

**Randomised controlled trial comparing impact on platelet reactivity of twice-daily with once-daily aspirin in people with type 2 diabetes**

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**Title:** Randomised controlled trial comparing impact on platelet reactivity of twice-daily with  
once-daily aspirin in people with type 2 diabetes

**Running Title:** Twice-daily aspirin decreases platelet reactivity

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**Novelty Statement:** Aspirin is an established intervention for the secondary prevention of cardiovascular disease, but has a relative lack of efficacy for the primary prevention of cardiovascular disease in people with diabetes. This study demonstrates that aspirin 100mg given twice-daily was more effective than 100mg given once-daily. These data should help inform the design of future large-scale trials evaluating the potential risks and benefits of aspirin for primary cardiovascular prevention in people with type 2 diabetes.

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For Peer Review

## Abstract

**Aims:** Reduced aspirin efficacy has been demonstrated in people with type 2 diabetes (T2DM). As increased platelet reactivity and/or turnover are postulated mechanisms, we examined whether higher and/or more frequent aspirin dosing could reduce platelet reactivity more effectively.

**Methods:** Participants with T2DM (n=24) but without known cardiovascular disease were randomized in a three-way crossover design to 2-week treatment periods with aspirin 100mg once-daily, 200mg once-daily or 100mg twice-daily. The primary outcome was platelet reactivity, assessed by the VerifyNow™ ASA method. Relationships between platelet reactivity and aspirin dosing were examined using generalized linear mixed models with random subject effects.

**Results:** Platelet reactivity decreased from baseline with all doses of aspirin. Modelled platelet reactivity was more effectively reduced with aspirin 100mg twice-daily *versus* 100mg once-daily, but not *versus* 200mg once-daily. Aspirin 200mg once-daily did not differ from 100mg once-daily. Aspirin 100mg twice-daily was also more effective than once-daily as measured by collagen/epinephrine stimulated platelet aggregation and urinary thromboxane levels, with a similar trend measured by serum thromboxane levels. No episodes of bleeding occurred.

**Conclusions:** In T2DM, aspirin 100mg twice-daily reduced platelet reactivity more effectively than 100mg once-daily, and numerically more than 200mg once-daily. Clinical outcome trials evaluating primary cardiovascular disease prevention with aspirin in T2DM may need to consider using a more frequent dosing schedule.

*Clinical Trial Registration:* 2011-003123-35 (<https://www.clinicaltrialsregister.eu/>)

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**Introduction**

Aspirin inhibits platelet aggregation and is used clinically to prevent cardiovascular events. In type 2 diabetes (T2DM), it is a proven therapy for secondary cardiovascular prevention, but for primary prevention the risk of major bleeding may outweigh potential benefits (1, 2). Randomised controlled primary cardiovascular prevention trials have shown only modest or no significant reduction in cardiovascular risk with aspirin therapy (1) (3-5).

Reduced aspirin efficacy has been demonstrated in people with T2DM but the underlying mechanisms are poorly understood. A prothrombotic state may exist, characterized by increased platelet reactivity and turnover, increased hepatic synthesis of fibrinogen and plasminogen activator inhibitor (PAI-1), quantitative changes in the glycation and oxidation of clotting factors, (6-8) or increased reactive oxygen species. Accordingly, it is possible that higher or more frequent doses of aspirin might be needed to overcome these metabolic barriers.

Placebo-controlled trials of aspirin for CVD prevention have used doses ranging from 50 to 1300mg *per* day (9). Evidence, primarily from secondary cardiovascular prevention trials, suggests that using “high dose” aspirin (>100mg daily) increases bleeding risk without enhancing efficacy. Small-scale studies have suggested twice-daily aspirin regimens reduce platelet aggregation more effectively (10) (11-13), but no large-scale outcome trials have examined twice-daily dosing to assess CVD prevention or bleeding risks.

This study of people with T2DM but without known CVD examines three aspirin regimens to determine whether a higher dose (200mg), given once-daily or split 50:50 twice-daily, can more effectively reduce platelet reactivity than a once-daily dose of 100 mg.

