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ABSTRACT

Purpose: This study aimed to investigate how genotype and caffeine habituation influence the acute effects of caffeine ingestion on exercise-induced hemostatic responses in individuals with obesity. **Methods:** Using a randomized, double-blind, placebo-controlled crossover design, 40 physically inactive young men with obesity (age, 22.2 ± 2.3 years; BMI, 34.1 ± 2.7 kg/m^2) completed two moderate-to-high-intensity concurrent exercise sessions following ingestion of caffeine (3 mg/kg) or placebo. Blood samples were collected at baseline, after exercise, and after 60 minutes of recovery. Statistical analysis was performed by repeated measures MANOVA. **Results:** Acute exercise increased platelet count and aggregation, fibrinogen, F1+2, tPA antigen, D-dimer, and clot lysis time, regardless of genotype or caffeine habituation status ($P < 0.05$). PAI-1 antigen remained unchanged after exercise ($P > 0.05$) but decreased following recovery ($P < 0.01$). Caffeine resulted in a greater increase in platelet aggregation, fibrinogen, F1+2, and clot lysis time, alongside a blunted increase in tPA antigen levels post-exercise in naïve consumers ($P < 0.05$). In contrast, habitual caffeine consumers exhibited a mitigated increase in clot lysis time and a greater post-recovery reduction in PAI-1 antigen following caffeine ingestion ($P < 0.001$). Caffeine's impact on hemostatic responses to exercise was unaffected by genotype ($P > 0.05$). **Conclusions:** Moderate-to-high-intensity concurrent exercise induces a transient prothrombotic state in physically inactive individuals with obesity. Acute caffeine supplementation at a moderate dose modulates the hemostatic responses depending on caffeine habituation status rather than *CYP1A2* genotype: it exacerbates the prothrombotic response in naïve consumers but attenuates it in habitual consumers.

Key Words: CAFFEINE; CONCURRENT EXERCISE; OBESITY; PLATELET AGGREGATION; COAGULATION; FIBRINOLYSIS

INTRODUCTION

Obesity, a global health epidemic currently affecting over one billion individuals (1), is characterized by chronic low-grade inflammation and metabolic dysregulation (2). It is closely associated with disruptions in hemostatic balance, including platelet hyperreactivity, a procoagulant state, and impaired fibrinolysis (2-4). These hemostatic abnormalities not only increase the risk of thrombosis but also act synergistically with other risk factors to further accelerate the development of atherosclerotic disease (5). A growing body of evidence underscores this link, as individuals with obesity exhibit a markedly increased incidence of both arterial and venous thrombotic events compared to their lean counterparts (6, 7).

Numerous investigations have documented the antithrombotic benefits of regular exercise training, revealing that exercise attenuates coagulation potential while augmenting fibrinolytic activity in both healthy individuals and patients with cardiovascular disease (8, 9). However, the hemostatic consequences of a single bout of exercise are somewhat complex. It has been shown that acute exercise transiently stimulates platelet activation and coagulation pathways to a greater extent than fibrinolysis (10-12). This condition may be exacerbated in people with obesity and increase the risk of thrombosis and related cardiovascular events.

Caffeinated beverages, such as coffee and tea, are some of the most frequently consumed beverages in the world. Caffeine has been shown to provide various beneficial effects on human health. Habitual intake of low-to-moderate caffeine doses has been associated with improved hemostatic balance, mainly through reduced platelet aggregability, and presents a protective effect against cardiovascular disease (13-16). Moreover, observational data indicate that higher dietary caffeine consumption is associated with a more favorable metabolic profile—lower low-density lipoprotein cholesterol, reduced systolic blood pressure, improved insulin sensitivity, and elevated high-density lipoprotein

concentrations—in individuals who are overweight or obese (17). Chronic caffeine intake may also promote weight, body mass index (BMI) and body fat reduction according to a meta-analysis by Tabrizi et al. (18). In addition, beyond its metabolic effects, caffeine is a well-established ergogenic aid that enhances performance across a spectrum of exercise modalities (19). Consequently, the beneficial properties of caffeine make it an appropriate substance for overweight and people with obesity. However, the acute effect of caffeine on blood hemostasis, particularly under exercise stress, remains limited and controversial. While some studies indicated that acute caffeine ingestion has procoagulatory effects at baseline (20-22) or following exercise (23, 24), which may be associated with an elevated risk of thrombosis, others demonstrated that acute caffeine intake resulted in no impact (25, 26), or even a favorable hemostatic effect at baseline (27, 28) or following exercise (28), which may be beneficial for prevention of thrombosis. These discrepancies, which may be due to differences in caffeine dose and source, measurement method, genetic variation, caffeine habituation status, and small sample sizes, emphasize the need for further research in this area, especially in high-risk populations such as individuals with obesity.

To the best of our knowledge, no studies have investigated the interplay between caffeine and exercise on blood hemostasis in people with obesity. Therefore, in this study, the acute effects of caffeine ingestion on platelet aggregation, coagulation, and fibrinolysis systems in individuals with obesity were determined at baseline, immediately after exercise and following recovery. Possible differences in hemostatic responses between naïve and regular caffeine consumers as well as between C allele carriers and AA homozygotes in the cytochrome P450 1A2 (*CYP1A2*) gene, were also assessed.

METHODS

Participants

Following outreach in public spaces, laboratories, and social networks, 117 young adult men with obesity volunteered to participate in this study. Among them, 40 participants were eligible for the study entry criteria. The inclusion criteria were as follows: young adult male, 18–25 years, $30 \geq$ BMI $\geq 39.99\,kg/m^2$, daily caffeine consumption < 400 mg, physically inactive over the past six months (not participating in < 150 min/week of moderate intensity or < 75 min/week of vigorous intensity, or an equivalent combination of the two different intensities (29)), having no history of cardiovascular or endocrine disorders, hemostatic abnormalities, diabetes, cancer, hyperlipidemia, hypertension, kidney, or liver disease, and no history of surgery, caffeine allergy, smoking, alcohol consumption, herbal or therapeutic diets, taking any medications or supplements for the past 3 months. Exclusion criteria included joint ailments, physical disabilities, suffering from any pathology or injury, failure to complete the study trials, or failure to adhere to the recommendations. Volunteers were encouraged to adhere to their habitual bedtime/wake-up schedule. Participants were also instructed to refrain from exercise for 72 hours before the study began until the end of the study. A list of all foods and beverages containing caffeine was given to the participants, and they were asked to minimize their daily caffeine intake to less than 80 mg two weeks before the experiments. The adherence to the recommendations was checked via "MyFitnessPal" software. All the participants signed written informed consent after being fully informed about the potential risks involved in the study experiments.

Sample size was determined a priori using G*Power 3.1.9.7 (University of Dusseldorf, Dusseldorf, Germany) for repeated-measures multivariate analysis of variance (MANOVA) (within-between interaction) with $\alpha = 0.05$, power = 0.80, and effect size (ES) = 0.50. This ES was chosen based on study examining the effect of caffeine on hemostatic

markers in adult men (23). The calculation accounted for 3 repeated measures and 2 groups, indicating that the minimum required sample size was 34 participants. However, we recruited 40 participants to factor in possible dropouts.

Study Design

A randomized, double-blind, placebo-controlled crossover study design was employed to investigate the acute effects of caffeine on blood hemostasis responses to exercise in individuals with obesity (Figure 1). Participants attended the study site for four sessions with a seven-day interval between sessions. Prior to the main experiments, all the participants completed a familiarization session. Participants underwent muscular strength and aerobic endurance measurements in the next session to determine one-repetition maximum (1RM) and maximal oxygen uptake ($\dot{V}O_2max$), respectively. The participants were then randomly assigned (Microsoft Excel 365, Washington, DC, USA) to ingest either caffeine or placebo before exercise in the two experimental sessions. The trials took place in an indoor gym with a dry temperature of 21.4 ± 0.6 °C and a relative humidity of 35 ± 10%. To avoid the potential effect of diurnal variation on the results (12), all measurements were carried out between 7:00 and 10:30 a.m. This study was conducted according to the latest version of the Declaration of Helsinki and received approval from the Ethics Committee of International Science and Technology University (202503-01), where the study was performed. The study was registered with the Iranian Registry of Clinical Trials (IRCT20231004059615N2).

