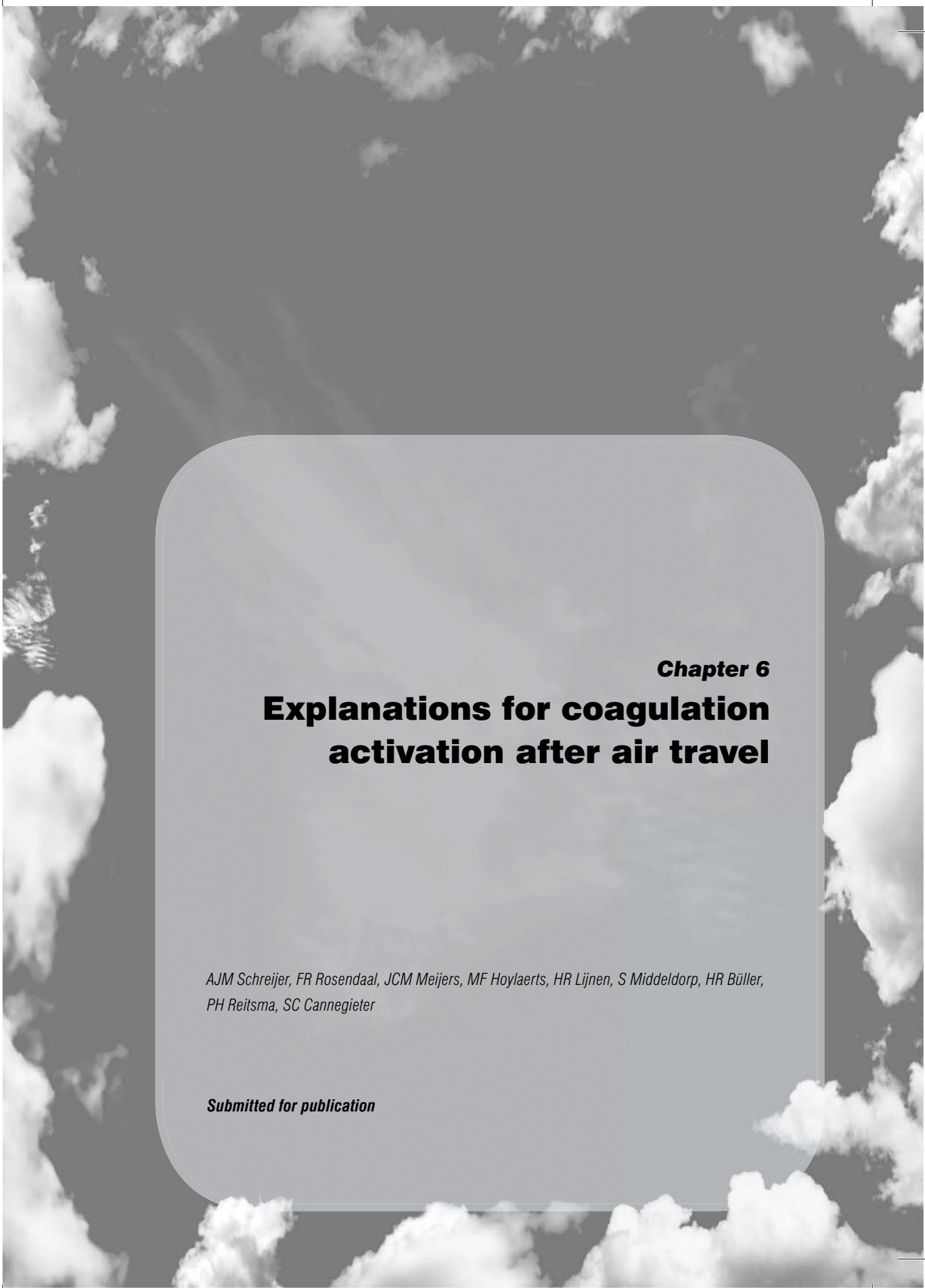
Air travel and venous thrombosis. Results of the WRIGHT study. Part II: Mechanism

Schreijer, A.J.M.



Chapter 6

Explanations for coagulation activation after air travel

AJM Schreijer, FR Rosendaal, JCM Meijers, MF Hoylaerts, HR Lijnen, S Middeldorp, HR Büller, PH Reitsma, SC Cannegieter

Submitted for publication

Abstract

It is unknown whether venous thrombosis after air travel is exclusively attributable to immobilisation or that other triggers also play a role. We compared surrogate markers for several hypothetical pathways in seventy-one healthy volunteers who were exposed to an 8-h flight, 8 h of immobilisation at ground level, and 8 h of regular daily activities. During the flight we had observed 11 (17%) volunteers with an activated clotting system, as evidenced by thrombin production (increases in thrombin antithrombin complexes, TAT).

We determined whether one or more of the following mechanisms was involved: hypoxia due to low cabin pressure (Plasminogen Activator Inhibitor 1, PAI-1), stress (coagulation factor VIIIc and a questionnaire), inflammation through exposure to air pollution (neutrophil elastase), or viral infection (interleukin-8, IL-8).