## Patients and Methods

This single-centre, randomized, prospective, double-blind study received ethical approval from the South East London Research Ethics Committee 3. It complied with The World Medical Association Declaration of Helsinki (1964) and its amendments and clarifications, the European Union Clinical Trials Directive (2001/20/EC), and International Conference on Harmonisation Harmonized Tripartite Guideline for Good Clinical Practice (CPMP/ICH/135/95). Follow-up concluded on the 18<sup>th</sup> of January 2013. The authors are solely responsible for the design, conduct, and analysis of this study, the drafting and editing of the manuscript, and its final contents.

### *Participants*

Twenty-four people with T2DM provided written informed consent and were enrolled between November 30, 2011 and October 8, 2012 from local primary care clinics and research recruitment registers. Eligible participants were  $\geq 18$  and  $< 55$  years of age, without documented history of CVD, on stable doses of oral antihyperglycaemic agents for at least 3 months, with  $\text{HbA}_{1c} \leq 64$  mmol/mol (8%) and triglycerides  $\leq 2$  mmol/l. To facilitate recruitment, age, medication, and  $\text{HbA}_{1c}$  criteria were amended on September 7, 2012 to:  $\geq 18$  and  $\leq 75$  years of age, on stable therapy with diet or oral antihyperglycaemic agents for at least 3 months, and  $\text{HbA}_{1c} < 86$  mmol/mol (10%). Exclusions were: pregnant or lactating women; peptic ulcer disease or other gastro-intestinal disorder; blood pressure  $> 150/100$  mmHg; bleeding disorder; evidence of severe hepatic disease or  $\text{ALT} > 3$ -times the upper limit of normal; severe renal disease or  $\text{eGFR} < 40 \text{ ml/min/1.73m}^2$ ; currently taking or with a contraindication to treatment with aspirin; taking any dose of aspirin, nonsteroidal anti-inflammatory drugs, antiplatelet agents or antithrombotic drugs within the last 30 days.

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*Study design*

In this 3-way crossover study, participants were randomly allocated in blocks of 6 to the 6 possible treatment sequences (Supplemental Figure 1). In all treatment periods they received two tablets twice-daily containing aspirin (acetylsalicylic acid, Bayer Pharma AG, Germany) or matching placebo, in order to deliver active therapy 100mg once-daily, 200 mg once-daily or 100 mg twice-daily in double-blind fashion. Each 2-week treatment period was followed by a 2-week washout. Aspirin doses were chosen to approximate those used in clinical practice and to facilitate the use of a double-blinded placebo control.

Fasting venous blood and urine samples were taken at baseline and after each treatment period. Adherence was assessed by pill count. Since the platelet inhibitory effect of aspirin is mediated primarily through the irreversible inhibition of the COX-1 enzyme, only platelets circulating during aspirin’s pharmacological half-life are affected. In order to minimize the immediate impact of aspirin administration, circadian effects on platelet function, and approximate a “steady state” for platelet inhibition, all samples were taken between 8am and noon, equating to 12 or 24 hours after the ingestion of the last aspirin dose depending on the allocated treatment regimen. Samples were hand transported to the laboratory in upright tubes at room temperature, with platelet function testing performed within 4 hours, consistent with British Committee for Standards in Haematology guidelines (14).

The primary objective was to compare changes from baseline in platelet reactivity between aspirin regimens, as measured by the VerifyNow™ ASA Point-of Care test (Accumetrics, CA, USA). It was selected as the most convenient method for assessing the impact of aspirin on platelet reactivity in large-scale trials. Test results, based on turbidimetric optical detection of platelet aggregation in whole blood, are expressed in aspirin reactive units (ARUs), with a lower limit of normal (LLN) >550 ARU.



Secondary objectives were to assess changes in platelet function using a variety of COX-1 dependent and COX-1 independent platelet function tests. Light transmission aggregometry (LTA) was measured with a PAP-4 aggregometer with 0.5 mg/ml arachadonic acid (AA) and 10  $\mu$ M adenosine diphosphate (ADP) agonists with unadjusted Platelet Rich Plasma counts in line with recent guidelines (15). Results are reported as maximal platelet aggregation after 10 minutes with LLN  $\geq 20\%$  for AA and  $\geq 70\%$  for ADP.