Familiarization

The participants attended the gym following overnight fasting. After a 30-minute sitting rest in a quiet, temperature-controlled room, resting heart rate was measured (Beurer, BM 77 Bluetooth, Germany). Subsequently, they were medically screened by a cardiologist.

Then, the volunteers were familiarized with the gym environment and experimental procedures. Next, height (Seca, Halmburger, Germany), body mass and percent of body fat (PBF; InBody 970 Body Composition Analyzer, South Korea) were measured. BMI was calculated as kilograms per square meter (kg/m^2). To familiarize with the exercise protocol, participants completed a similar exercise program to the experiments, but with a lower volume and intensity, including a 10-minute warm-up, a 36-meter all-out sprint, 8 repetitions utilizing a resistance of 10 kg for each of the chest press machine, the lat pulldown machine, the leg press machine, and the overhead shoulder press machine exercises, and a 10-minute treadmill exercise with a speed of 3 km/h at an incline of 0%.

Muscular Strength & Aerobic Endurance Measurements

Following overnight fasting, the participants arrived at the gym. First, participants performed a 10-minute warm-up. They then completed a single set of 15 repetitions using a light weight. After 5 minutes of rest, the load was adjusted to ensure that fewer than 10 repetitions could be achieved. Then, 1RM was estimated by the following equation for the chest press, lat pulldown, leg press and overhead shoulder press exercises: 1RM = weight lifted / 1.0278 – (0.0278 × repetitions) (30). All resistance exercises were performed with the weight machines (Technogym, Italy).

After a 10-minute active rest, the assessment of $\dot{V}O_2max$ and maximum heart rate (HRmax) was conducted using a modified Bruce protocol on a motorized treadmill (Varilant, Lode, The Netherlands) within a controlled environment. This protocol has been previously documented in studies involving people with overweight and obesity (31). The protocol begins with a five-minute warm-up at a speed of 1.3 km/h with a 0% incline, followed by a requirement for participants to run on a treadmill for as long as possible, during which the speed and gradient are progressively increased every three minutes: Stage 1: 2.7 km/h at 0%

incline; Stage 2: 2.7 km/h at 5% incline; Stage 3: 2.7 km/h at 10% incline; Stage 4: 4 km/h at 12% incline; Stage 5: 5.3 km/h at 14% incline; Stage 6: 6.6 km/h at 16% incline; Stage 7: 8 km/h at 18% incline. The test was continued until the participant reached volitional exhaustion. Ventilation ($\dot{V}E$), oxygen uptake ($\dot{V}O_2$) and carbon dioxide output ($\dot{V}CO_2$) were measured at 10-second intervals using online computer-assisted circuit spirometry (Ganshorn Medizin Electronic GmbH Power Cube-Ergo, Germany), which was calibrated before each participant according to the manufacturer's instructions. The highest 10-second $\dot{V}O_2$ value during the incremental test was recorded as the $\dot{V}O_2$max value if it coincided with at least three of the following criteria: (a) a plateau in $\dot{V}O_2$ despite an increase in running speed; (b) a respiratory exchange ratio (RER) higher than 1.20; (c) peak heart rate at least equal to 90% of the age-predicted maximum; and/or (d) visible subject exhaustion. The physiological data obtained from the test were used for estimation of the relative workload corresponding to 70% $\dot{V}O_2$max for each volunteer (32).

Experimental Procedure

Participants arrived at the study site at 7:00 a.m. after an overnight fast of 9-10 hours and immediately consumed the supplement or placebo. The order of the sessions was random and counterbalanced. After 60 minutes of rest (30 minutes in a lying position and 30 minutes in a seated position, respectively), the baseline blood sample was taken. Then, they started the exercise protocol at 8:00 a.m., which lasted about 70 minutes. The second blood sample was taken immediately after exercise. The third blood sample was also collected after 60 minutes of rest in a seated position (inactive recovery). Participants were only allowed to drink plain water before and during the exercise as well as during the recovery period. Volunteers were asked to record their diet 24 hours before the first experiment and replicate it exactly before

the second experiment. Standardized verbal encouragement was provided continuously throughout each trial.

Exercise Protocol

While most studies conducted in the field of hemostasis system responses have used a single mode of exercise (10, 23, 28), an optimal exercise session designed by exercise experts and trainers with various goals, including weight loss, typically incorporates multiple modalities. Accordingly, we designed a concurrent exercise protocol consisting of high-intensity interval exercise (HIE), resistance, and aerobic exercises, with a 2-minute rest between. Also, given that some evidence points to the effect of caffeine on hemostasis responses to exercise being mediated by the ergogenic effects of caffeine on increasing performance time in exhausting exercise (33), the current research utilized exercises of identical volume.

A standardized warm-up was performed, including 5 minutes of jogging and 5 minutes of supervised dynamic exercises (walking lunges, heel flicks, leg swings, jumping jacks, and stretching). Next, 6 bouts of 36-meter all-out sprints, separated by a 1-minute active recovery, were performed. The resistance exercise consisted of chest press, lat pulldown, leg press, and overhead shoulder press was then performed for 2 sets of 12 repetitions at 70% 1RM intensity. All resistance exercises were performed with the weight machines at a constant speed (1 second eccentric and 1 second concentric), separated by a 1-minute active recovery. Subsequently, the aerobic exercise protocol consisted of 5 minutes of gradual speed increases in which the workload gradually increased to 70% of each participant's $\dot{V}O_2$max and 15 minutes at a constant workload corresponding to 70% of $\dot{V}O_2$max on the treadmill. The exercise protocol ended with a 5-minute cool-down, including walking and static stretches.

Caffeine Assessment & Supplementation

An adapted version of the Food Frequency Questionnaire (FFQ) proposed by Bühler et al. (34) was used to assess daily caffeine consumption. Daily caffeine consumption <25 mg/day is defined as a naïve consumer, whereas ingestion ≥25 mg/day is defined as a habitual consumer (35), based on the past 4 weeks' data.

On the experimental days, participants consumed 3 mg/kg of caffeine (Caffeine®, Olimp Laboratories, Poland) or placebo (250 mg of cellulose) in opaque capsules with a glass of water, 60 minutes before exercise began. During recovery, participants were given a questionnaire to see if they could tell which substance they thought they had consumed. The answers for both experiments were as follows: mean values (%) true = 34.9 ± 8.9%, false = 36.1 ± 12.5%, unable = 29.1 ± 13.9%.

Blood Analysis

Venous blood samples were collected from the antecubital vein and placed into vacutainer tubes containing citrate and ethylenediaminetetraacetic acid (EDTA) as anticoagulants. The blood samples in tubes containing EDTA were used to determine hemoglobin, hematocrit, platelet count and genotype, while the samples in tubes containing citrate were handled to analyze platelet aggregation, coagulation and fibrinolytic parameters.

Platelet count & aggregation. Platelet count, hemoglobin and hematocrit was measured using the cell counter system (Sysmex K-1000, Germany). Percent changes in plasma volumes were calculated from the hematocrit and hemoglobin values using the Dill and Costill method (36). Platelet count was expressed as corrected for changes in plasma volume. For the Platelet aggregation assessment, samples were centrifuged (180 g, 15 min, 23 °C) to preparation of platelet-rich plasma (PRP). After PRP was separated, the platelet-poor plasma

(PPP) was obtained by further centrifugation (2000 g, 10 minutes, 23 °C). The number of platelets in the PRP was standardized at 300×10^3 platelets/ml by dilution with PPP. The light transmission aggregometry (APACT 4004, LABiTec, Germany) was used to determine platelet aggregation using the following agonists: 5 μM adenosine diphosphate (ADP) and 3 μg/ml collagen. Platelet aggregation was expressed as a percentage of maximal aggregation.

