aggregation returned to baseline values in patients with CAD but remained slightly elevated by 75 AU × min (95%CI: 24;125, $p = 0.001$) in healthy individuals. Similar changes were observed for TRAP-induced platelet aggregation. Immediately after exercise, TRAP-induced platelet aggregation increased by 77 AU × min (95%CI: 46;107) in patients with CAD and by 153 AU × min (95%CI: 75;232) in healthy individuals (both $p < 0.001$). After 2 h, TRAP-induced platelet aggregation remained elevated in patients with CAD by 53 AU × min (95%CI: 22;84) and by 140 AU × min (95%CI 62;219) in healthy individuals (both $p < 0.001$). Although the changes in the two groups were similar for all platelet parameters, the changes over time were significantly more pronounced in healthy individuals (Table S1, group × time $P < 0.05$ for platelet count, ADP-, ASPI- and TRAP-induced aggregation). We performed further subgroup

Table 1
Baseline characteristics of patients with CAD and healthy individuals.

Characteristics	CAD ($n = 164$)	Healthy individuals ($n = 25$)	*P*-value
Age (years)	67 ± 9	52 ± 7	<0.001
Sex (male/female)	137/27 (84 %/16 %)	6/19 (24 %/76 %)	<0.001
BMI, kg/m^2	29.5 ± 4.7	27.0 ± 3.4	0.002
Systolic blood pressure*	144 ± 19	129 ± 18	<0.001
Diastolic blood pressure*	83 ± 10	83 ± 12	0.88
Smoking			0.02
Current	14 (9 %)	3 (12 %)	
Previously	106 (65 %)	9 (36 %)	
Never	43 (26 %)	13 (52 %)	
Biochemistry			
Haemoglobin [♀7.3–9.5, ♂8.3–10.5 mmol/L]	8.8 ± 0.8	8.6 ± 0.7	0.13
Creatinine [60–105 μmol/L]	80 ± 21	68 ± 11	<0.001
eGFR [>60 mL/min/1.73m^2]	79 ± 14	87 ± 6	<0.001
Platelet count [♀165–400, ♂145–350 × 10^9/L]	210 ± 56	228 ± 59	0.17
APTT [20–29 s]	27 ± 7	26 ± 2	0.32
INR [<1.2]	1.0 ± 0.08	1.0 ± 0.1	0.15
Medications			
ASA	164 (100 %)	0 (0 %)	<0.001
Beta-blockers	110 (67 %)	0 (0 %)	<0.001

Continuous variables are presented as means ± standard deviations and dichotomous variables are expressed as numbers and percentages. APTT: activated partial thromboplastin time. BMI: body mass index. CAD: coronary artery disease. eGFR: estimated glomerular filtration rate. INR: international normalised ratio.

* Resting blood pressure pre intervention.

Table 2
Cardiorespiratory fitness, maximum heart rate and peak power after strenuous exercise on a bicycle ergometer.

	CAD ($n = 164$)	Healthy individuals ($n = 25$)	*P*-value
VO_{2peak} (mL/kg/min)	23 ± 5	34 ± 7	<0.001
VO_{2peak} (mL/min)	2058 ± 569	2558 ± 692	0.002
HR_{max} (bpm)	140 ± 22	170 ± 13	<0.001
Peak power (watt)	161 ± 54	205 ± 62	0.002
Exercise time (min)	11.0 ± 2.6	16.7 ± 2.1	<0.001

Continuous variables are presented as means ± standard deviations. CAD: coronary artery disease. HR_{max}: maximum heart rate. VO_{2peak}: peak oxygen uptake.

