

Effect of Hypobaric Hypoxia, Simulating Conditions During Long-Haul Air Travel, on Coagulation, Fibrinolysis, Platelet Function, and Endothelial Activation

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VENOUS THROMBOEMBOLISM after long-haul air travel was first reported more than 50 years ago.¹ Subsequent case reports and epidemiological studies have provided further evidence of an association but it remains unclear whether this is due to the effects of prolonged sitting, as in other modes of travel, or whether there is a causal relationship to some other specific factor in the airplane environment.²

One putative factor, which has attracted attention, is hypoxia associated with the decreased cabin pressure. At cruising altitude during a long-haul flight, cabin pressure is typically equivalent to that at an altitude of 1524 to 2134 m, although international regulations permit a maximum

For editorial comment see p 2297.

Context The link between long-haul air travel and venous thromboembolism is the subject of continuing debate. It remains unclear whether the reduced cabin pressure and oxygen tension in the airplane cabin create an increased risk compared with seated immobility at ground level.

Objective To determine whether hypobaric hypoxia, which may be encountered during air travel, activates hemostasis.

Design, Setting, and Participants A single-blind, crossover study, performed in a hypobaric chamber, to assess the effect of an 8-hour seated exposure to hypobaric hypoxia on hemostasis in 73 healthy volunteers, which was conducted in the United Kingdom from September 2003 to November 2005. Participants were screened for factor V Leiden G1691A and prothrombin G20210A mutation and were excluded if they tested positive. Blood was drawn before and after exposure to assess activation of hemostasis.

Interventions Individuals were exposed alternately (≥ 1 week apart) to hypobaric hypoxia, similar to the conditions of reduced cabin pressure during commercial air travel (equivalent to atmospheric pressure at an altitude of 2438 m), and normobaric normoxia (control condition; equivalent to atmospheric conditions at ground level, circa 70 m above sea level).

Main Outcome Measures Comparative changes in markers of coagulation activation, fibrinolysis, platelet activation, and endothelial cell activation.

Results Changes were observed in some hemostatic markers during the normobaric exposure, attributed to prolonged sitting and circadian variation. However, there were no significant differences between the changes in the hypobaric and the normobaric exposures. For example, the median difference in change between the hypobaric and normobaric exposure was 0 ng/mL for thrombin-antithrombin complex (95% CI, -0.30 to 0.30 ng/mL); -0.02 nmol/L for prothrombin fragment 1 + 2 (95% CI, -0.03 to 0.01 nmol/L); 1.38 ng/mL for D-dimer (95% CI, -3.63 to 9.72 ng/mL); and -2.00% for endogenous thrombin potential (95% CI, -4.00% to 1.00%).

Conclusion Our findings do not support the hypothesis that hypobaric hypoxia, of the degree that might be encountered during long-haul air travel, is associated with prothrombotic alterations in the hemostatic system in healthy individuals at low risk of venous thromboembolism.

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cabin altitude equivalent of 2438 m under normal operating conditions.^{3,4} At 2438 m, the reduced partial pressure of oxygen in inspired air typically results in a decrease in arterial oxygen saturation to about 93% in healthy resting individuals, although there is considerable variability in the response.⁵ Larger reductions may be expected in older individuals and those with cardiac or pulmonary disease.⁶

There is experimental evidence from studies of cultured human venous endothelial cells that exposure to hypoxia may increase procoagulant activity⁷ and inhibit fibrinolysis.⁸ In addition, a small uncontrolled study of healthy volunteers showed a transient increase in markers of coagulation activation during exposure to hypobaric hypoxia.⁹

We performed a controlled study (from September 2003 to November 2005) to assess the effects of hypoxia, similar to that which might be encountered during commercial air travel, on a comprehensive range of markers of activation of the hemostatic system.

