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Chapter 4

Effect of aspirin intake on
awakening or at bedtime on
circadian rhythm of platelet
reactivity in healthy subjects:
a randomized cross-over trial

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ABSTRACT

Background

The risk of acute cardiovascular events is highest during morning hours, and platelet activity peaks during morning hours. The effect of the timing of aspirin intake on circadian rhythm and morning peak of platelet reactivity is not known.

Methods and Results

A randomized open-label cross-over trial in healthy subjects (n=14) was conducted. Participants used acetylsalicylic acid (80mg) for two periods of two weeks on awakening and at bedtime, or the opposite order. At the end of both periods, participants were admitted and blood was drawn every 3 hours to measure cyclo-oxygenase-1 (COX-1)-dependent (VerifyNow-Aspirin; Serum Thromboxane B₂ [STxB₂]) and COX-1-independent (flow cytometry surface CD62p expression; microaggregation) platelet activity.

Mean VerifyNow platelet reactivity over the whole day was similar with intake on awakening and at bedtime (mean difference: -9 [95% confidence interval (CI) -21 to 4]). However, the morning increase in COX-1-dependent platelet activity was more efficiently reduced by intake of aspirin at bedtime compared with on awakening (mean difference VerifyNow: -23 Aspirin Reaction Units [CI -50 to 4]; STxB₂: -1.7 ng/ml [CI -2.7 to -0.8]). COX-1-independent assays were not affected by aspirin intake or its timing.

Conclusions

Low-dose aspirin taken at bedtime compared with intake on awakening reduces COX-1-dependent platelet reactivity during morning hours in healthy subjects. Future clinical trials in larger patient groups are required to investigate whether simply switching to aspirin intake at bedtime reduces the risk of cardiovascular events during the high risk morning hours and offers an overall benefit.

INTRODUCTION

Low-dose aspirin is one of the most used drugs worldwide, and a cornerstone in the prevention of cardiovascular disease (CVD).¹ It reduces the risk of recurrent CVD with about 25%.² Although not supported by evidence, aspirin is usually taken on awakening. However, it may be more beneficial to take aspirin at bedtime. Aspirin's preventive action is based on the inhibition of platelet aggregation and the formation of arterial thrombi, which plays an important role in the pathogenesis of acute CVD.^{3, 4} Platelet aggregation and activation surface markers follow a circadian rhythm, with a peak between 6 AM to noon.^{5, 6} This might play a role in the observed peak of acute CVD during morning hours, which is present in patients with- and without previous CVD.⁷⁻¹⁰ Moreover, patients with a myocardial infarction during morning hours have larger infarct size than those with events during the rest of the day, which has a worse prognosis.^{11, 12} Therefore, it could be useful to develop interventions specifically aimed at reducing the morning peak of CVD.

Due to its short half-life, aspirin only inhibits platelets which are present at the time of intake, while new platelets are released at a rate of 10%/day in healthy subjects.^{13, 14} This turnover rate is even higher in patients with atherosclerotic disease.^{15, 16} Newly released platelets are more reactive and are uninhibited just before the next aspirin intake.¹⁷ This is supported by a recent study, which showed that platelet aggregation was insufficiently inhibited 24 hours after morning aspirin intake in 25% of the patients with established CVD.¹⁸ Because it is desirable to achieve optimal inhibition of platelet aggregation during the high risk morning hours, it might be beneficial to take aspirin at bedtime.^{19, 20} However, the effect of the timing of aspirin intake on the morning peak and circadian rhythm of platelet reactivity during 24 hours has not been investigated. Therefore, the aim of this study was to assess whether aspirin intake at bedtime compared with intake on awakening alters the circadian rhythm and reduces the morning peak of platelet reactivity in healthy subjects.

METHODS

Subjects

Eligible participants were all healthy adults aged 18 or older, who had the capacity to give informed consent (IC). Exclusion criteria were: active chronic disease, use of any other medication, allergy to salicylates, platelet count $< 150 \times 10^9/L$, Verifynow Aspirin Reaction Units (ARU) < 550 , pregnancy, current smoking shift work < 2 months, history of major bleeding events, known bleeding diathesis, cardiovas-

cular disease, malignancy and extreme awakening- or bedtime hours, defined as regular (>2 days a week) bedtime <10 PM or >midnight and/or awakening <6 AM or >9 AM. Participants were recruited through local advertisements in the Leiden University Medical Center (LUMC, Leiden, the Netherlands).