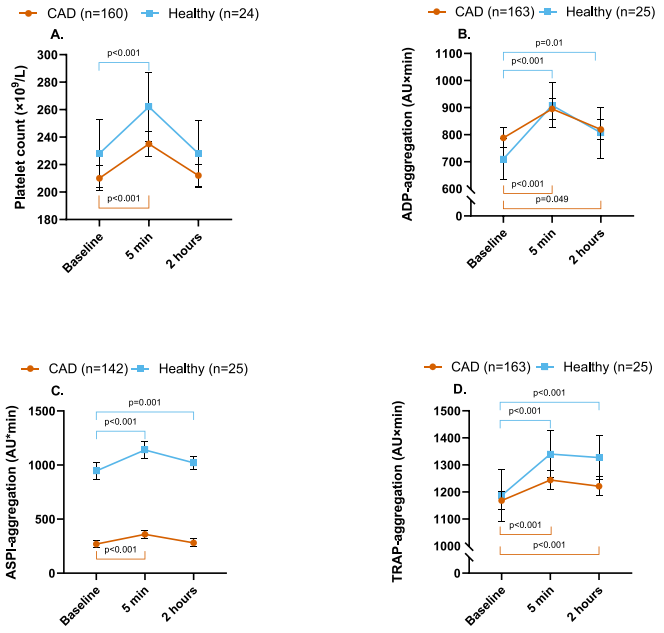

Fig. 1. Response in platelet count and platelet aggregation to acute strenuous exercise.
ADP: Adenosine diphosphate. ASPI: Arachidonic acid. TRAP: Thrombin receptor activating peptide.

analyses on females with CAD compared with healthy individuals (Table S2), CAD females *vs.* males (Table S3), age stratified CAD patients (<65 years *vs.* ≥65 years, Table S4), age-matched CAD patients compared with healthy individuals (Table S5) and CAD patients stratified to smoking status (Table S6). In aspirin-naïve healthy individuals, ASPI-induced platelet aggregation was more pronounced over time compared with females with CAD (group × time $P \leq 0.02$, Table S2) and in age-matched CAD patients (group × time $P = 0.01$, Table S5). Overall, the fluctuations in platelet aggregation were lower when adjusted for platelet count, however, platelet aggregation remained increased 2 h post exercise, especially in healthy individuals (Table S7).

3.2. Thrombin generation and prothrombin fragment 1 + 2

Immediately after exercise, lag time was 0.4 s (95%CI: 0.2;0.6) longer and tt-peak 0.7 s (95%CI: 0.4–1.0) longer compared to baseline in patients with CAD (both $p < 0.001$). Both returned to baseline after 2 h. Immediately after exercise, F1 + 2 increased from 224 pmol/L (95%CI: 209;240) to 267 pmol/L (95%CI: 249;286) in patients with CAD ($p < 0.001$), but remained unchanged in healthy individuals. ETP was not affected. Two hours after acute strenuous exercise, ETP was reduced below baseline from 1309 nM × min (95%CI: 1263;1356) to 1238 nM × min (95%CI: 1195;1282) ($p = 0.009$). F1 + 2 similarly decreased below baseline values 2 h after exercise in patients with CAD from 224 pmol/L (95%CI: 209;240) to 207 pmol/L (95%CI: 193;222), $p = 0.006$. All results are illustrated in Fig. 2 and Table S1. Between-group comparisons