METHODS

Study Design

A crossover study design was used to compare the effects of prolonged sitting in a hypobaric hypoxic environment with those of sitting for the same period in a normobaric normoxic environment on hemostatic function. The study was per-

formed within the framework of the WRIGHT (World Health Organization Research Into the Global Hazards of Travel) project, which is a multicenter collaborative study carried out under the auspices of the World Health Organization. The WRIGHT project was designed to provide key information on the frequency of venous thrombosis, the magnitude of its association with air travel, and the causal mechanisms involved, as well as clues on possible preventive strategies, and to lay scientific groundwork for future international research in the area. The hypobaric and normobaric exposures were performed in hypobaric chambers at the Royal Air Force Centre of Aviation Medicine, Henlow, and the University of Aberdeen. The study was approved by the Leicestershire Health Authority Research Ethics Committee, the Grampian Research Ethics Committee, and the Royal Air Force Experimental Medicine Ethics Committee. All individuals provided written informed consent.

Healthy Volunteers

The healthy volunteers were recruited by local advertisement and assigned to 1 of 3 groups (TABLE 1). Group 1 was made up of individuals between the ages of 18 and 40 years who did not have known risk factors for venous thromboembolism. Group 2 was made up of women between the ages of 18 and 40 years who were taking a combined oral contraceptive pill. Group 3

was made up of individuals aged 50 years or older. Individuals were excluded if they or a first-degree relative had a history of venous thromboembolism, or if they had taken anticoagulant or antiplatelet medication in the 2 weeks before the study. Individuals were screened for factor V Leiden G1691A and prothrombin G20210A mutation and excluded if they tested positive. Air travel was not permitted within 1 week before each hypobaric and normobaric exposure.

Hypobaric Chambers

The hypobaric facilities were 2-compartment chambers of approximately 2-m internal diameter with seating for 6 to 8 individuals (Henlow facility: Aeroform, Poole, England; Aberdeen facility: Magpie Engineering, Fraserburgh, Scotland). The temperature was thermostatically controlled (mean range, 17°C-22°C). Humidity was determined by the ambient conditions and airflow (mean range, 34%-56%).

Experimental Protocol

Individuals were seated in the chamber and exposed for 8 hours to normobaric normoxia or hypobaric hypoxia, equivalent to the prevailing atmospheric conditions at ground level (54 m and 70 m above mean sea level at Henlow and Aberdeen, respectively) or an altitude of 2438 m, respectively. Each individual was exposed to both conditions, at least 1

Table 1. Demographic and Clinical Characteristics

	All Individuals (n = 73)	Individuals by Order of Exposure		Individuals by Group		
		HH Followed by NN (n = 37)	NN Followed by HH (n = 36)	1: Young (n = 49)	2: OCU (n = 12)	3: Older (n = 12)
Age, mean (SD), y	29.1 (13.5)	24.2 (7.1)	34.1 (16.5)*	23.5 (5.2)	23.8 (6.0)	57.0 (5.0)
Sex, No. (%)						
Male	31 (42.5)	54 (74.0)	50 (68.5)	27 (55.1)	0	4 (33.3)
Female	42 (57.5)	19 (26.0)	23 (31.5)	22 (44.9)	12 (100.0)	8 (66.6)
Height, mean (SD), cm	172.1 (10.5)	173.6 (10.4)	170.6 (10.6)	175.5 (10.2)	166.9 (6.4)	164.0 (8.7)
Body mass index, mean (SD)†	24.1 (3.0)	24.2 (2.9)	24.0 (3.2)	23.9 (3.1)	23.3 (1.9)	25.9 (3.1)
Level of SaO ₂ , mean (SD)						
During normobaria	96.2 (1.1)	96.2 (1.1)	96.3 (1.2)	96.1 (1.1)	97.2 (0.7)	95.9 (1.0)
During hypobaria	92.6 (1.6)	93.0 (1.5)	92.3 (1.7)	92.9 (1.3)	93.5 (1.7)	90.7 (1.4)

Abbreviations: HH, hypobaric hypoxia; NN, normobaric normoxia; OCU, oral contraceptive user; SaO₂, arterial oxygen percent saturation.