Design

This study was designed as a prospective randomized open-label blinded-end-point (PROBE) two-period crossover trial.²¹ A computer-generated randomization code was prepared by an independent person at the department of Clinical Epidemiology of the LUMC, which was inaccessible to the investigators to guarantee treatment allocation concealment. The study was designed and reported in accordance with the CONSORT guidelines for randomized, controlled trials and registered at www.clinicaltrials.gov/ct2/show/NCT01900639. The study was approved by the LUMC Ethics Committee and all participants gave written informed consent.

After a screening visit, participants were randomized into two groups. One group took aspirin on awakening during two weeks and subsequently at bedtime during two weeks, whereas the other group received the same interventions in opposite order. The intervention periods were separated by a washout period of 4 weeks. Subjects were instructed to take 80 mg of effervescent acetylsalicylic acid (TEVA Pharmaceuticals, Amsterdam, the Netherlands) in the morning between 7 and 10 AM or in the evening between 10 PM and midnight, respectively. Participants visited the research site at the beginning of each 2-week intervention period for instructions and new study medication for the subsequent period. At the end of both intervention periods, subjects were admitted for 24 hours to the research department after an overnight fast (Table 1). Thus, each subject was admitted twice for 24 hours, both after intervention with aspirin on awakening and after intervention with aspirin at bedtime.

All study medication was prepared by the pharmacy of the LUMC. Medication compliance was registered and optimized with electronic pill boxes (Evalan, Amsterdam, the Netherlands), which sends a text message by phone when subjects do not open the box within the pre-specified time window. Additionally, a pill count was performed after both intervention periods. Noncompliance was defined as an adherence of <90% as registered by the electronic pill box, as a remaining pill count of ≥ 3 , or the subject's acknowledgement of noncompliance. At each study visit, we registered possible adverse events and side effects of aspirin use by structured questionnaires.

Table 1. Day scheme of admission at visit 2 and 4

Time	Action*
Day 1	
8:30	Insertion intravenous catheter
9:00	Blood sampling
9:05	Intake of aspirin when on awakening
9:30	Breakfast
12:00	Blood sampling
12:05	Lunch
15:00	Blood sampling
18:00	Blood sampling
18:05	Diner
21:00	Blood sampling
23:00	Blood sampling (+STxB ₂)
23:05	Intake of aspirin when at bedtime
Day 2	
00:00	Blood sampling – Lights off
03:00	Blood sampling
6:00	Blood sampling (+STxB ₂ + Flow cytometry)
8:00	Lights on - awakening
8:15	15 minutes ambulating
9:00	Blood sampling (+STxB ₂ + Flow cytometry)
9:05	Intake of aspirin when taking it at awakening
9:30	Breakfast
12:00	Blood sampling (+STxB ₂ + Flow cytometry)

* At every blood sampling moment a VerifyNow tube was drawn.

STxB₂: serum thromboxane B₂. The grey area represents the morning peak of platelet reactivity at day 2.

Twenty-four-hour Admission Periods

After each intervention period, subjects were admitted from 8 AM to noon the next day. An overview of the schedule during admission is given in table 1. Because the absorption of aspirin after intake could be affected by food intake, the timing of breakfast, lunch, and dinner, these variables were fixed for all subjects.²² Subjects were allowed to sleep from midnight to 8 AM, during which the lights were turned off. After awakening, subjects were asked to ambulate for 15 minutes to resemble normal morning activity. During the rest of the day, subjects behaved according to their personal preference, but were not allowed to perform physical exercise.

Using an 18 gauge antecubital venous catheter (VC) (Becton Dickinson Inc., Franklin Lakes, NJ, USA), blood was sampled every 3 hours. This sampling rate is analogous to a previous study on the circadian rhythm of platelet aggregation.²³ Additional samples were drawn at 9 AM and 11 PM, just before each supervised

aspirin intake. Each first 5 mL was discarded, after which blood for platelet function testing was drawn, followed by infusion of saline to keep the VC patent. Additional tubes for determination of serum thromboxane B₂ (STxB₂) were drawn at 11 PM on day 1, and 6 AM, 9 AM and 12 AM on day 2.

Laboratory measurements

At baseline and at the beginning of each 24-hour admission period, hematologic variables were determined according to standard procedures. Platelet activity was evaluated with COX-1-dependent and COX-1-independent assays.