PAI-1 increased by 4.2 ng/ml (CI95 of the median: -49.5 to 6.5) in volunteers with an activated clotting system whereas it decreased in volunteers without clotting activation (median individual change -20.0 ng/ml, CI95: -33.2 to -14.0). Also, factor VIIIc levels rose more in individuals with clotting activation (median individual change 18.0 %, CI95: -1.0 to 33.0) than in those without (2.0, CI95: -2.0 to 5.0). The increases in FVIIIc were not associated with stress, which appeared unrelated to clotting activation. There was no difference in changes in levels of neutrophil elastase or interleukin-8 between the subjects with and without clotting activation.

In conclusion, clotting activation after air travel was related to increases in factor VIIIc and PAI-1 pointing to hypoxia as a possible trigger. Our results do not support the hypotheses that stress, infection, or air pollution are involved in the development of a prothrombotic state in air travellers.

Introduction

Air travel is an established risk factor for venous thrombosis. The risk of thrombosis is 2- to 4-fold increased after air travel, and is higher when other risk factors for venous thrombosis, such as the use of oral contraceptives or the factor V Leiden mutation are present[1-3]. The overall absolute risk for air travel related thrombosis is approximately 1 in 4500 passengers[4].

Which triggering mechanisms could underlie the increased risk? Several studies have investigated the effect of air travel, or one of its specific aspects (e.g. immobilisation and hypoxia) on the coagulation system. As recently summarised in a review on this subject, results in all three settings (immobilisation, hypoxia and air travel) have been conflicting, possibly due to differences in participant characteristics, duration of exposure, type of exposure and statistical analyses[4]. Also, most studies summarised their results on a group level which will dilute any individual effects that are present when only some individuals are susceptible to coagulation activation during these circumstances.

Previously, in a cross-over study in 71 volunteers we found evidence for coagulation activation in 17% of individuals after a long distance flight, which occurred less often during immobilisation for 8 hours (movie marathon) or during daily activities. Hence, we suggested that there is an additional mechanism to immobilisation underlying air travel related thrombosis. Further analyses showed that the clotting activation was not related to fluid loss or dehydration[5] and did not affect endothelial cell function, but it was found to be related to moderate platelet activation[6]. The aim of this present study was to explore other triggers that could underlie coagulation activation after exposure to air travel. To this aim we compared surrogate markers for several hypothetical pathways in individuals with and without an activated clotting system (based on increases in TAT) after the flight. The mechanisms that we explored were: the effect of hypoxia due to low cabin pressure (reflected by plasminogen activator inhibitor 1, PAI-1); stress due to fear of flying (reflected by a questionnaire on mood state in combination with plasma factor VIII coagulant activity (FVIIIc); viral infection shortly before or during the flight from other passengers (reflected by interleukin-8, IL-8); inflammation in the lungs through exposure to air pollution on the airport or during the flight (reflected by neutrophil elastase).

Material and methods

Procedures

Between May 24 and July 10, 2004, we performed a crossover study in 71 healthy volunteers, 41 (58 %) of whom had risk factors for thrombosis (i.e. were carriers of the factor V Leiden mutation or users of the oral contraceptive pill). All participants were exposed to an 8-h flight, 8 h of immobilisation (movie marathon), and 8 h of regular daily activities, separated by 2 weeks or more. Blood was drawn before, during, and after each exposure, at the same time of day. The medical ethics committee of the Academic Medical Center, Amsterdam, approved the study, and all participants gave written informed consent.

Flight

For the flight exposure situation, we chartered a Boeing 757 for a non-stop day flight of 8 h from and to Schiphol airport, The Netherlands. Volunteers were instructed not to smoke, use drugs, drink alcohol, or take any prophylactic measures to prevent thrombosis (e.g., heparin or aspirin use, or wearing of elastic stockings). Also, they were asked to keep a structured record of fluid intake and to remain seated as much as possible. Furthermore, volunteers were asked to fill in a questionnaire containing questions on their mood (Profile Of Mood State, POMS[7]). Blood was sampled by experienced technicians (each time by fresh venepuncture) who also recorded when blood was obtained and any problems that arose during sampling. Blood was collected between 08.00 and 08.30 h (after an overnight fast), around noon, and between 16.30 h and 17.30 h into Vacutainer tubes (Becton Dickinson, Oxford, England) containing EDTA (4.5 mg) and trisodium citrate (3.2% wt/vol).

Assays

We centrifuged citrated blood within 15 min of venepuncture at 2500 g for 15 minutes at 15°C. This was repeated after which plasma was frozen and immediately stored at -80°C. We extracted DNA from EDTA-anticoagulated blood and stored it at 4°C. We did the assays (all in duplicate except for the DNA tests) after all participants had completed the study.