Coagulation & fibrinolytic system. Blood samples were centrifuged for 20 min at 3000 g and 4°C. The PPP, with residual platelets less than 7×10^9/L, was aliquoted in plastic tubes, frozen by immersion in liquid nitrogen, and stored at −80°C until analyses. Blood fibrinogen concentration was evaluated via the modified Clauss method (Merck KGaA, Germany).

The lysis time of a tissue factor-induced clot by exogenous tissue-type plasminogen activator (tPA) was studied with a turbidimetric assay as previously described (37), with minor modifications. Briefly, plasma sample, thromboplastin, tPA, and TBS were added to microplate wells. The clotting reaction was started by adding CaCl2. The plate was incubated at 37°C, and the alteration in optical density at 405 nm was measured every minute in a microplate reader (Multiskan™ FC; Thermo Fisher Scientific, USA). Clot lysis time was defined as the interval between the midpoint of the clear to maximum turbidity transition and the midpoint of the maximum turbidity to clear transition. For each sample, the Clot lysis time assay was determined in duplicate.

Commercially available ELISA kits were used to determine plasma concentrations of prothrombin fragment 1 + 2 (F1 + 2) (E-EL-H1793), D-dimer (ab315310), tPA antigen (EL-H2106), and plasminogen activator inhibitor-1 (PAI-1) antigen (EL-H2104), according to the manufacturer's instructions.

Genotype. DNA was obtained from whole blood samples using the illustra™ blood genomicPrep Mini Spin Kit (GE Healthcare Life Sciences, Buckinghamshire, UK), and genotyping was performed via restriction fragment length polymorphism-polymerase chain reaction, as previously described (Cornelis et al. 2004). DNA was amplified by thermal cycling using the HotStar DNA Polymerase Kit (Qiagen, Mississauga, Canada) with specific primers (forward primer (5'-CTCATCCTCCTGCTACCTGG-3') and reverse primer (3'-ACTCTCAGGGAAGTGCTGTC-5')) to generate a 920 bp fragment of the *CYP1A2* gene. PCR conditions included an initial denaturation at 95°C for 15 min followed by 35 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 30 s, with a final extension at 72°C for 7 min. Half of each PCR product was digested by the restriction enzyme *ApaI*. All PCR products were analyzed by electrophoresis in a 2% agarose gel stained with ethidium bromide, and DNA bands were visualized under UV light. A 920 bp fragment indicated the A/A genotype, while 709 bp and 211 bp fragments indicated the C/C genotype. Participants were categorized as C-allele carriers (CC homozygotes and CA heterozygotes), due to a preliminary analysis showing very similar phenotyping responses in these individual subjects (38), or homozygous for the A-allele (AA homozygotes). A genetic specialist outside the study performed genotyping.

Statistical Analysis

The data are presented as mean ± standard deviation (SD). The Shapiro–Wilk test was used to assess the normality of the distribution. The Levene test was used to evaluate the equality of variances. The sphericity assumption was also tested by using Mauchly's test. If the sphericity assumption presented a probability of $p < 0.05$, the Greenhouse-Geisser correction was used. Repeated measures MANOVA was conducted with time (baseline, post-exercise, after 60 minutes recovery) and condition (placebo, caffeine) as within-subjects

factors and caffeine habituation (naïve consumer, habitual consumer) and genotype (C allele carriers, AA homozygotes) as between-subjects factors. When the analysis revealed a significant effect, the Bonferroni test was performed for the post hoc comparisons. ES were calculated using partial eta squared (η_p^2) (< 0.1, 0.10-0.24, 0.25-0.39 and ≥ 0.40 for trivial, small, moderate and large effects, respectively) to quantify the magnitude of the observed effects (Cohen, 2013). SPSS (version 27, Chicago, IL, USA) was used to analyze the data at the significance level $p < 0.05$.

RESULTS

Out of 40 participants who entered the study, 36 met the study criteria and were included in the analysis, while 4 participants were excluded due to their inability to complete the trials. Participant characteristics were presented in Table 1. Apart from the mean daily caffeine consumption, which was significantly higher in habitual consumers than in naïve consumers ($P < 0.001$), there were no significant differences between the groups in age, body mass, BMI, PBF, resting heart rate and $\dot{V}O_2max$ ($P > 0.05$).

Neither the main effects nor the interactions of genotype (*time* × *genotype*, *condition* × *genotype*, *genotype* × *habituation*, *time* × *condition* × *genotype*, *time* × *genotype* × *habituation*, *condition* × *genotype* × *habituation*, *time* × *condition* × *genotype* × *habituation*) for the study variables reached significance ($P > 0.05$, trivial to small ESs; Supplemental Table 1, Supplemental Digital Content).

There was a main effect of *time* for platelet count ($F = 476.469$, $P < 0.001$, $ES = 0.94$), as it increased significantly from baseline to post-exercise in both the placebo ($P < 0.001$; habitual: 18.8 ± 4.7%; naïve: 17.5 ± 6%) and caffeine ($P < 0.001$; habitual: 18.6 ± 6.3%; naïve: 18.3 ± 5.7%) conditions, and returned to the baseline values after 60 minutes recovery

(P > 0.05). There were no significant main effects of *condition* or *habituation* and no significant interaction effects on platelet count (P > 0.05, trivial ESs; Table 2).

The main effects of *time* (F = 365.370, P < 0.001, ES = 0.92) and *condition* (F = 12.175, P < 0.001, ES = 0.28), as well as interactions of *condition* × *habituation* (F = 27.589, P < 0.001, ES = 0.46) and *time* × *condition* × *habituation* (F = 4.177, P < 0.05, ES = 0.12) for the maximum ADP-induced platelet aggregation were detected. ADP-induced platelet aggregation increased following exercise in both the placebo (P < 0.001; habitual: 24.1 ± 7.1%; naïve: 23.2 ± 5.6%) and caffeine (P < 0.001; habitual: 22 ± 6.6%; naïve: 28.9 ± 8.9%) conditions, and returned to the baseline after recovery (P > 0.05). However, baseline (P < 0.001), post-exercise (P < 0.01) and post-recovery (P < 0.01) values for ADP-induced platelet aggregation in naïve consumers were significantly higher in the caffeine condition than in the corresponding placebo condition. ADP-induced platelet aggregation immediately after exercise was also significantly higher in naïve caffeine consumers than in habituated ones in the caffeine condition (P < 0.01), but not in the placebo condition (P > 0.05) (Figure 2A).

Likewise, there were main effects of *time* (F = 127.340, P < 0.001, ES = 0.8) and *condition* (F = 9.051, P < 0.01, ES = 0.22) as well as interactions of *condition* × *habituation* (F = 16.322, P < 0.001, ES = 0.34) and *time* × *condition* × *habituation* (F = 3.428, P < 0.05, ES = 0.1) for maximum platelet aggregation induced by collagen. Collagen-induced platelet aggregation increased immediately after exercise in both the placebo (P < 0.001; habitual: 14.9 ± 9.1%; naïve: 12.4 ± 11.2%) and caffeine (P < 0.001; habitual: 14.6 ± 7.9%; naïve: 19.4 ± 7.8%) conditions, and returned to the baseline after recovery (P > 0.05). Caffeine significantly increased collagen-induced platelet aggregation in naïve consumers compared to the corresponding placebo condition at post-exercise (P < 0.001) and post-recovery (P < 0.05). In the caffeine condition (P < 0.01), but not in the placebo condition (P > 0.05),

collagen induced-platelet aggregation was significantly higher in naïve caffeine consumers compared to habitual consumers (Figure 2B).