COX-1-dependent assays

Platelet reactivity was measured with the VerifyNow Aspirin Assay (Accumetrics, San Diego, CA, USA) as described previously.²⁴ The VerifyNow Aspirin System converts measured platelet aggregation into Aspirin Reaction Units (ARU). Higher ARU values correspond with higher platelet reactivity. Although manufacturer's instructions allow measurement of platelet reactivity between 30 min – 4 hours after blood draw, we measured all samples 3 hours after each blood draw, to prevent time-dependent changes of platelet reactivity within the determination window.²⁵ STxB₂ is a stable metabolite of thromboxane A₂ (TxA₂), which directly reflects platelet COX-1 enzyme capacity, and is pharmacologically the most specific assay to evaluate the effect of aspirin on platelets.^{14, 26} Whole blood was allowed to clot at 37°C in non-anticoagulated tubes for 1 hour, after which serum was separated by centrifugation and stored at -80°C until analysis. STxB₂ was measured with an enzyme immuno-assay according to manufacturers' instructions (Thromboxane B₂ Express, Cayman Chemicals, Ann Arbor, MI, USA).

COX-1-independent assays

For COX-1-independent assays, blood was drawn at 6 AM, 9 AM and 12 AM of day 2. Four weeks after the last aspirin intake blood was drawn for controls. Platelet reactivity was determined by agonist induced platelet surface CD62P (P-selectin) expression and platelet microaggregation. As agonists, thrombin receptor-activating peptide (TRAP; activates the thrombin receptor proteinase-activated receptors-1)²⁷, adenosine diphosphate (ADP; activates P2Y₁, P2Y₁₂, and P2X₁ receptors)²⁸, phorbol 12-myristate 13-acetate (PMA; activates protein kinase C which in turn indirectly activates αIIbβ₃ integrin)²⁹ and ristocetin (binds von Willebrand factor, changes its conformation and subsequently activates platelets through glycoprotein Ib binding) were used.³⁰ Induced platelet surface CD62p expression was determined as described previously.³¹ Concentration series ranged from 625 μmol/L – 38 nmol/L (TRAP; H-2936, Bachem, Weil am Rhein, Germany), 125 μmol/L – 8

nmol/L (ADP; 01897, Sigma-Aldrich, Zwijndrecht, the Netherlands) and 8 μ mol/L – 0.5 nmol/L (PMA; P8139, Sigma-Aldrich), Platelet microaggregation was performed as described previously.³⁰ In short, citrated blood was labelled with either FITC Mouse Anti-Human CD31 (1:100; 555445; BD Pharmingen™, San Diego, CA, USA) or Alexa Fluor® 647 Mouse anti-Human CD31 (1:100; 558094, BD Pharmingen™). The differently labeled platelets were mixed and subsequently activated with TRAP (250 μ mol/L), ADP (50 μ mol/L), PMA (160 nmol/L) or ristocetin (700 μ mol/L; R7752, Sigma-Aldrich). Samples were taken at 0s, 30s, 60s, 120s, 180s, 300s and 480s, measured by flow cytometry (Coulter FC-500-MPL, Beckman Coulter, Fullerton, CA, USA) and analyzed with Kaluza Analysis Software (Beckman Coulter). CD62P expression was determined in the CD61 positive population. The CD62P-positive gate was established using unlabelled cells and isotype controls. The mean percentage of CD62P-positive in the eight concentrations was calculated. Microaggregation was quantified using a quadrant in the dot plot of non-stimulated samples. The percentage double positive samples were calculated relative to the total amount of positive events. Platelet microaggregation increased over time and after 30 s for TRAP, ADP and ristocetin and after 180 s for PMA. The largest discrimination was observed and these values are reported in this manuscript.