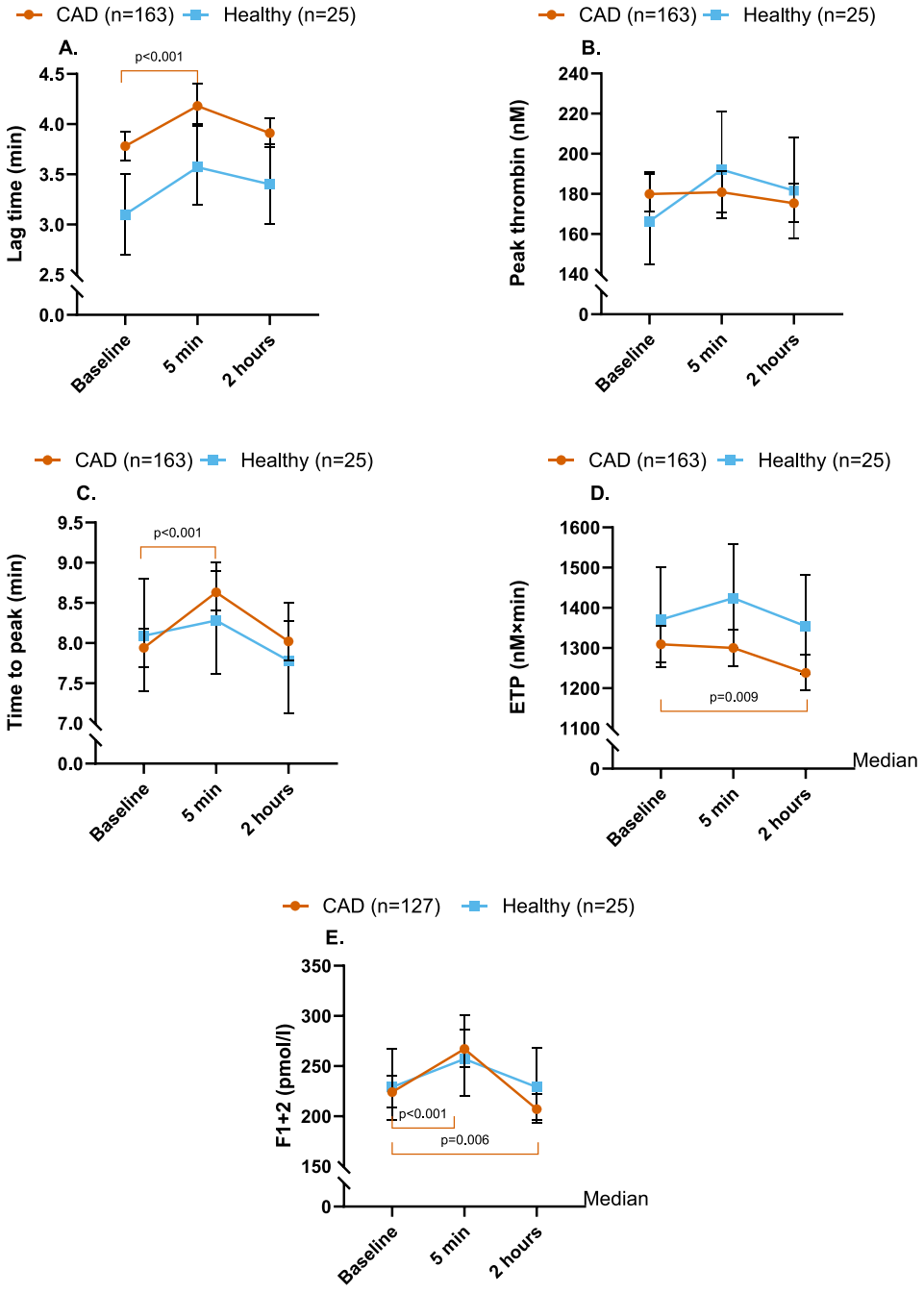

Fig. 2. Effect of acute strenuous exercise on thrombin generation.
ETP: Endogenous thrombin potential. F1 + 2: Prothrombin fragment 1 + 2.

over time (Table S1) were also without changes in ETP and F1 + 2. However, in a subgroup analysis on age-matched patients with CAD compared with healthy individuals, changes in F1 + 2 were more pronounced in age-matched CAD patients with immediate increase in F1 + 2, and a decrease after 2 h after strenuous exercise (Table S5).