Whole blood aggregometry (WBA) was performed using a 5-channel Multiplate™ aggregometer (Verum Diagnostica GmbH, Munich, Germany) with 0.5 mM AA and 6.5  $\mu$ M ADP agonists. Results are expressed as area under the curve (AUC) with LLN  $> 706$  for AA and  $> 113$  for ADP. The platelet function analyser (PFA-100 (Siemens Diagnostics, IL, USA) used either collagen and epinephrine (CEPI) or collagen and adenosine diphosphate (CADP) cartridges to measure platelet aggregation. Measures are reported as closure time in seconds with upper limit of the normal range  $> 164$  seconds for CEPI and  $> 112$  seconds for CADP.

Pharmacologic effects of aspirin were assessed by measuring serum thromboxane B2 (TxB2) and urinary 11-dehydro-thromboxane B2 (dTxB2). Serum TxB2 was assayed by ELISA (R&D systems, Abingdon, UK) according to the manufacturer's instructions with LLN 10 ng/mL. Urinary dTxB2 was measured using ELISA (AspirinWorks Test kit, Corgenix, Peterborough, UK) according to the manufacturer's instructions with LLN  $\geq 1500$  pg/mg urinary creatinine. Immature platelet fraction was measured using the Sysmex XE-2100 haematology analyser (Sysmex, Kobe, Japan) with normal range 0.83% to 6.17% (mean = 3.5%).

### *Statistical Analysis*

Sample size estimates, based on previous VerifyNow™ ASA Point-of Care studies, suggested that 17 participants were required to detect a clinically-significant 27% effect size between

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any two aspirin regimens, with 90% power at the 5% significance level. Twenty-four participants were recruited to allow for potential dropouts.

Data were checked for normality and equality of variances. Summary statistics for categorical variables are provided as n (%) and continuous variables as mean and standard deviation or median and interquartile range, as appropriate.

Efficacy analyses were conducted using a modified intent-to-treat principle where data for any specific treatment period could be excluded for pre-specified protocol violations expected to influence platelet function test results, *i.e.* concomitant use of insulin, washout period <10 days, <80% adherence to study medication, study drug not taken in the 24 hours prior to assessment, participant missed 2 consecutive days of study medication in the week prior to assessment, or treatment period <12 days. Generalized linear mixed effect models with random subject effects were used for both primary and secondary objectives to estimate changes in platelet reactivity by aspirin regimen, with adjustment for possible period and sequence interactions.

*Randomization*

Randomisation sequences were generated by an independent statistician and assigned to each participant at recruitment by the trial management system. All participants and study personnel were blinded to treatment allocation. Unblinding codes were released by the independent statistician after the database had been locked.

**Results**

*Participant Characteristics*

Participant baseline characteristics are summarised in Table 1. Nineteen participants were treated with metformin, 6 with sulfonylurea and 2 with thiazolidinediones. None were

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3 treated with other glucose-lowering therapies at any time during the study. Fourteen were  
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5 taking a statin.  
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9 Data from 2 of 72 treatment periods were excluded *per* protocol from primary and secondary  
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11 analyses because study medication was not taken within 24 hours of assessment (1 aspirin  
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13 200mg once-daily period, 1 aspirin 100mg twice-daily period). Urinary dehydro-  
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15 thromboxane B2 analyses were limited to the 67 treatment periods with the requisite data  
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17 available.  
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#### 20 21 *COX-1 dependent tests*

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24 VerifyNow™ ASA platelet reactivity decreased significantly (Figure 1, Table 2) from  
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26 baseline  $650 \pm 19$  ARUs to  $448 \pm 68$  with aspirin 100mg once-daily,  $430 \pm 65$  with 200mg once-  
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28 daily and  $416 \pm 39$  with 100mg twice-daily ( $p$  all  $< 0.0001$ ). Modelled platelet reactivity was  
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30 reduced to a greater extent with aspirin 100mg twice-daily versus 100mg once-daily  
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32 ( $p=0.043$ ), but not versus 200mg once-daily ( $p=0.44$ ). Aspirin 200mg once-daily did not  
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34 differ from 100mg once-daily ( $p=0.20$ ). Results were similar when the model was adjusted  
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36 for treatment period, showing no evidence of a treatment carry-over effect (data not shown).  
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42 Other COX-1 dependent platelet function tests (Table 2) also showed significant decreases  
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44 from baseline with all three aspirin regimens ( $p$  all  $< 0.0001$ ). Compared to aspirin 100mg  
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46 once-daily, aspirin 100mg twice-daily produced statistically significant reductions from  
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48 baseline for all COX-1 dependent platelet function tests except LTA-AA. There was no  
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50 statistical difference for COX-1 dependent tests between aspirin 100mg twice-daily and  
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52 200mg once-daily. Aspirin 200mg once daily more effectively reduced Multiplate™-AA than  
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54 100mg once-daily.  
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*COX-1 independent tests*