Factor VIIIc (plasma factor VIII coagulant activity) was measured by a one-stage clotting assay with factor-VIII-deficient plasma (Dade Behring, Marburg, Germany). We determined factor V Leiden and prothrombin G20210A status by PCR. Plasma plasminogen activator inhibitor-1 (PAI-1) antigen levels were measured with a specific home-made ELISA[8]. Neutrophil elastase activity in plasma was determined using the highly neutrophil elastase–specific chromogenic

substrate N-methoxysuccinyl-Ala-Ala-Pro-Val p-nitroanilide (Sigma, St. Louis, MO). Briefly, samples were incubated in 0.1 M Tris-HCl buffer (pH 8.0), containing 0.5 M NaCl and 1 mM substrate, for 70 hours at 37°C. After incubation, p-nitroaniline was measured spectrophotometrically at 405 nm, and absorbance, corrected for baseline activity, was taken as an index of neutrophil elastase activity. The plasma concentration of Interleukin-8 (IL-8) was measured by use of a validated cytometric bead array kit (BD Biosciences Pharmingen, San Diego, Ca).

During the exposures we took 639 samples. For FVIIIc 14 measurements samples (2%) were unavailable: ten were visibly haemolytic, three were missing because of unsuccessful blood collection and in one results were technically unreliable. For PAI-1 there were 49 samples (8%) missing: 7 samples were unavailable and of 42 samples the results were technically unreliable. For neutrophil elastase 15 samples were unavailable and 64 results were missing because of technically unreliable results (12% missing samples). For IL-8, 2 samples were missing due to unsuccessful blood collection.

Profile of Mood States Questionnaire

Volunteers filled in a questionnaire (Profile Of Mood States, POMS) before each blood draw containing 32 questions on their mood of which six concerned stress. Volunteers gave scores to the applicability of several mood states for that moment on a scale of 0 to 4. The mean score of these questions reflects the amount of stress experienced for that moment, with a higher score reflecting a higher level of stress[7]. All questions were filled out by all volunteers before and during the flight.

Statistical analyses

General characteristics of the volunteers are shown as means and ranges. We calculated absolute changes in the variables for each individual by subtracting the pre-exposure value from the post-exposure value. We calculated the medians (CI95) of these individual changes. We identified high responders for each assay by using as cut-off points the 85th percentile for the absolute change in that assay during the daily life situation. Previously, we identified individuals with and without an activated clotting system[9]. Based on the relative change in thrombin antithrombin complexes (TAT) during the flight, 66 volunteers (in 5 volunteers the change in TAT was missing) could be divided into volunteers with (n=11) and without (n=55) an activated clotting system. Of the 11 volunteers with clotting activation 8 had risk factors for venous thrombosis (i.e. oral contraceptive use, factor V Leiden mutation or both). For the flight situation we compared the absolute change for each parameter in volunteers with an activated clotting

system to that of volunteers without clotting activation by Mann-Whitney U test. We compared the frequency of high responders in volunteers with and without an activated clotting system with Fisher's exact test and by calculating odd ratios and their 95% confidence intervals. Also relative risks were calculated. Means were compared by T-test. Relations between variables were investigated using linear regression after viewing the data in scatter plots.

Results

General

The flight took place on May 24th 2004, between 08.30 and 16.19 hrs and was uneventful. 71 healthy volunteers aged 20-39 years participated in the study, 56 (79%) of whom were women (Table 1). Of these 56 women, 15 used oral contraceptives, 11 were carriers of the factor V Leiden mutation and 15 women had both risk factors. The 15 men did not have risk factors for venous thrombosis. Each volunteer returned for each of the three stages in the study as planned. There were no episodes of venous thrombosis. Mean BMI was 23.0 kg/m^2 (range: 18 to 33) and one volunteer was obese (BMI: 33 kg/m^2).

Table 1. Absolute changes in parameters after the flight in volunteers with (n=11) and without (n=55) an activated clotting system.*

	All (n=66)	Volunteers with an activated clotting system (n=11)	Volunteers without an activated clotting system (n=55)	P-value
PAI-1	-18.0 (-30.8 to -12.0)	4.2 (-49.5 to 6.5)	-20.0 (-33.2 to -14.0)	0.02
FVIIIc	3.0 (0.0 to 7.0)	18.0 (-1.0 to 33.0)	2.0 (-2.0 to 5.0)	0.01
Elastase	0.05 (0.03 to 0.07)	0.06 (0.02 to 0.14)	0.05 (0.01 to 0.07)	0.12

* *Absolute changes (medians and 95% confidence intervals). P-values calculated by Mann-Whitney U test. PAI-1= plasminogen activator inhibitor 1 (ng/ml), Neutrophil elastase (x1 µmol of p-nitroanaline/ml sample), FVIIIc = clotting factor VIII (%).*

Levels around exposures and differences in volunteers with and without coagulation activation