3.3. Clot lysis time, PAI-1, fibrinogen and D-dimer

Changes in clot lysis time were different between patients with CAD and healthy individuals from baseline to 5 min and 2 h post exercise (group × time $P = 0.04$, Table S1). Immediately after acute exercise, clot lysis time increased by 9 % (95%CI: 1;17, $p = 0.02$) in patients with CAD and by 26 % (95%CI: 8;45, $p = 0.002$) in healthy individuals. After 2 h, clot lysis time returned to baseline for healthy individuals, whilst it fell below baseline by 19 % (95%CI: −27;-11, $p < 0.001$) in patients with CAD. There were similar changes in a subgroup analysis in age-matched patients with CAD and healthy individuals (group × time $P = 0.007$, Table S5).

As depicted in Fig. 3 and Table S1, PAI-1 decreased in both groups, however, more pronounced in healthy individuals (group × time $P < 0.001$). Immediately after exercise, PAI-1 decreased from 12 ng/mL (95%CI: 11;14) to 10 ng/mL (95%CI: 9;12) in patients with CAD and from 11 ng/mL (95%CI: 8;16) to 7 ng/mL (95%CI: 5;9) in healthy individuals (both $p < 0.001$). After 2 h, PAI-1 further decreased to 7 ng/mL (95%CI: 6;8) in patients with CAD and to 4 ng/mL (95%CI: 3;6) in healthy individuals (both $P < 0.001$ compared with baseline). Similar outcomes were observed when healthy individuals were compared with age-matched CAD patients, where effects were also more noticeable in healthy individuals (group × time $P = 0.01$).

In patients with CAD, there was an immediate response to acute exercise, where fibrinogen increased by 0.7 μmol/L (95%CI: 0.5;0.9, $P < 0.001$) and D-dimer increased by 23 % (95%CI: 14;31, $P < 0.001$). Both parameters returned to baseline values after 2 h. No changes were observed for fibrinogen or D-dimer in healthy individuals. There were no between-group differences over time between patients with CAD and healthy individuals for D-dimer and fibrinogen. However, a subgroup analysis comparing age-matched CAD patients and healthy individuals revealed that changes in D-dimer were significantly different over time and driven by an increase in D-dimer 5 min after strenuous exercise in age-matched patients with CAD (group × time $P = 0.04$).

4. Discussion

The present study demonstrated an acute increase in platelet aggregation and thrombin generation in response to strenuous exercise in patients with CAD and in healthy individuals, thus confirming the primary study hypothesis. Platelet aggregation remained slightly elevated 2 h after exercise. On the other hand, the changes after 2 h showed a downregulated pro-thrombotic profile demonstrated by reduced thrombin generation in patients with CAD and increased fibrinolysis in both patients with CAD and healthy individuals.

The immediate increase in platelet aggregation and thrombin generation following strenuous exercise must be considered an unfortunate combination and may be explanatory factors for the increased risk of acute myocardial infarction and even sudden cardiac death after strenuous exercise [2], especially in a vulnerable atherosclerotic vessel with an increased wall stress [21]. We observed that immediately post-exercise, platelet count and platelet aggregation were elevated in both CAD patients and healthy individuals. Despite aspirin therapy, acute exercise still caused an increase in ASPI-induced platelet aggregation in CAD patients; however, the increase was relatively small compared with the levels of ASPI-induced platelet aggregation in healthy individuals not taking aspirin. In accordance with our findings, several previous studies reported increased platelet aggregation in patients with CAD following acute strenuous exercise, whereas others report no changes in healthy individuals [10–12]. Whilst two studies employed acute strenuous exercise [11,12], the participants in the study by Andreotti et al. [10] only performed mild exercise, which may explain the lack of response in the healthy population group. This theory is supported by a review suggesting that exercise intensity rather than durability seems important for changes in platelet activity, thrombin generation and fibrinolysis [22]. Yet, another three studies did not report any effect on platelet aggregation following acute strenuous exercise, neither in patients with CAD nor in healthy individuals [12,23,24]. In our study, platelet aggregation remained elevated for at least 2 h after strenuous exercise training in patients with CAD (ADP and TRAP) and the healthy individual group (ADP, ASPI and TRAP). Aspirin treatment in patients with CAD may explain why ASPI-induced platelet aggregation returned to baseline within 2 h post-exercise in this group. These findings contrast previous studies which observed that eventual increased platelet aggregation returned to baseline values within 1–3 h [10,23]. The lack of upregulated platelet aggregation after 1–3 h in previous studies may be explained by a less potent initial response due to the execution of mild exercise.