Neither the PFA-100 CADP nor the Multiplate™-ADP platelet function tests showed any significant reduction from baseline. Aspirin 100mg twice-daily produced a greater effect on PFA-100 CEPI compared to 100mg once-daily, but no such difference was seen with other COX-1 independent tests. There were no statistical differences for COX-1 independent tests between aspirin 100mg twice-daily and 200mg once-daily.

*Pharmacologic effects*

Adequate pharmacologic suppression of COX-1 activity compared to baseline was demonstrated at all aspirin doses, as measured by decreased serum TxB2 and urinary dTxB2 (Table 2). A greater pharmacologic effect was seen with aspirin 100mg twice-daily compared to 100mg one-daily for urinary dTxB2 ( $p=0.048$ ) with a similar non-significant trend for serum TxB2. All analyses examined the possibility of carryover effects by adjusting for period and period-by-treatment interaction. No significant effects were seen ( $p>0.05$  in all instances).

*Immature Platelets*

There was no evidence of increased platelet turnover as measured by IPF. No change was seen regardless of aspirin dose, and no interactions were identified between aspirin dose response and IPF value for any of the primary or secondary endpoints.

*Adverse events*

Eleven participants experienced a total of 17 adverse events, none of which were serious. Three participants reported heartburn or indigestion, and 3 reported bruising. No major or minor episodes of bleeding were reported. The remaining events were of unlikely

relationship to the study drug: headache in 4 participants (6 events), local skin infection, cough, upper respiratory infection, elbow fracture, pain in great toe.

## Discussion

This study shows, in people with well-controlled short duration T2DM but no history of CVD, that aspirin 100mg twice-daily reduced platelet reactivity more effectively than 100mg once-daily, and more than 200mg once-daily. It was also the most effective regimen based on the pharmacologic effect assessed by serum and urinary thromboxane levels, and for reducing platelet reactivity measured by a variety of platelet function tests (VerifyNow™ ASA, AA induced Multiplate™ aggregation, PFA-100™ CEPI). The lack of evidence for increased platelet turnover suggests that this is not the mechanism by which twice-daily aspirin dosing confers additional efficacy, but this study was probably underpowered to address this issue. Previous studies have demonstrated associations between age, poor glycaemic control, hyperlipidaemia, and smoking with increased platelet turnover, but these findings have been inconsistent (16). Resistance to aspirin in people with T2DM is associated with lipid disorders and history of current smoking. (17) Our study provides no additional mechanistic clues to indicate why twice-daily aspirin dosing reduces platelet reactivity most effectively.

We also demonstrated that aspirin 200mg once-daily was not consistently better than 100mg once-daily for platelet inhibition. These findings are consistent with those of Lemkes et al. who demonstrated that aspirin 300mg once-daily does not further improve platelet suppression as compared to 100mg once-daily (18) and with the finding of the ASPECT secondary prevention trial where aspirin 325mg once-daily was not superior to 162mg once-daily for reductions in platelet reactivity in a subset of people with diabetes (19).

The significant increase in platelet suppression seen here with aspirin 100mg twice-daily extends the observations of Rocca *et al.* (10) who investigated the response to aspirin in 100

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people with T2DM with (46%) or without (64%) CVD. Rocca showed that when compared to 100mg or 200 mg once-daily, only 100 mg twice-daily aspirin dosing completely reversed the abnormally fast recovery of thromboxane B<sub>2</sub>. However, no difference was seen between treatment groups in inhibition of platelet aggregation measured by the VerifyNow™ ASA assay. Our study resembles the cross-over trial by Spectre et al. (12) but with smaller effect sizes. This study compared aspirin 75mg once-daily, 75mg twice-daily and 320mg once-daily in 25 people with T2DM and macrovascular or microvascular complications and demonstrated that aspirin 75mg twice-daily significantly reduced whole blood platelet aggregation as compared with once-daily, whilst aspirin 320mg once-daily did not. Similar to our results, they found no relationship between platelet turnover and benefits of twice-daily aspirin. Capodanno *et al.* also showed a dose-dependent effect of aspirin 81mg twice-daily as assessed by serum TxB2 concentrations and the VerifyNow™ ASA assay in 20 people with diabetes and CVD (11).