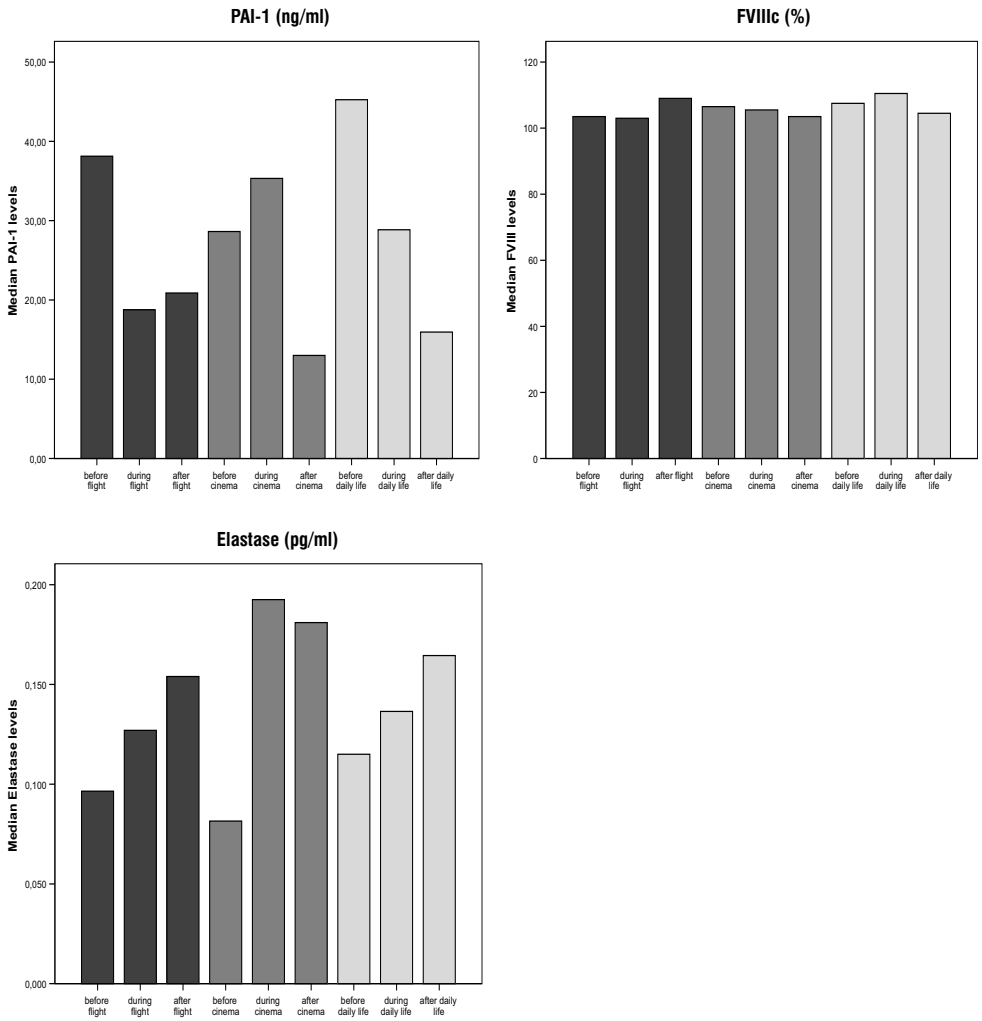
Based on the change in thrombin antithrombin complexes (TAT) during the flight, 11 volunteers were identified as individuals with an activated clotting system[9]. Table 1 shows the differences in absolute changes for the parameters between the two groups.

PAI-1 values were highest in the morning before the start of the exposure (Figure 1) which is consistent with its circadian rhythm[10]. PAI-1 levels were higher after the flight than after the other situations. While overall PAI-1 decreased during the flight (median individual change -18.0 ng/ml; CI95: -30.8 to -12.0), PAI-1 increased by 4.2 ng/ml (CI95: -49.5 to 6.5) in volunteers with an activated clotting

system and decreased in volunteers without clotting activation (-20.0, CI95: -33.2 to -14.0, table 1). For PAI-1, the flight caused a high-response in 9 volunteers (cut off: -4.7 ng/ml), 6 of whom had clotting activation as measured by TAT levels (OR: 28.0, CI95: 4.6 to 171.9).

Median levels of FVIIIc were higher after the flight than after the daily life situation, whereas baseline levels of FVIIIc were lowest before the flight (Figure 1). Overall, FVIIIc increased during the flight (median individual increase 3.0 %, CI95: 0.0 to 7.0). This increase was 9-fold higher in individuals with clotting activation (18.0 %, CI95: -1.0 to 33.0) than in those without an activated clotting system (2.0 %, CI95: -2.0 to 5.0, table 1). We identified 26 high-responders in FVIIIc, (cut off: 6.7 %), 8 of whom also had an activated clotting system as measured by TAT levels (OR 5.5, CI95: 1.3 to 23.2).

Figure 1. Absolute median levels of the markers before during and after exposures (n=71).