Taken together, our findings are consistent with earlier observations that acute strenuous exercise increases platelet aggregation in both patients with CAD [10–12] and healthy individuals [22]. The extent and duration of the change in aggregation has not previously been well described but may depend on the total exercise load (intensity and volume), which should be elucidated in future research. On the contrary, the association between platelet count and platelet aggregation did not emerge as expected after 2 h [10,23]. We have previously shown that platelet aggregation measured in whole blood is dependent on platelet count in patients with CAD [25]. In the present study, platelet count, and platelet aggregation increased to the same extent immediately post exercise, whereas, 2 h after exercise, platelet aggregation remained elevated despite normalisation of platelet count. Furthermore, platelet aggregation increased even after adjusting for platelet count, particularly in healthy individuals and 2 h after exercise. This may suggest that there are separate mechanisms underlying the elevated platelet count and platelet aggregation following acute strenuous exercise. During acute strenuous exercise, a large portion of plasma is transferred from the blood stream to muscle tissue, but it is redistributed to baseline levels within 30 min [26,27]. Thus, this may account for the increase in concentration of blood cells immediately after acute strenuous exercise, shown in our study (Table S1).

Thrombin plays a key role in the formation of a blood clot. It is an enzyme with multiple effects, and one of its most important roles is to convert fibrinogen to fibrin, which stabilises the blood clot and eventually blocks a narrow atherosclerotic artery [28]. Overall, our findings suggest a slightly increased *in vivo* thrombin generation immediately following strenuous exercise, but following 2 h of recovery, both *ex vivo* and *in vivo* thrombin generation were reduced in patients with CAD. Aspirin treatment may reduce thrombin generation parameters [29], but the effect seems to be more evident on aspirin dosage above 100 mg daily [30]. In our study, ETP and F1 + 2 did not differ between CAD patients and healthy individuals; however, 2 h after strenuous exercise, ETP and F1 + 2 decreased in CAD patients alone. It has been well-

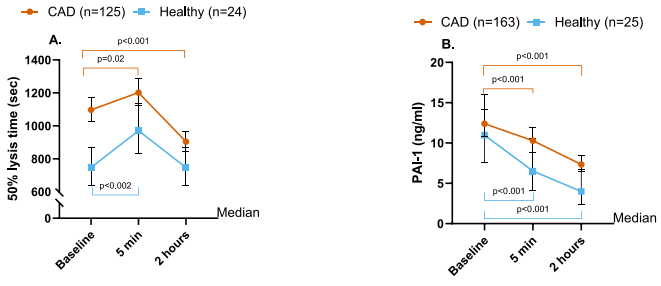

Fig. 3. Effect of acute strenuous exercise on clot lysis time and PAI-1.
PAI-1: Plasminogen activator inhibitor-1.