*In group 3, 11 of the 12 individuals were exposed first to normobaria, which resulted in some imbalance with respect to age.

†Calculated as weight in kilograms divided by the height in meters squared.

week apart, in a quasi-random order (individual availability was matched to pre-assigned dates on which the type of exposure was determined by the chamber staff according to operational considerations). Studies were performed at the same time of day to control for circadian changes in hemostatic markers. Individuals were not told of the assigned exposure, although blinding could not be ensured due to possible perception of otitic pressure changes during the hypo-

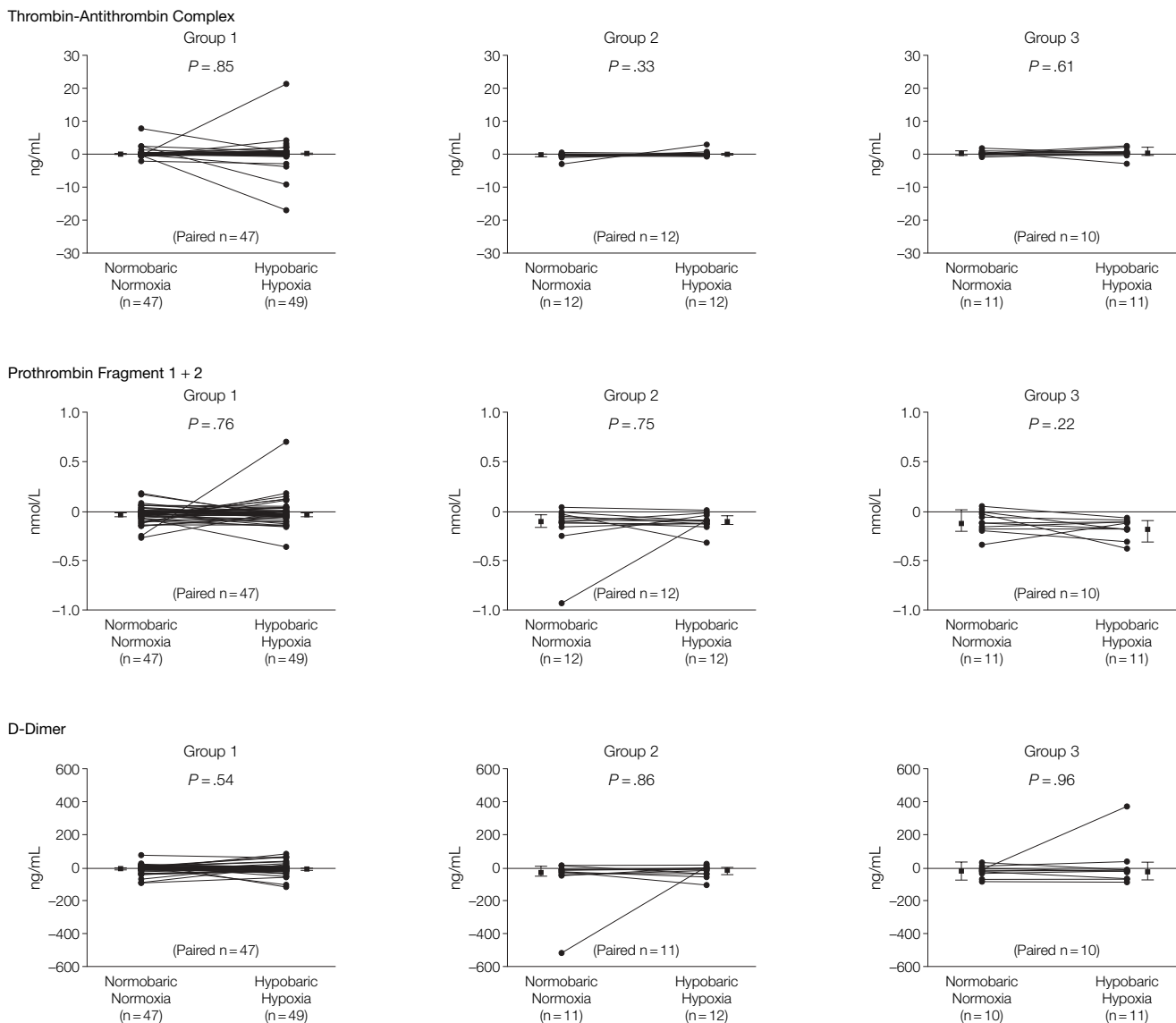
baric exposure. Depressurization and repressurization were both gradual over 10 to 20 minutes. Individuals were permitted to stand and move about for up to 5 minutes per hour. Continuous visual surveillance was maintained and arterial oxygen saturation was monitored by pulse oximetry and recorded hourly. Individuals were permitted to drink non-alcoholic beverages and were given a light midday meal and snacks. Individuals were asked to report any symptoms or