There was a main effect of *time* ($F = 96.739$, $P < 0.001$, ES = 0.75) and interactions of t*ime* × *habituation* ($F = 13.620$, $P < 0.001$, ES = 0.3), *condition* × *habituation* ($F = 7.066$, $P < 0.05$, ES = 0.18), *time* × *condition* ($F = 20.006$, $P < 0.001$, ES = 0.38) and *time* × *condition* × *habituation* ($F = 19.026$, $P < 0.001$, ES = 0.37) for fibrinogen levels. Fibrinogen increased immediately after exercise in both placebo ($P < 0.001$; habitual: 3.1 ± 3%; naïve: 3.7 ± 1.9%) and caffeine ($P < 0.001$; habitual: 3.2 ± 3.3%; naïve: 12.1 ± 5%) conditions, and returned to baseline upon recovery ($P > 0.05$). Caffeine caused a significant increase in fibrinogen levels in naïve consumers compared with the corresponding placebo condition only after exercise (<0.001). Fibrinogen level was also significantly higher in naïve caffeine consumers compared to habitual consumers in the caffeine condition ($P<0.001$; Table 2).

In F1 + 2, the main effects of *time* ($F = 392.549$, $P < 0.001$, ES = 0.92) and *condition* ($F = 7.423$, $P < 0.01$, ES = 0.19), as well as the interactions of *condition* × *habituation* ($F = 13.912$, $P < 0.001$, ES = 0.3), *time* × *condition* ($F = 9.952$, $P < 0.05$, ES = 0.24) and *time* × *condition* × *habituation* ($F = 3.478$, $P < 0.05$, ES = 0.1) were observed. F1 + 2 increased after exercise in both the placebo ($P < 0.001$; habitual: 10.2 ± 2.3%; naïve: 9.5 ± 3.3%) and caffeine ($P < 0.001$; habitual: 9.8 ± 3.7%; naïve: 12.8 ± 5.1%) conditions, and returned to the baseline after recovery ($P > 0.05$). In naïve consumers, caffeine led to a significantly greater increase in F1+2 than the corresponding placebo condition post-exercise ($P < 0.001$). F1 + 2 after exercise was significantly higher in naïve consumers than in habitual consumers in the caffeine condition ($P < 0.05$), but not in the placebo condition ($P > 0.05$) (Table 2).

The main effects of *time* ($F = 295.347$, $P < 0.001$, ES = 0.9) and *condition* ($F = 4.550$, $P < 0.05$, ES = 0.12) and the interactions of *time* × *habituation* ($F = 3.923$, $P < 0.05$, ES = 0.11), *time* × *condition* ($F = 3.561$, $P < 0.05$, ES = 0.1) and *time* × *condition* × *habituation* (F

= 3.666, P < 0.05, ES = 0.1) on tPA antigen were identified. The tPA antigen increased immediately after exercise in both the placebo (P < 0.001; habitual: 31.6 ± 9.6%; naïve: 32 ± 13.5%) and caffeine (P < 0.001; habitual: 33.6 ± 15.4%; naïve: 23.8 ± 14.3%) conditions, and returned to the baseline after recovery (P > 0.05). In the caffeine condition, post-exercise and post-recovery tPA levels were significantly lower in naïve subjects than in the corresponding placebo condition (P < 0.05). In contrast, post-exercise tPA antigen was higher in habitual subjects compared to the corresponding placebo condition (P < 0.01). In the caffeine (P < 0.01) but not the placebo condition (P > 0.05), post-exercise tPA levels were also significantly higher in the habitual caffeine consumers than in the naïve consumers (Table 2).

We found the main effect of *time* (F = 69.9, P < 0.001, ES = 0.69) and the interaction effect of *time* × *condition* (F = 4.612, P < 0.05, ES = 0.13) on PAI-1 antigen. There were no significant changes in PAI-1 antigen levels immediately after exercise (P > 0.05). However, it was significantly decreased after recovery in both placebo (habitual: P < 0.01, -19.1 ± 11.9%; naïve: P < 0.001, -19.2 ± 6.3%) and caffeine (P < 0.01; habitual: -22.9 ± 12.4%; naïve: -21.1 ± 17.7%) conditions. After recovery, habitual consumers experienced a significantly greater reduction in PAI-1 antigen levels compared to the corresponding placebo condition (P < 0.001; Table 2).

The main effect of *time* was observed on D-dimer (F = 267.743, P < 0.001, ES = 0.89), which increased significantly from baseline to post-exercise in both the placebo (P < 0.001; habitual: 8.2 ± 3.1%; naïve: 7.6 ± 3%) and caffeine (P < 0.001; habitual: 8.8 ± 3.6%; naïve: 9 ± 4.3%) conditions, and returned to the baseline values after 60 minutes of recovery (P > 0.05). However, there were no significant main effects of *condition* or *habituation* and no significant interaction effects on D-dimer (P > 0.05, trivial ESs; Table 2).

The main effect of *time* (F = 209.874, P < 0.001, ES = 0.87) and interactions of *time* × *habituation* (F = 4.321, P < 0.05, ES = 0.12), *condition* × *habituation* (F = 8.385, P < 0.01,

ES = 0.21) and *time* × *condition* × *habituation* (F = 4.269, P < 0.05, ES = 0.12) for the clot lysis time were identified. Clot lysis time increased following exercise in both the placebo (P < 0.001; habitual: 16.8 ± 7.5%; naïve: 16.5 ± 13.7%) and caffeine (P < 0.001; habitual: 8.9 ± 5.9%; naïve: 18.7 ± 10.8%) conditions, and returned to the baseline after recovery (P > 0.05). Habitual caffeine users exhibited a significantly larger decrease in post-exercise clot lysis time when consuming caffeine versus placebo (P < 0.001). In addition, clot lysis time immediately after exercise was significantly longer in the naïve than in habitual consumers in the caffeine condition (P < 0.001; Figure 3).

DISCUSSION

Increased platelet activity, coagulation, and fibrinolysis following acute exercise are well-documented in the scientific literature (10-12). However, little is known about the hemostatic responses of individuals with obesity to concurrent exercise or the potential impact of caffeine supplementation on these responses in the obesity situation. The primary aim of this study was to evaluate the effect of acute caffeine supplementation on hemostatic responses to moderate-to-high-intensity concurrent exercise in adults with obesity, as determined by measures of platelet function, coagulation, and fibrinolysis parameters at baseline, immediately post-exercise, and following 60 minutes of recovery. The secondary aim was to assess whether differences in hemostatic responses exist between naïve and habitual caffeine consumers, as well as between C allele carriers and AA homozygotes of the *CYP1A2* gene. Findings showed that acute exercise triggered ADP and collagen-induced platelet aggregation, coagulation, and fibrinolysis, with a greater tendency toward a prothrombotic state, regardless of genotype or caffeine habituation status. After 60 minutes of recovery, most altered variables returned to baseline values, except for PAI-1, whose antigen levels remained unchanged immediately after exercise but decreased following recovery.

Acute caffeine supplementation, however, produced a stronger prothrombotic profile in naïve consumers, showing greater ADP and collagen-induced platelet aggregation at baseline, post-exercise, and post-recovery, as well as elevated levels of fibrinogen, F1+2, and clot lysis time, and less increased tPA antigen levels post-exercise. On the other hand, acute caffeine administration reduced post-exercise prolongation in clot lysis time and led to a larger decrease in post-recovery PAI-1 antigen levels among habitual caffeine users, suggesting improved fibrinolysis. Finally, caffeine's impact on hemostatic responses to exercise was unaffected by genotype.