documented that F1 + 2 rises with age [31]; nevertheless, it is challenging to compare the groups because CAD patients were around 15 years older than the healthy individuals. However, in a subgroup analysis, we found that age-matched CAD patients had lower F1 + 2 compared with healthy individuals. Moreover, F1 + 2 decreased below baseline values 2 h after strenuous exercise in patients with CAD only (Table S5). This positive shift in CAD patients compared with healthy individuals is probably explained by aspirin treatment. Our results corroborate the findings of three previous studies, which demonstrated an elevated *in vivo* thrombin generation immediately after strenuous exercise reflected by an increased F1 + 2 [13–15]. On the other hand, our results did not support the findings of Cwikiel et al., who reported that ETP increased immediately following strenuous exercise in both patients with CAD and controls without angiographycally verified CAD [14]. Similarly, a recent study showed an increased overall haemostatic potential and overall coagulation potential in CAD patients in dual antiplatelet therapy after performing a single bout of either moderate- or high-intensity exercise [32]. Both parameters returned to baseline after 1 h recovery [32]. One other study reported on thrombin generation during the recovery period of acute strenuous exercise [13]. Our results are in complete agreement with their findings immediately after exercise, which showed an increased *in vivo* thrombin generation in both patients with CAD and aspirin-treated controls without CAD. But in direct contrast with our results, F1 + 2 remained elevated in patients with CAD after 2 h of recovery in the study by Acil et al. [13] The baseline characteristics of the patients in these two investigations vary somewhat. The CAD patients in the current investigation had a higher percentage of males and a higher BMI, and were around ten years older. However, for thrombin generation, we obtained the same results using both *ex vivo* and *in vivo* analyses, which has to be considered a significant strength.

Consistent evidence has demonstrated that fibrinolysis increases immediately after strenuous exercise, which must be considered very appropriate if platelet aggregation and thrombin generation increases at the same time [33]. In the present study, the immediate fibrinolytic response to acute strenuous exercise was ambiguous in both groups; clot lysis time increased, whilst PAI-1 decreased. PAI-1 is released from activated platelets and is one of the most important inhibitors of tissue plasminogen activator (t-PA), and thus of fibrinolysis. However, the clot lysis time provides an overall overview of the fibrinolysis, since it is an *ex vivo* measure of a dynamic process that include all fibrinolytic process components including inhibition of fibrinolysis [34].

Several studies have reported an increased fibrinolytic response following strenuous exercise in patients with CAD with decreased PAI-1 [35–40] and increased t-PA [35–39,41,42]. Three studies included a group with healthy controls, and all results were similar to the CAD group [37,38,40]. In accordance with our findings, Khanna et al. showed that euglobulin lysis time increased in both patients with CAD and healthy individuals after submaximal exercise, however, with a significantly greater response in healthy individuals [43]. In contrast, Acil et al. reported an increased global fibrinolytic capacity immediately after strenuous exercise in patient with CAD and aspirin-treated controls without CAD [13]. Overall, t-PA and PAI-1 have been thoroughly investigated immediately after acute strenuous exercise in patients with CAD, and indicate an elevated fibrinolysis, which is partially in agreement with our results.

Research on the duration of enhanced fibrinolysis following strenuous exercise is scarce. In the present study, fibrinolysis increased considerably at 2 h after acute strenuous exercise. PAI-1 decreased markedly and clot lysis time changed from being prolonged to being shortened in patients with CAD and returned to baseline in healthy individuals. To the best of our knowledge, only three previous studies have investigated fibrinolytic changes in the recovery period after acute strenuous exercise [13,32,36]. Two of these found that the fibrinolytic capacity reverted to baseline values after 1–2 h in both patients with CAD and aspirin-treated controls without CAD [13,32]. However, Dejong et al. reported that t-PA returned to the baseline value, whereas PAI-1 levels remained low 1 h after an acute bout of resistance training in patients with CAD [36]. Collectively, our findings and those of Dejong et al. [36] suggest that fibrinolysis remains increased after 1–2 h following exercise, which may offer some protection against thrombotic events. Overall, the results on fibrinolysis parameters evolved similarly in patients with CAD and healthy individuals following acute strenuous exercise. From a clinical view, elevated fibrinolysis will ward off thrombotic episodes and shorten the duration of occlusion of blood flow. Future research should investigate the duration of the increased fibrinolysis response, and thus if it may be an important protective feature of regular exercise.