Statistical Analysis

To achieve a power of 90% with a 95% confidence level to find a reduction of 65 ARU at the morning peak with aspirin intake at bedtime, 12 subjects were needed in this cross-over study³². For this calculation, we used an intra-individual standard deviation of 46.85 ARU, as extracted from a previous study³³. 65 ARU corresponds with 1.5 times the intra-individual standard deviation, which we regarded as a possibly clinically relevant difference. Anticipating drop-out of approximately 10%, 14 subjects were randomized. The primary analysis was guided by the intention to treat principle. Second, a per-protocol analysis was performed in which participants were excluded who started smoking, started using other medication, or who did not take aspirin in the pre-specified time windows during the study. Third, a sensitivity analysis was performed, in which only measurements without the use of a tourniquet were analysed. Tourniquet use during blood draws results in higher intravascular pressure and can induce a turbulent blood flow which subsequently activates platelets and thereby influence the results.³⁴ Linear mixed models were used to compare platelet reactivity over the whole 24 hour (VerifyNow) and during morning hours on day 2 (6 AM – noon; all platelet activity measurements). Additionally, the area under the curve (AUC) during morning hours on day 2 was calculated for each participant using the linear trapezoidal method, which uses the following equation: $\sum (x_n - x_{n-1})(y_n + y_{n-1})/2$. Paired t-tests were used to test differences in AUCs.

SPSS statistics 20 (IBM Corp., USA) and GraphPad Prism 6 (GraphPad Software Inc., USA) were used for statistical calculations and graphical presentation of results.

RESULTS

Recruitment and study population

From June 2013 to October 2013, a total of 44 subjects were screened for eligibility, of whom 14 subjects refused to participate and 8 subjects did not meet the inclusion criteria. A total of 3 subjects were excluded because of chronic medication use

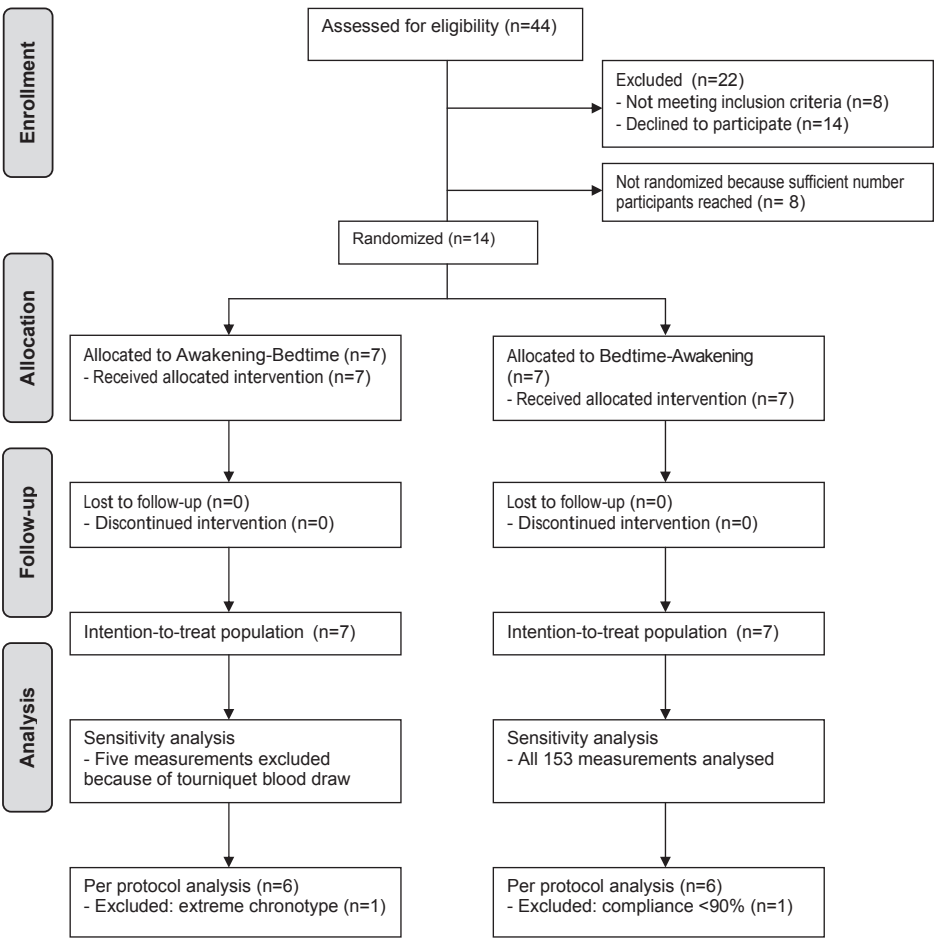


Figure 1 – Flowchart of study patients.

and 2 because of a known bleeding diathesis. Another 3 subjects were excluded because of extreme awakening- or bedtime hours, shift work in the preceding two months or current smoking. In addition, 8 subjects were meeting the inclusion criteria but were not randomized because sufficient number of participants had already been included. Finally, 14 subjects were randomized (Figure 1). Baseline characteristics of the randomized subjects are listed in table 2. Compliance, validated by electronic pill boxes, was high during the whole study, and similar with intake on awakening and at bedtime (96.5% and 97.2%, respectively).