signs of venous thromboembolism during or within a week after each hypobaric and normobaric exposure.

Blood Sampling and Processing

Blood was taken by clean venipuncture, using a 21-gauge butterfly needle, before each exposure (between 7:30 AM and 9 AM) and within 20 minutes after leaving the chamber (between 3:30 PM and 5 PM). Blood was collected into Vacutainer tubes (Becton Dickinson,

Figure 1. Paired Changes in Thrombin-Antithrombin Complex, Prothrombin Fragment 1 + 2, and D-Dimer After the Normobaric and Hypobaric Exposures



Unpaired data are included for individuals in whom data were only available for 1 of the 2 conditions. Squares indicate medians (95% confidence intervals) for each group.

Oxford, England) containing EDTA (4.5 mg) for a full blood count, CTAD (citrate, theophylline, adenosine, and dipyridamole) for measurement of plasminogen activator inhibitor type-1 and β -thromboglobulin, and trisodium citrate (3.2% wt/vol). Samples were processed immediately for whole blood flow cytometry or centrifuged for plasma separation. For preparation of plasma, blood samples were double spun, first at 1550g and 4°C for 30 minutes (except samples for factor VII assay, which were spun at room temperature), followed by 9300g for 10 minutes. Plasma was immediately divided into aliquots, frozen, and stored at -80°C.

Flow Cytometry

Flow cytometry was performed near the chamber using a Beckman Coulter XL-MCL flow cytometer (Beckman Coulter Ltd, High Wycombe, England), which was standardized daily with Flow-Check beads (Beckman Coulter Ltd).

Monocyte-platelet aggregates were analyzed as a marker of in vivo platelet activation,¹⁰ and the platelet response to agonist stimulation (adenosine diphosphate 1×10^{-6} mol and thrombin receptor agonist peptide SFLLRN 1×10^{-6} mol; both from Sigma Chemical Co Ltd, Poole, England), measured as the binding of fibrinogen to glycoprotein IIb/IIIa, was analyzed as previously described.¹¹ Activation was expressed as the

percentage of monocytes with platelets bound or the platelets positive for fibrinogen binding, respectively.

Analysis of Plasma Samples

Coagulation Activation. Prothrombin fragment 1 + 2 and thrombin-anti-thrombin complex (TAT) were measured by enzyme-linked immunosorbent assay (ELISA; Dade Behring, Marburg, Germany). Factors VII and VIII, prothrombin time, and activated partial thromboplastin time were determined using clotting assays on a Behring Coagulation System with reagents and protocols from the manufacturer (Dade Behring). Plasma concentrations of factor VIIa were de-

Table 2. Effect of Normobaric Normoxia on Markers of Coagulation Activation, Fibrinolysis, Platelet Activation, and Endothelial Cell Activation*