The advantage of twice daily dosing could also be related to the timing of aspirin administration. Platelet aggregation and activation surface markers follow a circadian rhythm which peaks between 6AM and noon (20) and has been correlated with the risk and severity of myocardial infarctions, also observed at peak frequency in the morning hours (21). Bonten et al (22) demonstrated in a randomised cross-over trial in 14 healthy participants that bedtime administration of 80mg aspirin was more effective than morning administration in suppressing COX-1 platelet reactivity during the morning hours. Larger clinical trials are necessary to determine whether once-daily bedtime, as opposed to morning, dosing might more effectively reduce subsequent risk of cardiovascular events.

*Clinical implications*

Current guidelines reflect uncertainty about using aspirin for primary cardiovascular

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3 prevention in people with T2DM (23). The 2013 American Diabetes Association guidelines  
4 recommend to “consider aspirin therapy (75–162 mg/day) as a primary prevention strategy in  
5 those with type 1 or type 2 diabetes at increased cardiovascular risk (10-year risk >10%)”  
6 (24). In contrast, guidelines from the European Society of Cardiology recommend against  
7 using aspirin for primary prevention for people with diabetes, citing little evidence for major  
8 cardiovascular risk reduction and inconsistent data relating to harm (25).  
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18 Bleeding is the major risk associated with the administration of aspirin. In a meta-analysis of  
19 randomized controlled trials of aspirin use (doses ranging from 325mg every other day to  
20 500mg daily) in primary cardiovascular prevention, the Antithrombotic Trialists’  
21 collaboration reported an absolute increase of 0.03% per year in major gastrointestinal and  
22 extracranial bleeds (1). Risks for bleeding, *e.g.* age, sex, diabetes, were the same as those for  
23 cardiovascular events, although diabetes itself did not appear to confer a disproportionate risk  
24 increase (26). When compared to a 12% risk reduction in serious vascular events, which  
25 includes MI, stroke, or vascular death (absolute risk reduction of 0.07% per year), the benefit  
26 of aspirin use does not sufficiently outweigh the risk. Twice-daily aspirin regimens have not  
27 been examined for bleeding risk or long-term cardiovascular outcomes.  
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43 In trials using platelet aggregation measurements as an outcome parameter, higher doses of  
44 aspirin administered once daily have not been superior to lower doses of 75-100 mg. Our data  
45 in people with T2DM but without known CVD show that twice-daily aspirin is more  
46 effective in reducing platelet reactivity than once-daily, with no episodes of minor or major  
47 bleeding events in this small study. Ongoing cardiovascular outcome trials are examining the  
48 use of aspirin in primary prevention but none are evaluating a twice-daily regimen. The  
49 Aspirin and Simvastatin Combination for Cardiovascular Events Prevention Trial in Diabetes  
50 (ACCEPT-D, ISRCTN48110081) is assessing the impact of aspirin 100mg once-daily on the  
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primary composite endpoint of cardiovascular death, non-fatal myocardial infarction, nonfatal stroke, and cardiovascular hospitalisations in 5170 Italian participants with diabetes. Results are expected in 2015 (27). A Study of Cardiovascular Events in Diabetes (ASCEND, NCT00135226) has recruited 15,480 participants with diabetes and randomised in a 2x2 factorial design to aspirin 100mg once-daily *versus* placebo and to 1 gram of omega-3 fatty acids *versus* placebo. The study’s primary outcome is a composite of non-fatal myocardial infarction, non-fatal stroke or vascular death assessed over median 7.5 years follow up. Results are expected in 2016. Although both trials will investigate the role of aspirin in primary cardiovascular prevention in diabetes, neither addresses whether an alternative dosing regimen might have a better safety or efficacy profile. Our data suggest that an aspirin 100mg twice-daily regimen may be more efficacious in a well-controlled T2DM population. Future large scale clinical outcome trials are required to confirm whether the risk of primary cardiovascular events can be reduced in more diverse T2DM populations, whether twice-daily dosing is a barrier to adherence, and to assess the bleeding risk.