Table 2. Baseline characteristics of 14 randomized subjects

Characteristic	Healthy Volunteers (n=14)
Age (years)	22.2 ± 1.8
Sex (female)	9 (64.3)
Body Mass Index (kg/m ²)	22.8 ± 2.7
Hemoglobin (mmol/L) males	9.3 ± 0.7
females	8.2 ± 0.7
Platelet count (x10 ⁹ /L)	236 ± 33
Mean platelet volume (fl)	10.8 ± 1.1
Platelet distribution width (µm)	13.2 ± 2.4
VerifyNow (Aspirin Reaction Units [ARU])	642 ± 20

Data are number (%) or mean ± SD

COX-1-dependent platelet reactivity

The effect of intake of low-dose aspirin on awakening or at bedtime on the circadian rhythm and morning peak of COX-1-dependent platelet assays are shown in figure 2. VerifyNow platelet reactivity reached its maximum at 6 AM with aspirin intake on awakening and shifted to noon with intake at bedtime. Mean VerifyNow platelet reactivity over the whole day was 438 (SD 54) ARU with intake on awakening and 430 (SD 59) ARU with intake at bedtime (mean difference: -9 [95% CI -21 to 4]; $p=0.190$). Mean VerifyNow platelet reactivity during the morning peak was 445 (SD 63) with intake on awakening, whereas this was 421 (SD 61) with aspirin intake at bedtime (mean difference -23 ARU [95% CI -50 to 4; $p=0.09$]). The mean VerifyNow AUC was higher during the morning peak with intake of aspirin on awakening ($882 \pm \text{SD } 107$) than with intake at bedtime ($851 \pm \text{SD } 105$), although this was not statistically significant (mean difference -31 [95% CI: -88 to 26] $P=0.256$). Aspirin intake at bedtime reduced STxB₂ mean and AUC during morning hours. Mean levels of STxB₂ during the morning peak with aspirin intake on awakening was 4.3 (SD 2.8) ng/ml with intake on awakening, whereas this was 2.6 (SD 1.3) ng/ml with intake at bedtime (mean difference -1.7 [95% CI -2.7 to -0.8]; $p=0.001$).