Overall, we found that our groups of patients with CAD and healthy individuals responded in a similar way to acute strenuous exercise although the response seemed more pronounced in the healthy group. All participants performed strenuous exercise until exhaustion, however, the healthy individuals were younger and fitter with higher peak oxygen consumption and peak power output than the CAD-group, which is a limitation for a direct comparison. Indeed, the healthy individuals exercised for an overall longer period of time, with both a longer warm-up phase and a greater peak power output. Thus, a longer duration of intense exercise and a higher absolute peak power output may account for the somewhat greater response in platelet aggregation and fibrinolysis in the group of healthy individuals.

4.1. Strengths and limitations

Most previous studies compared baseline values with parameters obtained immediately after exercise only. A strength of the present study is the additional blood sampling 2 h after the single bout of acute strenuous exercise. Other strengths include the relatively large sample size of patients with CAD, the standardised performance of acute strenuous exercise, and the use of a dynamic clot lysis assay, which considers all components of the plasma. The main limitation is that the patients with CAD were not age- and sex-matched with healthy individuals, and that CAD patients received aspirin. Another significant limitation is that platelet function was only evaluated by platelet aggregation and mean platelet volume in a subgroup of patients. The Multiplate® Analyzer evaluates an aggregation on whole blood, but only for the selected agonists. Thus, only a few of the platelet receptors are examined, making it impossible to determine whether other platelet activation mechanisms have changes, such as granule release and shape modification.

5. Conclusion

Immediately after acute strenuous exercise, platelet aggregation increased in patients with CAD and also in healthy individuals. Thrombin generation was also slightly increased. Results on fibrinolysis were ambiguous. Following 2 h of recovery, platelet aggregation remained slightly elevated, but thrombin generation decreased in patients with CAD, and fibrinolysis was considerably increased. Overall, the haemostatic balance was skewed toward a pro-thrombotic state immediately after acute strenuous exercise, whereas, after 2 h of recovery, it was skewed toward lower thrombin generation and increased fibrinolysis.

CRediT authorship contribution statement

Jacobina Kristiansen: Writing – review & editing, Writing – original draft, Visualization, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Erik L. Grove:** Writing – review & editing, Visualization, Validation, Supervision, Methodology, Funding acquisition, Conceptualization. **Tórur Sjúrðarson:** Writing – review & editing, Investigation. **Magni Mohr:** Writing – review & editing, Supervision,

Resources, Methodology, Funding acquisition. **Steen D. Kristensen:** Writing – review & editing, Supervision, Resources, Methodology, Funding acquisition, Conceptualization. **Anne-Mette Hvas:** Writing – review & editing, Visualization, Supervision, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

Erik Lerkevang Grove reports a relationship with AstraZeneca that includes: consulting or advisory, funding grants, and speaking and lecture fees. Erik Lerkevang Grove reports a relationship with Bayer AG that includes: consulting or advisory, funding grants, and speaking and lecture fees. Erik Lerkevang Grove reports a relationship with Boehringer Ingelheim GmbH that includes: consulting or advisory, funding grants, and speaking and lecture fees. Erik Lerkevang Grove reports a relationship with Bristol-Myers Squibb Company that includes: consulting or advisory and speaking and lecture fees. Erik Lerkevang Grove reports a relationship with Pfizer that includes: consulting or advisory and speaking and lecture fees. Erik Lerkevang Grove reports a relationship with Novo Nordisk that includes: consulting or advisory. Erik Lerkevang Grove reports a relationship with Organon & Co. Inc. that includes: speaking and lecture fees. Erik Lerkevang Grove reports a relationship with Lundbeck Pharma doo that includes: consulting or advisory. Erik Lerkevang Grove reports a relationship with Idorsia Pharmaceuticals Ltd. that includes: funding grants. Anne-Mette Hvas reports a relationship with CSL Behring GmbH that includes: funding grants. Steen Dalby Kristensen reports a relationship with Idorsia Pharmaceuticals Ltd. that includes: funding grants. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.thromres.2024.03.007.

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