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support from Merck and received compensation for advisory boards from NovoNordisk and Boehringer Ingelheim. H.S. receives research funding from Boehringer Ingelheim and Merck. P.H. is a consultant for Sysmex UK and received research funding from Siemens Diagnostics & Lilly.

For Peer Review

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**Table 1:** Participant characteristics at Baseline. Results for continuous variables are mean±1SD or median (IQR).

	n=24
Age (years)	51±7
Male (n)	12 (50%)
Body mass index (kg/m <sup>2</sup> )	31.4±7.2
Ethnicity (n)	
White	22 (92%)
Black or Black British	1 (4%)
Asian or Asian British	1 (4%)
Current smoker	3 (13%)
Waist circumference (cm)	104±14
Duration of diabetes (years)	2 (1, 3)
Systolic blood pressure (mmHg)	127±13
Diastolic blood pressure (mmHg)	78±10
Haemoglobin A <sub>1c</sub> (mmol/mol, [%])	47±7 [6.4±0.6 ]
Total cholesterol (mmol/l)	4.0±0.9
Low-density lipoprotein cholesterol (mmol/l)	2.3±0.9
High-density lipoprotein cholesterol (mmol/l)	1.14±0.27
Triglyceride (mmol/l)	1.05 (0.72, 1.49)
Platelet count (x10 <sup>9</sup> /l)	246±58

**Table 2:** Platelet function tests at baseline and following each aspirin regimen. Data are mean±1SD or median (IQR). P-values in columns A, B & C reflect change from baseline. Abbreviations: ARU aspirin reactive units; AUC area under the curve.

		(A)	(B)	(C)	p value		
	Baseline	Aspirin	Aspirin	Aspirin	A vs B	A vs C	B vs C
		100mg	200mg	100mg			
		once-daily	once-daily	twice-daily			
<i>COX-1 dependent tests</i>							
VerifyNowASA (ARU)	650±19	448±68	430±65	416±39	0.20	0.043	0.44
		p<0.0001	p<0.0001	p<0.0001			
Multiplate™ (AUC)	63±17	28±14	20±13	16±9	0.0043	<0.0001	0.19
with arachidonic acid		p<0.0001	p<0.0001	p<0.0001			

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Light transmission aggregometry (%)	76±8	10±14	10±15	8±3	0.17	0.16	0.98
with arachidonic acid		p<0.0001	p<0.0001	p<0.0001			
<i>COX-1 independent tests</i>							
Multiplate™ (AUC)	50±15	52±20	52±20	52±16	0.61	0.82	0.78
with adenosine diphosphate		p=0.41	p=0.79	p=0.56			
Light transmission aggregometry (%)	70±9	47±13	43±16	43±16	0.99	0.49	0.50
with adenosine diphosphate		p<0.0001	p<0.0001	p<0.0001			
Platelet function analyser 100 (seconds)	133±42	231±74	250±73	264±60	0.071	0.031	0.71
with collagen and epinephrine		p<0.0001	p<0.0001	p<0.0001			
Platelet function analyser 100 (seconds)	104±21	117±59	109±45	118±61	0.60	0.86	0.73
with collagen and adenosine diphosphate		p=0.08	p=0.40	p=0.14			

*Pharmacologic effects*

Urinary dehydro-thromboxane B2 (pg/mg urinary creatinine)	4463±2411	1076±601 p<0.0001	1037±641 p<0.0001	906±664 p<0.0001	0.47	0.048	0.21
Serum thromboxane B2 (ng/ml)	130.5±76.5	7.2±11.9 p<0.0001	5.3±7.9 p<0.0001	2.3±1.3 p<0.0001	0.39	0.055	0.28

*Immature Platelets*

Immature platelet fraction (%)	2.8 (1.7, 3.6)	2.8 (2.0, 3.2) p=0.51	2.8 (2.2, 3.3) p=0.87	2.7 (2.0, 3.2) p=0.61	0.69	0.93	0.76
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**Figure 1 Legend**

Platelet reactivity measured by the VerifyNow™ ASA Point-of Care test, at baseline and following each of three aspirin regimens. P values for possible differences between regimens are taken from generalized linear mixed effect models with random subject effects, with adjustment for possible period and sequence interactions.