The exercise-induced increases in platelet count and aggregation observed in the present study are consistent with previous studies reporting similar changes in healthy individuals (10-12) and in populations at elevated thrombotic risk (10). On the other hand, Lamprecht et al. (39) found that platelet indices were not affected by 30 minutes of aerobic running at 70% of $\dot{V}O_2$peak in women with obesity. This inconsistency is likely due to differences in exercise modality, intensity and duration (70 min of moderate-to-high-intensity concurrent exercise versus 30 min of moderate-intensity aerobic exercise), participant sex (male versus female), and age (22.2 ± 2.3 years versus 41.1 ± 3.8 years). Platelet reactivity transiently increases during and immediately after exercise, driven by elevated shear stress, oxidative stress, catecholamine release, and enhanced thromboxane A_2 synthesis—all of which favor aggregation (40). Moreover, the post-exercise increases in platelet count among our participants could potentially enhance the aggregation responses (41).

In this study, acute concurrent exercise increased plasma fibrinogen and F1+2 levels, indicating enhanced coagulation. This observation stands in contrast to the findings of Nagelkirk et al. (23), who reported no significant alteration in the plasma thrombin–antithrombin complex, a well-established marker of coagulation activation, following six sets of ten leg extensions performed at 70% of 1-RM in overweight female subjects (BMI, 25.3 ±

2.9). Such inconsistency may arise from differences in exercise modality and volume, as well as variations in participant sex and BMI.

Previous investigations have shown that individuals with obesity exhibit a blunted exercise-induced fibrinolytic response compared with normal-weight controls, a phenomenon attributed to elevated baseline coagulation and antifibrinolytic activity (23, 42). Post-exercise increases in tPA antigen and D-dimer levels in the current study verify activation of the fibrinolytic cascade. However, the simultaneous prolongation of clot lysis time, even with stable PAI-1 antigen levels, suggests a reduction in the general efficiency of the fibrinolysis system. An increase in fibrinogen and accelerated thrombin generation (as indicated by elevated F1+2) due to exercise is likely to alter the structure of the clot and make it denser and more resistant to enzymatic degradation. Moreover, upregulation of fibrinolysis inhibitors, such as thrombin-activatable fibrinolysis inhibitor (TAFI) or functionally active PAI-1, may further delay clot resolution (43). In addition, it was shown that high levels of active PAI-1 convert more tPA into tPA/PAI-1 complex, slowing the clearance of total tPA antigen (44). Together, these findings suggest that acute exercise in obesity induces a hemostatic profile favoring thrombin generation and clot stability, with compensatory—but limited—fibrinolytic activation.

Our results show that hemostatic responses to exercise are affected by caffeine habituation, which could also help to explain some of the discrepancies noted in the previous studies. In caffeine-naïve individuals, acute caffeine supplementation exacerbated platelet aggregation at baseline, immediately post-exercise, and after recovery. Along with lower tPA antigen levels, these participants also showed higher post-exercise fibrinogen, F1+2, and clot lysis time, all of which indicate an increased prothrombotic state. Conversely, regular caffeine users showed a smaller increase in clot lysis time after exercise and a more pronounced decrease in PAI-1 antigen levels during recovery under caffeine conditions, indicating a

relative improvement in fibrinolytic capacity. To the best of our knowledge, no previous studies have specifically examined the combined effects of acute caffeine ingestion and exercise on hemostatic responses in people with obesity, which makes it difficult to interpret our results. Nevertheless, Whittaker et al. (28) found that a single HIE session increased platelet function, whereas caffeine consumption (3 mg/kg) reduced the exercise-induced platelet activation and aggregation in physically active men. However, the lack of habitual caffeine intake assessment in that study limits direct comparison with our findings. Further, Nagelkirk et al. (23) found that caffeine consumption (6 mg/kg) prior to exhaustive cycling exercise augmented coagulation potential, as evidenced by elevated factor VIII levels, without a corresponding increase in fibrinolysis in healthy men with habitual moderate caffeine consumption. Similarly, Wassell et al. (24) observed that caffeine intake (6 mg/kg) before firefighting drills test led to a higher coagulation response in healthy male firefighters with habitual moderate caffeine use. Importantly, the higher caffeine doses administered in both of the latter studies may have elicited greater catecholamine release, thereby increasing coagulation responses (14). Additionally, the ergogenic properties of caffeine could potentially enhance exercise performance during exhaustive exercise and/or exercise testing, resulting in increased physical exertion and subsequent hemostatic alterations. These changes may result not from a direct physiological effect of caffeine on the coagulation system per se, but rather from the increased intensity and/or volume of physical activity it enables (33). Accordingly, in the present study, both the intensity and volume of concurrent exercise were carefully standardized across all participants. It is also important to note, as highlighted in the present study, that evaluation of isolated hemostatic markers may not fully capture the complexities of the coagulation and fibrinolytic systems.

The different responses between caffeine-naïve and habitual consumers are likely due to the interaction of caffeine with adenosine receptors, sympathetic stimulation, and

habituation-related physiological adaptations. Caffeine acts primarily as a non-selective adenosine receptor antagonist. In naïve consumers, this antagonism acutely increases sympathetic activity, which can enhance platelet reactivity, fibrinogen synthesis, and thrombin generation via catecholamine-mediated mechanisms (14). Under normal conditions, adenosine inhibits platelet aggregation through A_{2A} receptor signaling (45). By blocking this inhibition, caffeine further promotes aggregation, especially during exercise-induced catecholamine increases. Conversely, habitual caffeine intake induces adaptive alterations, such as upregulation of adenosine receptors, altered receptor affinity, and desensitization of sympathetic pathways, that blunt the acute prothrombotic effects of caffeine (14). Data from epidemiological studies suggest that habitual consumers of caffeinated coffee may also exhibit improved endothelial function and reduced systemic inflammation (46, 47), potentially modifying the hemostatic response to exercise. The mechanisms behind these modulations are still under study, but it seems enhanced endothelial nitric oxide production (48) and reduced certain inflammatory markers by regular caffeine ingestion (49) probably can dampen platelet activation and coagulation cascade amplification, as well as increase fibrinolysis during acute exercise. Collectively, these adaptations suggest that regular caffeine consumption at a moderate dosage may condition the hemostatic and vascular systems to mount a more balanced response to exercise-induced stress.

CYP1A2 is the primary enzyme responsible for metabolizing caffeine into paraxanthine, with its activity largely influenced by the -163C>A polymorphism. Individuals with the AA genotype are classified as “fast metabolizers,” whereas C-allele carriers exhibit slower caffeine metabolism (38). This genetic variation has been associated with differential physiological responses to caffeine, including alterations in blood pressure, cardiovascular risk, and exercise performance (38, 50, 51). However, our findings indicate that the *CYP1A2* genotype does not significantly modify the acute hemostatic response to caffeine during

exercise, at least at the moderate dose employed in this study. Several physiological and pharmacokinetic explanations may account for this lack of genotype effect. Acute exercise elicits vigorous hemostatic changes driven by shear stress, catecholamine increase, and endothelial activation (40), which may overshadow possible relatively minor interindividual differences in caffeine metabolism. The consistent post-exercise elevation in hemostatic markers across genotypes supports this interpretation. Furthermore, in people with obesity, the pre-existing prothrombotic state (2) may influence the overall hemostatic profile and attenuate any possible genotype-dependent modulation of caffeine's effects.