The mean stress score *before* the flight was 0.3 (range 0 to 2.2 out of 4). The score in volunteers with clotting activation (mean 0.2, CI95: 0.1 to 0.4) was not different from the score in volunteers without clotting activation (mean score 0.4, CI95: 0.2 to 0.5). *During* the flight, the mean stress score was 0.2 (range 0 to 1.3 out of 4) and the score in volunteers with clotting activation (mean score: 0.1, CI95: 0.0 to 0.2) was similar to the score in volunteers without clotting activation (0.2, CI95: 0.1 to 0.3).

The pattern of neutrophil elastase levels was similar for both the flight and the daily life situation. Neutrophil elastase levels were highest after the cinema (Figure 1). There was no difference in absolute changes in levels of neutrophil elastase between subjects with and without coagulation activation (Table 1). 10 volunteers were identified as high-responders in neutrophil elastase (cut off: 0.086 p-nitroanaline/ml), 4 of whom also had an activated clotting system (OR: 4.4, CI95: 0.9 to 21.3)

IL-8 was detectable (>3 pg/ml) in only six participants after the flight (range 47 to 469 pg/ml). In three of these individuals IL-8 was already detectable (with similar levels) before flying, indicating no effect of flying on IL-8 levels in these individuals. After the cinema IL-8 levels were detectable in 8 volunteers (range 44 to 369 ng/ml) four of whom already had detectable levels before the cinema. In nine individuals IL-8 were detectable after the daily life situation (range 9 to 417 ng/ml) of whom five already had detectable IL-8 levels at baseline. There was one individual with coagulation activation amongst the three volunteers in whom IL-8 became detectable after the flight.

Mechanistic pathways

Stress is known to increase levels of several coagulation parameters, such as factor VIII[11]. As we questioned whether stress could explain the increases in FVIIIc levels that we found in some volunteers, we investigated the relationship between changes in FVIIIc and the stress score before or during the flight. As described above, the stress experienced by volunteers during our flight was minimal. We found no relationship between levels of factor FVIII:c and the stress experienced either before the flight (regression coefficient: 2.0, CI95: -10.9 to 14.9) or during the flight (regression coefficient: -10.2, CI95: -29.2 to 8.8).

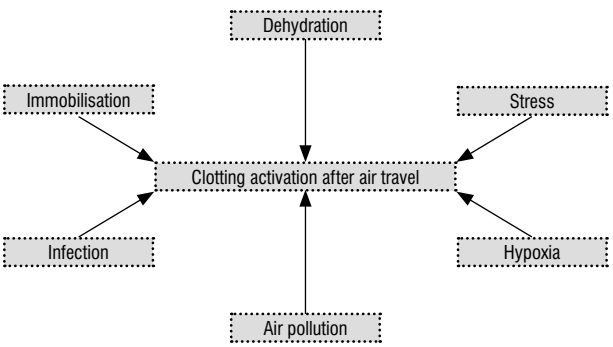
To investigate whether changes in FVIII could be explained by hypoxia, we investigated the relationship between FVIIIc and PAI-1. The increases in FVIIIc during the flight were not related to changes in PAI-1 (regression coefficient: -0.1, CI95: -0.1 to 0.3), nor were the values after the flight (regression coefficient: 0.1 (CI95: -0.2 to 0.7).

Inflammation (e.g. after exposure to air pollution) and thrombosis are related via interactions between leucocytes, platelets, the vasculature and the coagulation pathway[12,13]. To investigate the relationship between leucocytes and platelets we examined neutrophil elastase and P-selectin (a marker of platelet activation; results on P-selectin were reported previously[6]) in our study and we found that absolute changes in neutrophil elasatase levels were related to absolute changes in P-selectin during the flight (regression coefficient: 22.0, CI95: 2.6 to 41.5) while we did not find such a relationship during the cinema (regression coefficient: 2.9, CI95: -10.1 to 15.9) or daily life situation (regression coefficient: 2.6, CI95: -14.1 to 19.3).

Discussion

The aim of this study was to explore mechanisms (other than immobilisation and dehydration) that could be responsible for coagulation activation during air travel. Figure 2 gives an overview of mechanisms that could be involved. The middle box represents the coagulation activation that we found in 17% of volunteers after an 8-h flight[9]. This is the central point from which we currently explored mechanisms that are possibly involved. We found that changes in PAI-1 and factor VIII:c were clearly higher in individuals with an activated clotting system than in those without clotting activation after air travel.

Figure 2. Possible explanations for coagulation activation during air travel.