**Supplemental Figure 1 Legend**

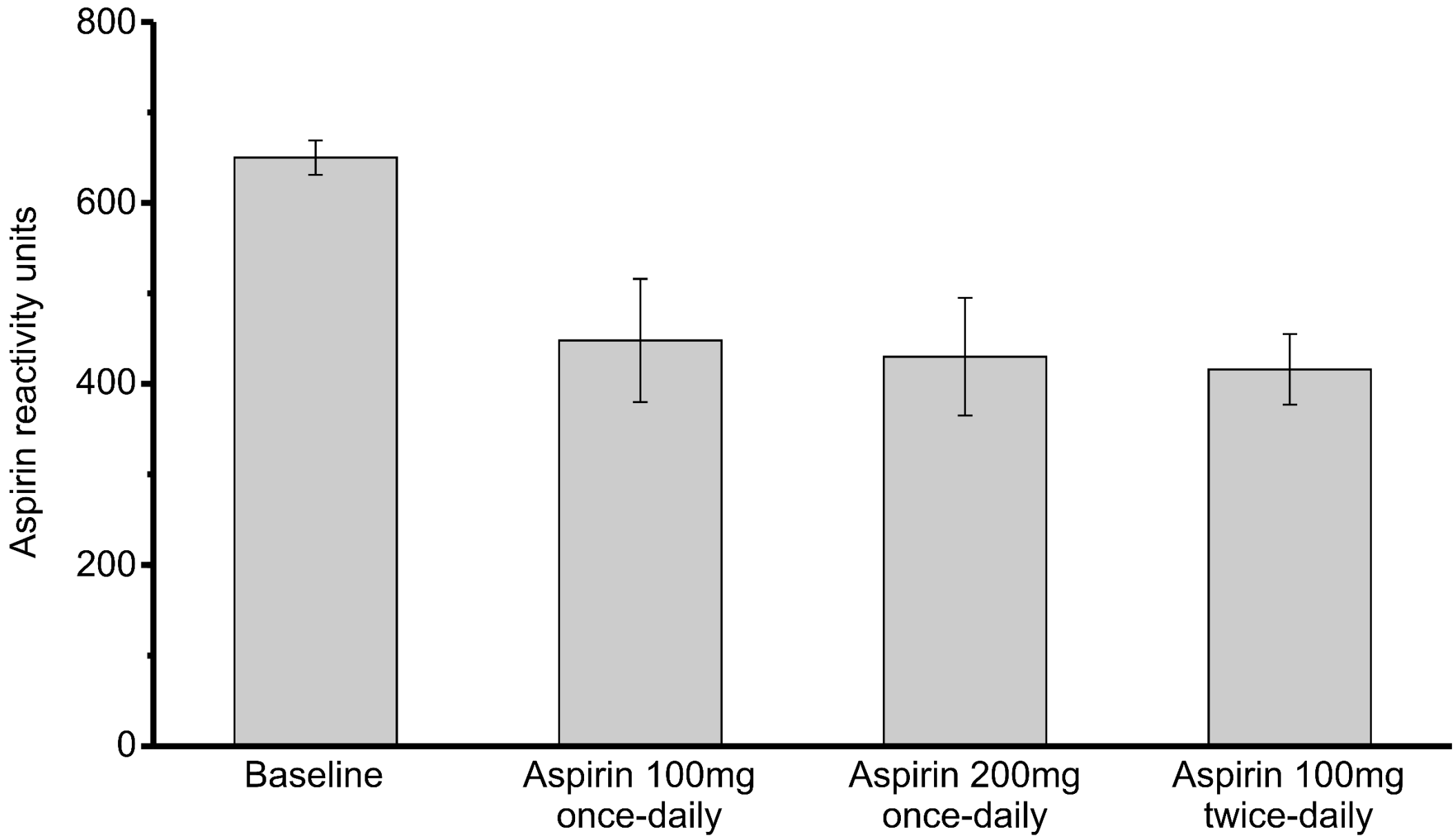
Study design. Participants were randomized (R) within one day of screening to one of six possible treatment sequences during which they were prescribed two tablets twice daily in order to deliver active drug doses of 100mg once-daily, 200 mg once-daily or 100 mg twice-daily in double-blind fashion. Platelet function tests were done at baseline and following each treatment period. Adverse events (AEs) were collected at all face to face visits and phone contacts. Visit types are denoted either by the picture of a human figure (face to face visits) or telephone (phone visit). A: active drug; P: placebo.