Limitations

Some limitations should be taken into account while considering the findings of this study. We recruited young adults with obesity who were relatively healthy. It is possible that common comorbidities with obesity may affect hemostatic responses to exercise and caffeine intake. Also, the dose of caffeine used (3 mg/kg) was equal to or slightly higher than the usual dose used in habituated participants, so higher doses (e.g., 6 mg/kg) may reverse caffeine's beneficial effects on modulating hemostatic responses to exercise (23, 24). Furthermore, this study was conducted in the morning, when the prothrombotic state at baseline and in response to exercise is predominant (12). Therefore, performing a similar protocol in the evening, when fibrinolytic system activity is increased (12), may diminish the negative effects of acute caffeine intake on hemostatic responses to exercise in naïve caffeine consumers. In addition, without healthy normal-weight controls, we cannot delineate obesity-specific effects on hemostatic responses. Finally, our study did not assess inflammatory mediators or markers of endothelial activation, which limits our ability to draw definitive conclusions about the contribution of inflammation to the observed hemostatic changes. Future research should investigate the complex interplay between caffeine, exercise,

hemostasis, and inflammation, particularly in the context of obesity, as this may yield valuable mechanistic insights.

CONCLUSIONS

In conclusion, moderate-to-high-intensity concurrent exercise induces a transient prothrombotic state in physically inactive individuals with obesity. Acute caffeine supplementation at a moderate dose can modulate the hemostatic responses depending on caffeine habituation status rather than *CYP1A2* genotype: it exacerbates the exercise-induced prothrombotic state in naïve consumers, whereas it promotes a more fibrinolytic profile in habitual consumers. Accordingly, caffeine intake before exercise should be customized based on habitual consumption to minimize potential prothrombotic risks, particularly in people with obesity, who are a vulnerable population for cardiovascular disease.

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FIGURE LEGENDS

Figure 1. CONSORT diagram and study design.

Figure 2. ADP- (A) and collagen- (B) induced platelet aggregation at baseline, immediately after exercise and after 60 minutes of recovery in placebo and caffeine conditions. Values are mean ± SD. [a] $P < 0.01$ different from baseline; [b] $P < 0.05$ different from corresponding placebo condition; [c] $P < 0.01$ different from habitual consumers.

Figure 3. Clot lysis time at baseline, immediately after exercise and after 60 minutes of recovery in placebo and caffeine conditions. Values are mean ± SD. [a] $P < 0.001$ different from baseline; [b] $P < 0.001$ different from corresponding placebo condition; [c] $P < 0.001$ different from habitual consumers.

SUPPLEMENTAL DIGITAL CONTENT

SDC 1: Supplemental Digital Content.docx

Figure 1

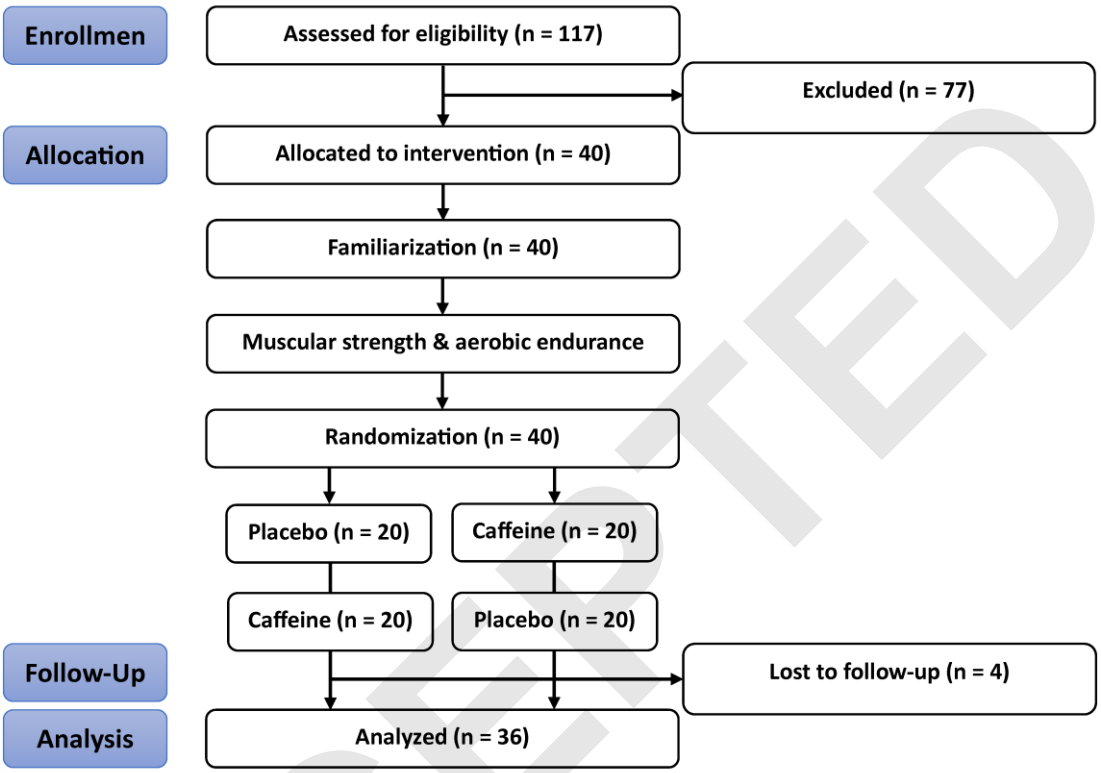

FIGURE 1.

Figure 2

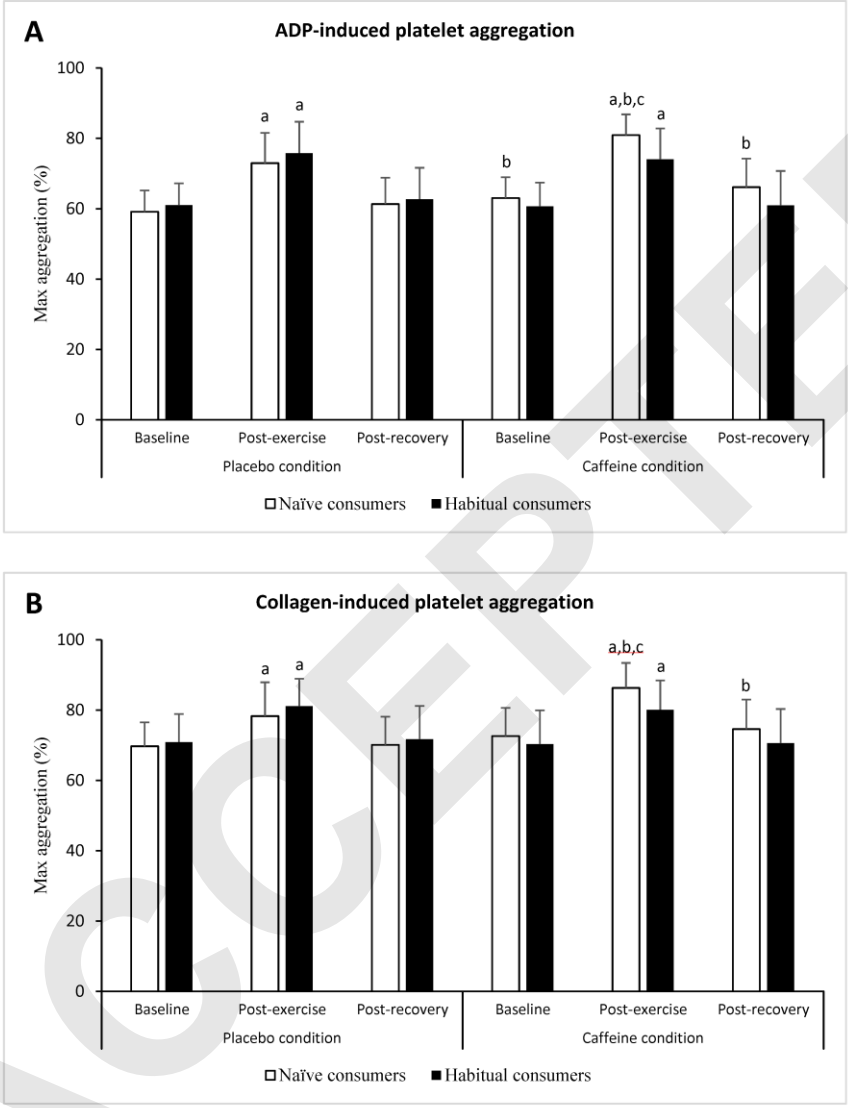

FIGURE 2.