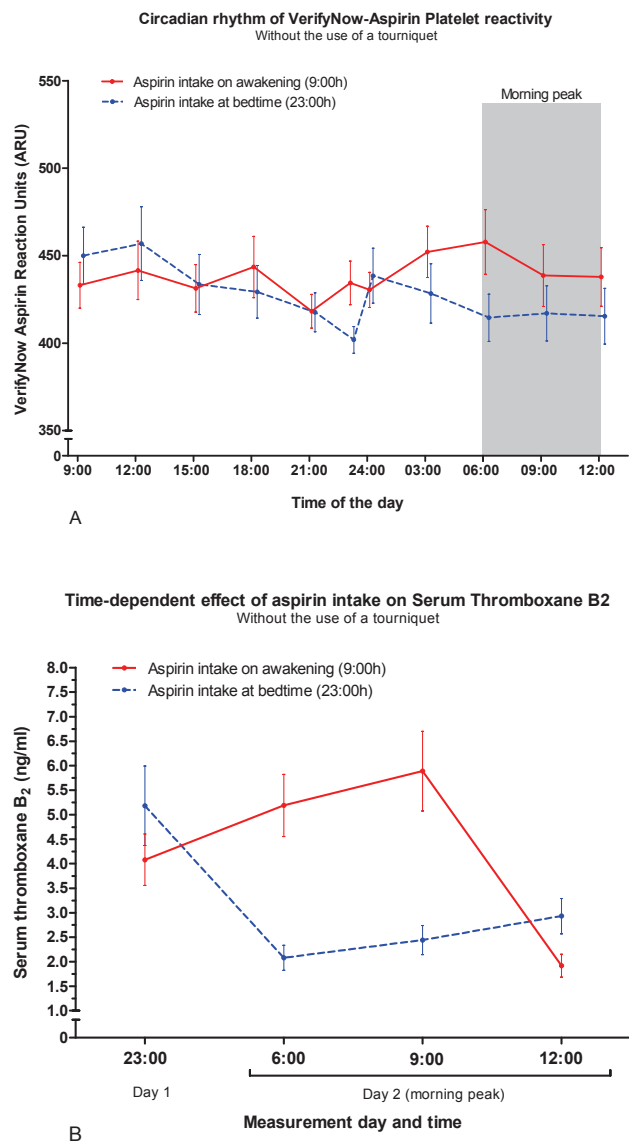


Figure 2 – Effect of aspirin intake on awakening or at bedtime on circadian rhythm and morning hour COX-1-dependent platelet activity. Panel **A**) VerifyNow-Aspirin platelet reactivity. Grey shaded area depicts the morning peak of platelet reactivity and cardiovascular events. Panel **B**) Serum Thromboxane B₂. Figures are the results of the sensitivity analysis, in which measurements after tourniquet blood draw (n=5 (1.6%)) were excluded. Values are depicted as means ± standard error.