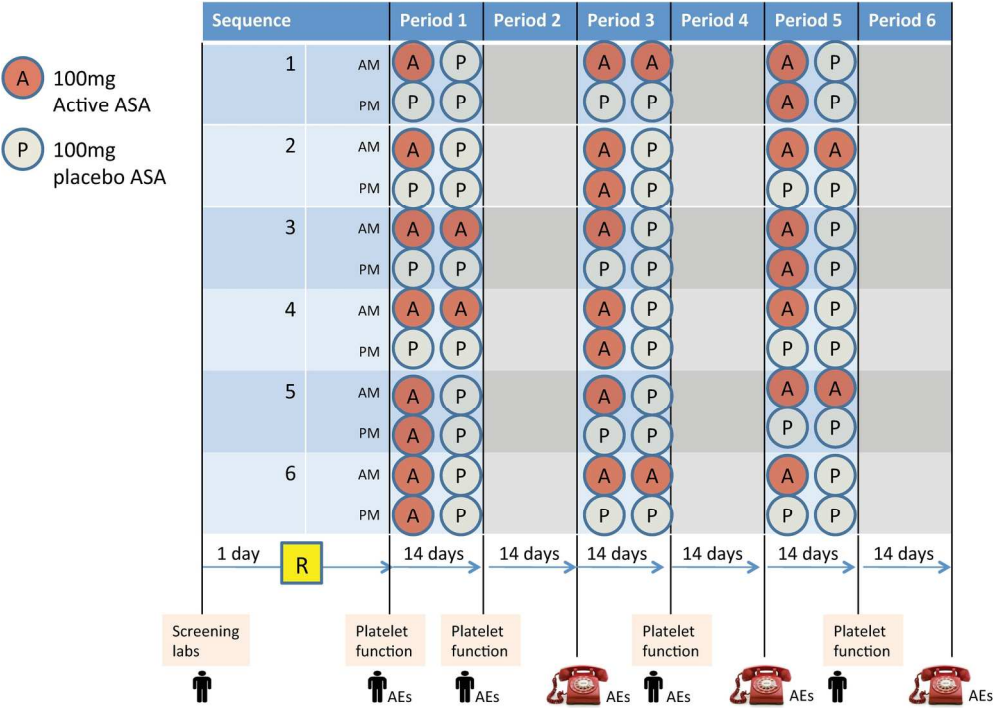


Section/Topic	Item No	Checklist item	Reported on page No
<b>Title and abstract</b>			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts <sup>21,31</sup> )	3
<b>Introduction</b>			
Background and objectives	2a	Scientific background and explanation of rationale	4
	2b	Specific objectives or hypotheses	7
<b>Methods</b>			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	6
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	6
Participants	4a	Eligibility criteria for participants	5-6
	4b	Settings and locations where the data were collected	5
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	6
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	6-8
	6b	Any changes to trial outcomes after the trial commenced, with reasons	NA
Sample size	7a	How sample size was determined	8
	7b	When applicable, explanation of any interim analyses and stopping guidelines	NA
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	9
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	9
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	9
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	9

Section/Topic	Item No	Checklist item	Reported on page No
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	6
	11b	If relevant, description of the similarity of interventions	NA
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	8-9
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	8-9
<b>Results</b>			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	9
	13b	For each group, losses and exclusions after randomisation, together with reasons	9
Recruitment	14a	Dates defining the periods of recruitment and follow-up	5
	14b	Why the trial ended or was stopped	NA
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	19
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	19
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	20-22
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	NA
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	20-22
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms <sup>28</sup> )	11
<b>Discussion</b>			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	11-12
Generalisability	21	Generalisability (external validity, applicability)	15

Section/Topic	Item No	Checklist item	Reported on page No
		of the trial findings	
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	11-15
<b>Other information</b>			
Registration	23	Registration number and name of trial registry	3
Protocol	24	Where the full trial protocol can be accessed, if available	NA
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	15





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