Figure 3

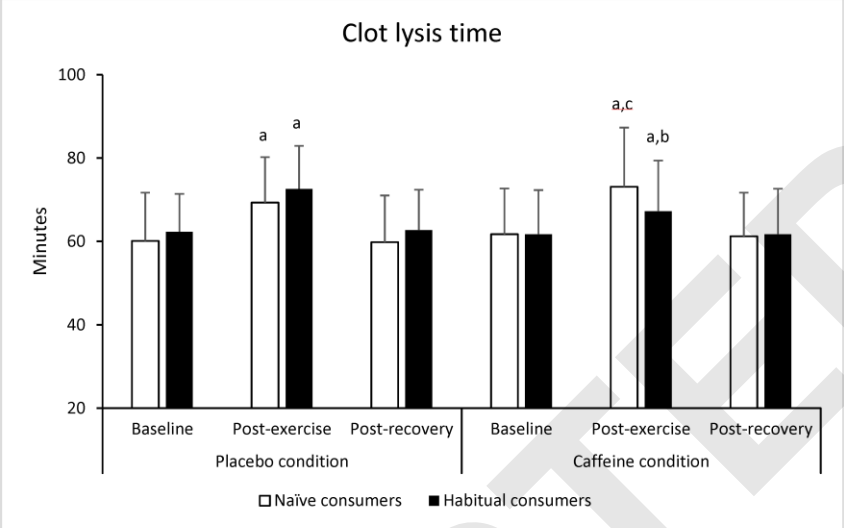

FIGURE 3.

TABLE 1. Participant characteristics.

	All (n=36)	Naïve (n=13)	Habitual (n=23)	C allele (n=20)	AA homozygotes (n=16)
Age (y)	22.2 ± 2.3	21.9 ± 2.5	22.3 ± 2.1	21.9 ± 2.3	22.4 ± 2.3
Body mass (kg)	114.9 ± 11.9	116.4 ± 13	114 ± 11.4	114.6 ± 11.5	115.2 ± 12.6
BMI (kg/m^2)	34.1 ± 2.7	34.5 ± 2.6	33.9 ± 2.8	34 ± 2.9	34.3 ± 2.5
PBF (%)	33.2 ± 2.9	33.7 ± 2.7	32.9 ± 3	32.8 ± 3.2	33.7 ± 2.4
Resting heart rate (b/min)	76.4 ± 2.7	76.8 ± 3	76.1 ± 2.4	75.7 ± 2.7	77.1 ± 2.5
$\dot{V}O_2$max (ml/kg/min)	28.8 ± 2.7	28.1 ± 3.1	29.2 ± 2.5	29.2 ± 3	28.3 ± 2.4
Caffeine intake (mg/day)	152.2 ± 136.7	16 ± 5.7[a]	229.2 ± 112	164 ± 139	137.4 ± 137

Values are mean ± SD.

[a] $P < 0.001$ different from habitual consumers.

BMI, body mass index; PBF, percent of body fat; $\dot{V}O_2$max, maximal oxygen consumption.

TABLE 2. Platelet count and fibrinogen, tPA, PAI-1, F1+2 and D-dimer levels at baseline, immediately after exercise and after 60 minutes of recovery in placebo and caffeine conditions.

		Baseline		Post-exercise		Post-recovery	
		Naïve	**Habitual**	**Naïve**	**Habitual**	**Naïve**	**Habitual**
platelet count	Placebo	260.9± 14	255.3 ± 12	306.3 ± 17[a]	303.2 ± 15[a]	260.4 ± 16	258.1 ± 12
($\times 10^9$/L)	Caffeine	260.7 ± 15	257.6 ± 13	308.2 ± 18[a]	305.3 ± 17[a]	265.1 ± 23	262.7 ± 19
Fibrinogen	Placebo	2.93 ± 0.39	3 ± 0.33	3.04 ± 0.39[a]	3.1 ± 0.36[a]	2.95 ± 0.38	3 ± 0.32
(g/L)	Caffeine	2.93 ± 0.36	2.99 ± 0.32	3.28 ± 0.34[a,b,c]	3.09 ± 0.37[a]	2.91 ± 0.33	2.97 ± 0.3
tPA antigen	Placebo	4.5 ± 1.4	4.6 ± 1	5.9 ± 1.5[a]	6 ± 1.2[a]	4.4 ± 1.1	4.7 ± 1
(ng/ml)	Caffeine	4.6 ± 1.3	4.7 ± 1.1	5.6 ± 1.2 [a,c]	6.2 ± 1.3[a]	4.7 ± 1.2	4.9 ± 0.9
PAI-1 antigen	Placebo	13.8 ± 2.6	13.6 ± 1.7	14 ± 2.4	13.5 ± 1.8	10.9 ± 2.3[a]	11 ± 2.1[a]
(ng/ml)	Caffeine	14.4 ± 2.2	13.5 ± 1.9	13.2 ± 2.3	12.7 ± 2.1	11.1 ± 2.2[a]	10.4 ± 2[a,b]
F1+2	Placebo	254.5 ± 17.1	252.1 ± 18.5	278.8 ± 19.4[a]	277.7 ± 18.6[a]	256.5 ± 17.9	252.9 ± 18.6
(pmol/L)	Caffeine	258.2 ± 17.5	253.3 ± 19.3	290.9 ± 19 [a,b,c]	278 ± 21.1[a]	257.3 ± 18	249 ± 19.8
D-dimer	Placebo	324.4 ± 14	319.7 ± 16.7	349 ± 17.9[a]	345.9 ± 18.4[a]	323.7 ± 11.9	323.6 ± 17.6
(ng/ml)	Caffeine	323.7 ± 11.9	320.3 ± 17.2	353.2 ± 24[a]	348.3 ± 18[a]	324.5 ± 18.3	320.5 ± 19.5

Values are mean ± SD.

[a] $P < 0.01$ different from baseline; [b] $P < 0.05$ different from corresponding placebo condition; [c] $P < 0.05$ different from habitual consumers.

PC, Platelet count; tPA, tissue-type plasminogen activator; PAI-1, plasminogen activator inhibitor-1; F1+2, prothrombin fragment 1 + 2.

Table S1. The main effect of genotype and its interaction with other independent variables on hemostatic responses.