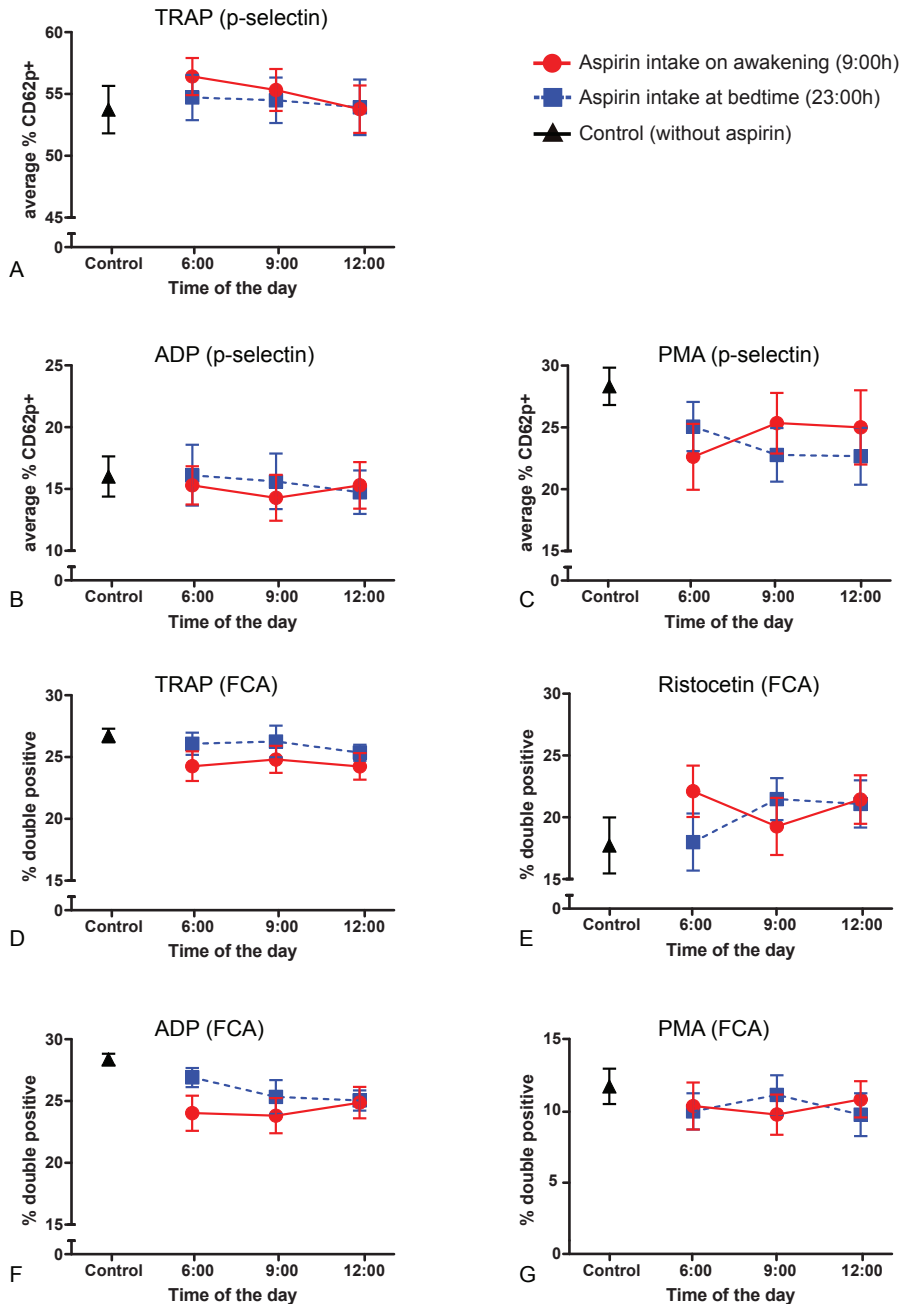


Figure 3 – Effect of aspirin intake on awakening or at bedtime on COX-1-independent platelet assays during morning hours. Panel A to C Flow cytometry CD62 (p-selectin) surface expression after activation with respectively Thrombin receptor agonist peptide (TRAP), adenosine diphosphate (ADP) and phorbol 12-myristate 13-acetate (PMA). Panel D to F Flow cytometry based aggregation (FCA) after activation with respectively TRAP, Ristocetin, ADP and PMA.

The mean AUC of STxB₂ levels during the morning peak was also lower with aspirin intake at bedtime (mean difference -4.7 [95% CI -6.9 to -2.5]).

Two subjects were excluded in the per-protocol analysis, because of compliance <90% and change of chronotype during the study. The results of the per protocol analysis for VerifyNow platelet reactivity and STxB₂ were similar to the intention to treat analysis (Appendix table). In our sensitivity analysis, which excluded tourniquet blood draws (5/304; 1.6%), aspirin intake at bedtime reduced morning VerifyNow platelet reactivity with 30 ARU (95% CI -56 to -3; p=0.03) compared with on awakening. Additionally, the mean difference in AUC during the morning peak was larger (-52 [95% CI: -105 to 2]; p=0.057) in this sensitivity analysis.

COX-1-independent platelet reactivity

The mean percentage platelet surface p-selectin expression during morning hours after activation with respectively TRAP, ADP and PMA was 55.5 (SD 6.0), 15.2 (SD 6.3) and 22.5 (SD 4.9) after aspirin intake on awakening and 56.0 (SD 6.6), 16.0 (SD 8.1) and 22.1 (SD 7.7) after aspirin intake at bedtime (Figure 3; Appendix table). In the per-protocol- and sensitivity analysis results were similar as the intention-to-treat analysis (Appendix table). So, the mean CD62p expression after stimulation with TRAP, ADP and PMA was not affected by the time of aspirin intake. Spontaneous and maximum platelet microaggregation did not differ between aspirin intake upon awakening or at bedtime (*data not shown*). The mean percentage of double positive events during morning hours after stimulation with TRAP, ADP, PMA and ristocetin was 25.1 (SD 3.6), 23.5 (SD 4.7), 9.6 (SD 5.0) and 22.5 (SD 4.9) after aspirin intake on awakening and 26.6 (SD 3.9), 25.4 (SD 3.8), 10.4 (SD 4.7) and 22.7 (SD 4.1) after aspirin intake at bedtime, respectively. Similar results were obtained in the per protocol and sensitivity analysis (Appendix table). Notably, both the platelet responsiveness- and microaggregation assay were not affected by aspirin intake at all, irrespective of time of intake (Figure 3).

DISCUSSION

The main finding of this study is that aspirin intake at bedtime compared with intake on awakening reduces COX-1-dependent platelet reactivity during the morning hours. Furthermore, aspirin intake at bedtime appeared to shift the peak of platelet reactivity away from the morning hours.

Comparison with previous studies

Previous authors suggested that the morning peak of platelet reactivity could be reduced by taking aspirin at bedtime instead of on awakening.^{19, 20, 35} However, to our knowledge, this has never been evaluated in a clinical trial. The Physicians Health Study showed that the protective effect of aspirin was most pronounced for morning myocardial infarctions, although the time of aspirin intake was not reported.³⁶ In the only study which specifically assessed the effect of aspirin intake on morning platelet reactivity, aspirin was taken at 9 PM.³⁷ In this study, aspirin intake at 9 PM compared with placebo abolished the morning peak of platelet aggregation, which is in line with our study. However, only one dose of 325 mg enteric coated aspirin was administered and no direct comparison with morning intake was made in that study. In our study, 80 mg of plain effervescent aspirin was taken for 14 consecutive days and the effect of intake on awakening and bedtime was directly compared. It is known that stable platelet inhibition by low-dose aspirin (80-100 mg) can take several days.¹⁴ Furthermore, recent clinical guidelines recommend a daily dose of 50 to 160 mg for CVD prevention.³⁸ Thus, our study most closely represents current clinical practice with respect to chronic daily use of low-dose aspirin for CVD prevention.