Hypoxia. During air travel, cabin pressure drops to 75.8 kPa, which is equivalent to an altitude of 2400 m above sea level. Consequently, oxygen saturation can drop as low as 90–93% and even to 80% in passengers who are asleep[14;15], which may cause acute mountain sickness[16]. Studies into the effect of both hypobaric as well as normobaric hypoxia on human coagulation have

been reviewed[4]. None of the controlled experiments found an effect of either 8 h of isocapnic hypoxia[17], short-term normobaric hypoxia[18] or 8 to 10 h of hypobaric hypoxia[19] compared to normobaric normoxia. Also markers of fibrinolysis remained unchanged during these controlled studies[17-20]. Previous research on fibrinolytic markers during air travel found an increase of PAI-1 during air travel[21]. One drawback of these studies is that they did not include participants with risk factors for venous thrombosis while it is conceivable that hypoxia has an effect in certain susceptible subjects only. One parameter that is sensitive to hypoxia is PAI-1. Besides its role in maintaining normal hemostasis by regulating the fibrinolytic system, PAI-1 plays a role in many other (patho)physiological processes[10].

We found that PAI-1 increased during the flight in the individuals with an activated clotting system whereas it decreased in those without coagulation activation. Furthermore, out of the 11 subjects with clotting activation, 6 were also high responders in PAI-1.

There are several mechanisms that could be proposed with respect to the relation between hypoxia, thrombin generation and increased PAI-1 levels. In the most parsimonious scenario, hypoxia leads to endothelial activation. This has several consequences, of which some or all may happen in an individual, i.e. release of PAI-1[10], tissue factor expression[22], and release of factor VIII. The tissue factor expression leads to clotting activation and, ultimately, production of thrombin and fibrin inactivation products, as TAT and D-Dimer, as well as platelet activation with P-selection release. The increased levels of PAI-1 and FVIII are markers of hypoxia and endothelial activation, but they play no role in the process. In a more complex hypothetical model, thrombin formation after tissue factor expression leads to increased PAI-1 levels via hypoxia inducible factor 1 (HIF-1)[23]. Increased factor VIII levels may also have occurred via other pathways, i.e. acute phase reactions, or partial activation. Given the small number of observations, it is not possible to firmly establish one or the other mechanism, although it is noteworthy that several individuals with increases in PAI-1 levels did not show signs of clotting activation, which argues against the second mechanism. Whatever the exact mechanism, these data support a role for hypoxia in air travel related thrombosis.

Stress. Individuals who are afraid of flying may experience anxiety when flying. Stress increases levels of several coagulation parameters, such as clotting factor VIII[12]. Therefore, it has been suggested that stress could underlie air travel related thrombosis. In a case-control study, stress increased the risk of venous thrombosis after air travel 2.5 fold[24]. In our study, volunteers with clotting activation after the flight were not more anxious than the others. Furthermore, changes in factor VIII:c were not related to anxiety experienced before or during the flight. So,

there seems to be no direct effect of stress on coagulation activation in our study. Still, we have to be cautious with interpreting these results since the levels of stress experienced during the flight were not very high, probably because people who are afraid of flying do not volunteer for a research project such as ours.

Air pollution. We questioned whether air pollution could play a role through inflammation in the lungs inducing clotting activation. Around airports, the air is polluted by aviation fuels and their combustion products. Combustion of aviation fuels results in CO_2, CO, C_e, NO_x, particles, and a large number of other organic compounds, among which a number of carcinogens[25;26]. Inflammation and thrombosis are related via interactions between leucocytes, platelets, the vasculature and the coagulation pathway[12;13]. For this purpose we measured neutrophil elastase in our study. Although neutrophil elastase was not different between the two groups, we did find a relationship between absolute changes in neutrophil elastase levels and levels of P-selectin only after the flight. This supports the theory that inflammation could play a role in platelet activation during air travel, but not directly in thrombin formation.

Infection. We hypothesized that passengers could be more at risk for infection, since they are placed together in a small space for a considerable time during air travel, allowing germs to spread. Mechanisms that could play a role are interactions between leucocytes, platelets, the vasculature and the coagulation pathway[12;13]. Results from a self-controlled case-series method in a large cohort study showed that acute infections are associated with a transient increased risk of venous thrombosis in a community setting[27]. In case control studies, detectable levels of IL-8 have been associated with a more than 2-fold increased risk for venous thrombosis[28], whereas cohort studies reported no effect[29]. In our study, IL-8 levels became only detectable in few volunteers and not more during air travel than during the other situations. Hence, we conclude that it is unlikely that infection plays a major role in clotting activation during air travel.

There are various general limitations to our study, which also have been described in our previous publication[9]. Although there is no evidence that our assays could be affected by haemolysis in vitro, we excluded all visibly haemolytic material. Also samples with technically unreliable data in other assays (TAT) were excluded as well as unreliable data in the current assays. Furthermore, the number of volunteers with clotting activation was small (n=11). However, as our parameters were of a continuous nature and were analysed in a pair-wise way, the power was still sufficient and clear effects in several parameters could be detected. Lastly, not all parameters used in our study fully cover the mechanism that we assigned to it. For example, stress was measured by a standard questionnaire filled in by the volunteers themselves. Possibly, not all questions

were answered accurately. For air pollution we used a parameter that is still experimental (neutrophil elastase).