Main Effect	*Time* × *Genotype*	*Time* × *Genotype* × *Habituation*	*Condition* × *Genotype*	*Condition* × *Genotype* × *Habituation*	*Time* × *Condition* × *Genotype*	*Time* × *Condition* × *Genotype* × *Habituation*
ADP-induced platelet aggregation						
F = 0.650 P = 0.426 ES = 0.02	F = 0.097 P = 0.908 ES = 0.003	F = 0.574 P = 0.566 ES = 0.05	F = 1.230 P = 0.276 ES = 0.037	F = 1.648 P = 0.208 ES = 0.049	F = 0.713 P = 0.494 ES = 0.022	F = 2.857 P = 0.065 ES = 0.082
Collagen-induced platelet aggregation						
F = 0.881 P = 0.355 ES = 0.027	F = 1.217 P = 0.303 ES = 0.037	F = 0.461 P = 0.502 ES = 0.014	F = 0.787 P = 0.382 ES = 0.024	F = 0.000 P = 0.990 ES = 0.000	F = 1.323 P = 0.274 ES = 0.040	F = 2.304 P = 0.108 ES = 0.067
Platelet count						
F = 0.256 P = 0.617 ES = 0.008	F = 0.899 P = 0.412 ES = 0.027	F = 2.746 P = 0.072 ES = 0.079	F = 0.102 P = 0.752 ES = 0.003	F = 2.478 P = 0.125 ES = 0.072	F = 0.110 P = 0.896 ES = 0.003	F = 0.531 P = 0.591 ES = 0.016
TPA antigen						
F = 1.033 P = 0.317 ES = 0.031	F = 1.514 P = 0.228 ES = 0.045	F = 1.441 P = 0.244 ES = 0.043	F = 0.307 P = 0.583 ES = 0.01	F = 1.638 P = 0.210 ES = 0.049	F = 0.495 P = 0.612 ES = 0.015	F = 0.759 P = 0.472 ES = 0.023
PAI-1 antigen						
F = 0.009 P = 0.927 ES = 0.0	F = 2.415 P = 0.124 ES = 0.07	F = 0.631 P = 0.456 ES = 0.019	F = 0.067 P = 0.797 ES = 0.002	F = 0.684 P = 0.414 ES = 0.021	F = 1.499 P = 0.233 ES = 0.045	F = 1.580 P = 0.219 ES = 0.047
Clot lysis time						
F = 0.271 P = 0.606 ES = 0.008	F = 1.545 P = 0.221 ES = 0.046	F = 2.069 P = 0.135 ES = 0.061	F = 1.980 P = 0.169 ES = 0.058	F = 0.060 P = 0.807 ES = 0.002	F = 0.412 P = 0.664 ES = 0.013	F = 1.354 P = 0.266 ES = 0.041
Fibrinogen						
F = 0.007 P = 0.936 ES = 0.0	F = 0.568 P = 0.523 ES = 0.017	F = 0.306 P = 0.738 ES = 0.009	F = 0.082 P = 0.777 ES = 0.003	F = 0.122 P = 0.729 ES = 0.004	F = 1.457 P = 0.242 ES = 0.044	F = 0.581 P = 0.535 ES = 0.018
F1+2						
F = 1.245 P = 0.273 ES = 0.037	F = 2.504 P = 0.09 ES = 0.073	F = 2.342 P = 0.104 ES = 0.068	F = 0.226 P = 0.638 ES = 0.007	F = 1.213 P = 0.279 ES = 0.037	F = 0.545 P = 0.583 ES = 0.017	F = 1.935 P = 0.153 ES = 0.057
D-dimer						
F = 0.068 P = 0.796 ES = 0.002	F = 0.845 P = 0.434 ES =	F = 0.401 P = 0.672 ES = 0.012	F = 0.229 P = 0.635 ES = 0.007	F = 1.312 P = 0.260 ES =	F = 0.280 P = 0.756 ES =	F = 1.014 P = 0.368 ES = 0.031

	0.026	0.039	0.009

ES, effect size.

Section/topic	No	CONSORT 2025 checklist item description	Reported on page no.
Title and abstract			
Title and structured abstract	1a	Identification as a randomised trial	1 & 2
	1b	Structured summary of the trial design, methods, results, and conclusions	2
Open science			
Trial registration	2	Name of trial registry, identifying number (with URL) and date of registration	4
Protocol and statistical analysis plan	3	Where the trial protocol and statistical analysis plan can be accessed	4
Data sharing	4	Where and how the individual de-identified participant data (including data dictionary), statistical code and any other materials can be accessed	15
Funding and conflicts of interest	5a	Sources of funding and other support (eg, supply of drugs), and role of funders in the design, conduct, analysis and reporting of the trial	16
	5b	Financial and other conflicts of interest of the manuscript authors	15 & 16
Introduction			
Background and rationale	6	Scientific background and rationale	3
Objectives	7	Specific objectives related to benefits and harms	3
Methods			
Patient and public involvement	8	Details of patient or public involvement in the design, conduct and reporting of the trial	20
Trial design	9	Description of trial design including type of trial (eg, parallel group, crossover), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	4
Changes to trial protocol	10	Important changes to the trial after it commenced including any outcomes or analyses that were not prespecified, with reason	-
Trial setting	11	Settings (eg, community, hospital) and locations (eg, countries, sites) where the trial was conducted	4
Eligibility criteria	12a	Eligibility criteria for participants	4 & 5
	12b	If applicable, eligibility criteria for sites and for individuals delivering the interventions (eg, surgeons, physiotherapists)	-
Intervention and comparator	13	Intervention and comparator with sufficient details to allow replication. If relevant, where additional materials describing the intervention and comparator (eg, intervention manual) can be accessed	-
Outcomes	14	Prespecified primary and secondary outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome	9 & 11
Harms	15	How harms were defined and assessed (eg, systematically, non-systematically)	4 & 5
Sample size	16a	How sample size was determined, including all assumptions supporting the sample size calculation	5

	16b	Explanation of any interim analyses and stopping guidelines	-
Randomisation:			
Sequence generation	17a	Who generated the random allocation sequence and the method used	4
	17b	Type of randomisation and details of any restriction (eg, stratification, blocking and block size)	4
			Reported on page no.
Allocation concealment mechanism	18	Mechanism used to implement the random allocation sequence (eg, central computer/telephone; sequentially numbered, opaque, sealed containers), describing any steps to conceal the sequence until interventions were assigned	4
Implementation	19	Whether the personnel who enrolled and those who assigned participants to the interventions had access to the random allocation sequence	4
Blinding	20a	Who was blinded after assignment to interventions (eg, participants, care providers, outcome assessors, data analysts)	4
	20b	If blinded, how blinding was achieved and description of the similarity of interventions	4
Statistical methods	21a	Statistical methods used to compare groups for primary and secondary outcomes, including harms	9
	21b	Definition of who is included in each analysis (eg, all randomised participants), and in which group	9
	21c	How missing data were handled in the analysis	9
	21d	Methods for any additional analyses (eg, subgroup and sensitivity analyses), distinguishing prespecified from post hoc	9
Results			
Participant flow, including flow diagram	22a	For each group, the numbers of participants who were randomly assigned, received intended intervention, and were analysed for the primary outcome	9 & 23
	22b	For each group, losses and exclusions after randomisation, together with reasons	9 & 23
Recruitment	23a	Dates defining the periods of recruitment and follow-up for outcomes of benefits and harms	-
	23b	If relevant, why the trial ended or was stopped	-
Intervention and comparator delivery	24a	Intervention and comparator as they were actually administered (eg, where appropriate, who delivered the intervention/comparator, how participants adhered, whether they were delivered as intended (fidelity))	5
	24b	Concomitant care received during the trial for each group	-
Baseline data	25	A table showing baseline demographic and clinical characteristics for each group	20
Numbers analysed, outcomes and estimation	26	For each primary and secondary outcome, by group: ● the number of participants included in the analysis ● the number of participants with available data at the outcome time point ● result for each group, and the estimated effect size and its precision (such as 95% confidence interval) ● for binary outcomes, presentation of both absolute and relative effect size	9 - 11
Harms	27	All harms or unintended events in each group	-

Ancillary analyses	28	Any other analyses performed, including subgroup and sensitivity analyses, distinguishing pre-specified from post hoc	9 - 11
Discussion			
Interpretation	29	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	12 - 14
Limitations	30	Trial limitations, addressing sources of potential bias, imprecision, generalisability, and, if relevant, multiplicity of analyses	14 - 15

Citation: Hopewell S, Chan AW, Collins GS, Hróbjartsson A, Moher D, Schulz KF, et al. CONSORT 2025 Statement: updated guideline for reporting randomised trials. BMJ. 2025; 388:e081123. https://dx.doi.org/10.1136/bmj-2024-081123

*We strongly recommend reading this statement in conjunction with the CONSORT 2025 Explanation and Elaboration and/or the CONSORT 2025 Expanded Checklist for important clarifications on all the items. We also recommend reading relevant CONSORT extensions. See www.consort-spirit.org.