We found time-dependent differences with COX-1-dependent-, but not with COX-1-independent platelet reactivity measurements. This can be explained by aspirin not affecting these COX-1-independent pathways, as indicated by our results in which COX-1-independent platelet activity was not affected by aspirin in general.

Strengths and limitations

The major strength of this study is that we used a cross-over design, which eliminated between-person variability and determinants of platelet reactivity.³⁹ We selected individuals with a pre-specified non-extreme chronotype, which optimized homogeneity of circadian biological rhythms between study participants. Furthermore, compliance during the intervention periods was optimally controlled with the combined use of electronic pill boxes and pill counts. Only 2 subjects were less than 90% compliant or changed their chronotype during the study, which did not materially affect our results as confirmed by the results of a per protocol analysis.

Platelet reactivity during morning hours is affected by physical activity.⁴⁰ Therefore, a limitation of our study is that participants were not admitted on the day before the start of our measurements to standardize behaviour and physical activity in the first measurement hours. This limitation is reflected by the difference in VerifyNow platelet reactivity during the morning hours on measurement day 1 and 2 (Figure 2). This could be explained by differences in physical activity necessary to

reach the study site that might have activated platelets. Behaviour and physical activity during admission were standardized, and therefore more reliable to assess treatment effects during the morning peak on day 2. Another limitation is that we only used the VerifyNow-Aspirin device to measure aspirin-related platelet reactivity, while several other devices are available. Although light transmission aggregometry (LTA) is the historical golden standard, LTA is more time consuming than the point-of-care VerifyNow, requires specialized laboratory technicians and additional blood manipulation. Of all alternatives, VerifyNow shows the strongest correlation with LTA.⁴¹ In addition, we measured serum levels of TxB_2 , which is pharmacologically the most specific test for aspirins' effect on platelets.²⁶

Finally, our study was conducted in healthy individuals and not in CVD patients. Yet, patients with CVD have higher platelet turnover, which would result in a higher proportion of uninhibited platelets 24 hours after aspirin intake. Therefore, we expect that the observed effects will be even larger in CVD patients.¹⁵ This is supported by a recent study, in which higher platelet turnover was associated with insufficient platelet inhibition 24 hours after morning aspirin intake in 25% of CVD patients.¹⁸

Clinical implications

The morning peak of platelet reactivity is likely to contribute to the morning peak of acute cardiovascular events.⁵ However, despite antiplatelet therapy, a morning peak is still present in patients with recurrent events.⁷ Aspirin intake at bedtime instead of on awakening might attenuate this morning peak by optimizing platelet inhibition during these high risk morning hours. Because acute arterial thrombosis is a multifactorial process and COX-1 only affects a part of total platelet reactivity, we do not expect that more efficient COX-1-dependent platelet inhibition abolishes the morning peak of cardiovascular events completely.⁴² For example, in a previous study in aspirin treated patients, poor clinical outcomes correlated with both high values of COX-1-dependent (STxB_2) and COX-1-independent assays.⁴³ The expected benefit of specifically reducing COX-1-dependent platelet reactivity could also be derived from previous studies, in which the risk of recurrent cardiovascular events was increased in patients with higher VerifyNow-Aspirin platelet reactivity.^{44, 45} Stable CVD patients with platelet reactivity >550 ARU had an absolute risk of 15.6% for developing the composite cardiovascular endpoint, whereas this was only 5.3% in patients with ARU values <550 .⁴⁵ In another study, the absolute risk for the primary endpoint (all-cause death and recurrent cardiovascular events) was 13.3% in patients >454 ARU and 5.9% in patients <454 ARU.⁴⁴ Although these observational studies suggest that already a modest reduction in COX-1- depen-

dent platelet reactivity could result in clinical benefit, future clinical trials should evaluate whether this indeed leads to a reduction of cardiovascular events.

CONCLUSION

This study suggests that low-dose aspirin taken at bedtime compared with intake on awakening reduces COX-1-dependent platelet reactivity during morning hours in healthy subjects. Future clinical trials in larger patient groups are required to investigate whether simply switching to aspirin intake at bedtime reduces the risk of cardiovascular events during the morning hours.

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