In summary, clotting activation after air travel was related to increases in factor VIIIc and PAI-1 in our study. The increases in FVIIIc were independent from stress. An obvious explanation for the increase in PAI-1 is hypoxia, but the increases in PAI-1 could also be a consequence of coagulation activation. None of the other parameters were related to clotting activation after air travel. Our results do not support the hypotheses that stress, infection or air pollution are involved in the prothrombotic state brought about by air travel. A role for hypoxia seems likely.

WRIGHT scientific executive committee

The study has been conducted as part of the WRIGHT project (World Health Organisation Research Into Global Hazards of Travel), which is an international research project under auspices of the World Health Organisation. The scientific committee consists of P Kesteven, Freeman Hospital, Newcastle upon Tyne, UK; W D Toff, University of Leicester, Leicester, UK; F Paccaud, Institute for Social and Preventive Medicine, Lausanne, Switzerland; M Greaves, University of Aberdeen, Aberdeen, UK; H R Büller, Academic Medical Centre, Amsterdam, Netherlands; F R Rosendaal, Leiden University Medical Centre, Leiden, Netherlands; S Mendis, WHO, Geneva, Switzerland.
The WRIGHT project monitoring group is chaired by
B M Psaty, University of Washington, Seattle,WA, USA.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments:

We thank all volunteers for their enthusiasm; the Julius Centre for Health Sciences and Primary Care, Utrecht, and especially A Algra and Y T van der Schouw, for providing us the contact details of the women with the factor V Leiden mutation that they identified for their AGENS study; Ph G de Groot for the use of laboratory facilities at University Medical Centre, Utrecht; the Emma Children's Hospital for use of the cinema; and J P Vandenbroucke for help with design of the study. We are grateful to many people for their generous help and practical contributions to the study: TNO Aerospace Medicine and Royal Netherlands Air Force; Centre for Man and Aviation, for their technical assistance in pilot work; the personnel of the department of Experimental Vascular Medicine and the Center for Experimental and Molecular Medicine of the Academic Medical Center, Amsterdam, and Clinical Epidemiology units of the Leiden University Medical Center; laboratory technicians

Wil Kopatz, Willy Morriën, Kamran Bakhtiari, Claudia van Rijn, Jeroen van der Meijden, Liesbeth Willems of Brilman-Tuinhof de Moed, Rob van Eck, Saskia Foeken, Kim van Rooden, Petra Noordijk, Koby Los, Astrid van der Niet, Claartje Koch, Carla van Dijk, Marry Bonnecroy, Marian Weijne, Annelies Hoenderdos, Arnoud Marquart, and Alinda Schimmel, the PhD students Yaël Nossent, Rick van Minkelen, Lois Brüggeman, Yvonne van Leeuwen, Saskia Kuipers, and also Lucie Timmers, Yvonne Souverein, and Agnes Vree, as well as Depex, especially Gert Kraaij, for making their centrifuges available, Rientjes and Partners, Top Systems, and Dutchbird. We kindly thank Baudewijntje Kreukels for her help with the POMS questionnaire. This study was done in the framework of the WRIGHT initiative (WHO Research Into Global Hazards of Travel), and was sponsored by the UK Government and the European Commission. The views expressed in this paper are those of the authors and do not necessarily represent the opinions of the other research institutions that participated in the project or the views or policies of WHO. This study was also supported by a grant from the Leducq Foundation, Paris, France for the development of Transatlantic Networks of Excellence in Cardiovascular Research. We thank the Center for Molecular and Vascular Biology of the Catholic University, Leuven, Belgium for performing the measurements of neutrophil elastase and PAI-1.

Reference List

(1) Cannegieter SC, Doggen CJM, van Houwelingen HC, Rosendaal FR. Travel-Related Venous Thrombosis: Results from a Large Population-Based Case Control Study (MEGA Study). PLoS Med 2006;3.

(2) Martinelli I, Taioli E, Battaglioli T, Podda GM, Passamonti SM, Pedotti P et al. Risk of venous thromboembolism after air travel: interaction with thrombophilia and oral contraceptives. Arch Intern Med 2003;163:2771-2774.

(3) Kuipers S, Cannegieter SC, Doggen CJM, Rosendaal FR. The Effect of Elevated Levels of Coagulation Factors on the Risk of Venous Thrombosis in Long Distance Travellers. Blood 2009; 113:2064-9.

(4) Kuipers S, Schreijer AJM, Cannegieter SC, Büller HR, Rosendaal FR, Middeldorp S. Travel and venous thrombosis: a systematic review. J Intern Med 2007; 262:615-634.

(5) Schreijer AJM, Cannegieter SC, Caramella M, Meijers JCM, Krediet RT, Simons RM et al. Fluid loss does not explain coagulation activation during air travel. Thromb Haemost. 2008 Jun;99: 1053-9.

(6) Schreijer AJM, Rosendaal FR, Meijers JCM, Middeldorp S, Reitsma PH, Büller HR et al. The effect of air travel on platelets and the vessel wall. Submitted.

(7) D.M.McNair, M.Lorr, L.F.Droppleman. Manual for the Profile of Mood States. Educational and Testing Service. San Diego: 1971.

(8) Declerck PJ, Verstreken M, Collen D. Immunoassay of murine t-PA, u-PA and PAI-1 using monoclonal antibodies raised in gene-inactivated mice. Thromb Haemost 1995; 74:1305-1309.

(9) Schreijer AJM, Cannegieter SC, Meijers JCM, Middeldorp S, Büller HR, Rosendaal FR. Activation of coagulation system during air travel: a crossover study. Lancet 2006; 367:832-838.

(10) Lijnen HR. Pleiotropic functions of plasminogen activator inhibitor-1. J Thromb Haemost 2005; 3:35-45.

(11) Kanel von R, Preckel D, Zgraggen L, Mischler K, Kudielka BM, Haeberli A et al. The effect of natural habituation on coagulation responses to acute mental stress and recovery in men. Thromb Haemost 2004; 92:1327-1335.

(12) Nemmar A, Nemery B, Hoet PH, Van RN, Hoylaerts MF. Silica particles enhance peripheral thrombosis: key role of lung macrophage-neutrophil cross-talk. Am J Respir Crit Care Med 2005; 171:872-879.

(13) Gale AJ, Rozenshteyn D. Cathepsin G, a leukocyte protease, activates coagulation factor VIII. Thromb Haemost 2008; 99:44-51.

(14) Simons R, Krol J. Jet leg, pulmonary embolism, and hypoxia. Lancet 1996; 348:416.

(15) Humphreys S, Deyermond R, Bali I, Stevenson M, Fee JP. The effect of high altitude commercial air travel on oxygen saturation. Anaesthesia 2005; 60:458-460.

(16) Muhm JM, Rock PB, McMullin DL, Jones SP, Lu IL, Eilers KD et al. Effect of aircraft-cabin altitude on passenger discomfort. N Engl J Med 2007; 357:18-27.

(17) Crosby A, Talbot NP, Harrison P, Keeling D, Robbins PA. Relation between acute hypoxia and activation of coagulation in human beings. Lancet 2003; 361:2207-2208.

(18) Hodkinson PD, Hunt BJ, Parmar K, Ernsting J. Is mild normobaric hypoxia a risk factor for venous thromboembolism? J Thromb Haemost 2003; 1:2131-2133.

(19) Toff WD, Jones CI, Ford I, Pearse RJ, Watson HG, Watt SJ et al. Effect of hypobaric hypoxia, simulating conditions during long-haul air travel, on coagulation, fibrinolysis, platelet function, and endothelial activation. JAMA 2006; 295:2251-2261.

(20) Schobersberger W, Mittermayr M, Fries D, Innerhofer P, Klingler A, Faulhaber M et al. Changes in blood coagulation of arm and leg veins during a simulated long-haul flight. Thromb Res 2007; 119:293-300.
(21) Schobersberger W, Fries D, Mittermayr M, Innerhofer P, Sumann G, Schobersberger B et al. Changes of biochemical markers and functional tests for clot formation during long-haul flights. Thromb Res 2002; 108:19-24.
(22) Tang H, Ivanciu L, Popescu N, Peer G, Hack E, Lupu C et al. Sepsis-induced coagulation in the baboon lung is associated with decreased tissue factor pathway inhibitor. Am J Pathol 2007; 171:1066-1077.
(23) Gorlach A, Diebold I, Schini-Kerth VB, Berchner-Pfannschmidt U, Roth U, Brandes RP et al. Thrombin activates the hypoxia-inducible factor-1 signaling pathway in vascular smooth muscle cells: Role of the p22(phox)-containing NADPH oxidase. Circ Res 2001; 89:47-54.
(24) Schreijer AJM, Cannegieter SC, Doggen CJM, Rosendaal FR. The effect of flight-related behaviour on the risk of venous thrombosis after air travel. Br J Haematol. 2009 Feb;144:425-9.
(25) Visser O, van Wijnen JH, van Leeuwen FE. Incidence of cancer in the area around Amsterdam Airport Schiphol in 1988-2003: a population-based ecological study. BMC Public Health 2005; 5:127.
(26) Tesseraux I. Risk factors of jet fuel combustion products. Toxicol Lett 2004; 149:295-300.
(27) Smeeth L, Cook C, Thomas S, Hall AJ, Hubbard R, Vallance P. Risk of deep vein thrombosis and pulmonary embolism after acute infection in a community setting. Lancet 2006; 367:1075-1079.
(28) van Aken BE, Reitsma PH, Rosendaal FR. Interleukin 8 and venous thrombosis: evidence for a role of inflammation in thrombosis. Br J Haematol 2002; 116:173-177.
(29) Christiansen SC, Naess IA, Cannegieter SC, Hammerstrom J, Rosendaal FR, Reitsma PH. Inflammatory cytokines as risk factors for a first venous thrombosis: a prospective population-based study. PLoS Med 2006; 3:e334.