	Median (IQR)		Change, Median (95% CI)
	Before Exposure	After Exposure	
Coagulation Activation			
Thrombin-antithrombin complex, ng/mL	1.9 (1.4 to 2.1)	1.9 (1.5 to 2.2)	0 (-0.10 to 0.10)
Prothrombin fragment 1 + 2, nmol/L	0.6 (0.4 to 0.6)	0.5 (0.4 to 0.6)	-0.05 (-0.07 to -0.03)†
Factor VIIa, mU/mL	76.0 (53.0 to 105.5)	78.0 (64.3 to 106.3)	5.00 (2.00 to 12.00)‡
Factor VIIc, %	107.0 (87.0 to 129.0)	110.0 (89.3 to 126.5)	2.00 (-1.00 to 7.00)
Factor VIIIc, %	92.0 (80.0 to 110.0)	100.0 (85.0 to 120.8)	2.00 (0 to 6.00)‡
Tissue factor pathway inhibitor, %	78.0 (66.0 to 95.0)	78.0 (65.0 to 95.0)	1.00 (-2.00 to 3.00)
Endogenous thrombin potential, %	99.5 (90.0 to 113.3)	98.0 (90.8 to 116.3)	1.00 (-1.00 to 3.00)
Activated protein C sensitivity ratio	0.8 (0.6 to 1.3)	0.7 (0.4 to 1.2)	-0.12 (-0.15 to -0.04)†
Prothrombin time, s	12.2 (11.6 to 12.8)	11.6 (11.2 to 12.1)	-0.30 (-0.40 to -0.30)†
Activated partial thromboplastin time, s	35.6 (33.0 to 38.6)	34.4 (31.8 to 37.8)	-0.95 (-1.40 to -0.50)†
Fibrinolysis			
Tissue plasminogen activator, ng/mL	6.7 (3.8 to 11.0)	5.2 (3.3 to 8.3)	-1.23 (-1.99 to -0.71)†
Plasminogen activator inhibitor type-1, ng/mL	15.9 (8.4 to 28.0)	4.0 (2.6 to 9.0)	-10.47 (-13.00 to -7.04)†
Plasmin-antiplasmin complexes, ng/mL	122.5 (60.0 to 204.5)	102.5 (40.0 to 148.3)	0 (0 to 5.00)
D-dimer, ng/mL	175.3 (104.9 to 250.7)	160.1 (105.2 to 233.5)	-5.52 (-13.54 to 0.66)
Platelet Activation			
β -Thromboglobulin, IU/mL	15.6 (13.0 to 21.0)	15.2 (11.9 to 19.9)	-0.83 (-1.79 to -0.14)‡
Soluble P-selectin, ng/mL	27.7 (22.8 to 34.9)	26.1 (22.1 to 32.8)	-0.63 (-2.49 to 0.94)
Monocyte-platelet aggregates, %	10.8 (9.1 to 13.7)	11.2 (9.9 to 13.6)	0.73 (0.20 to 1.20)§
Platelet fibrinogen binding, % positive			
With adenosine diphosphate	71.0 (60.0 to 78.5)	69.4 (61.1 to 78.3)	-0.75 (-1.75 to 1.30)
With thrombin receptor agonist peptide	2.9 (2.2 to 4.6)	3.3 (2.3 to 6.0)	0.21 (-0.11 to 0.62)
Endothelial Cell Activation			
Soluble E-selectin, ng/mL	31.4 (19.5 to 38.2)	31.4 (18.9 to 37.7)	-0.75 (-1.30 to 0.10)
von Willebrand factor, %	89.0 (69.0 to 107.0)	89.0 (67.5 to 107.0)	0 (-2.00 to 2.00)
Soluble thrombomodulin, ng/mL	34.7 (27.7 to 46.4)	33.4 (25.2 to 45.3)	-1.87 (-2.86 to 0)‡

Abbreviations: CI, confidence interval; IQR, interquartile range.

*Combined data from all participants.

†Significant at .001 level.

‡Significant at .01 level.

§Significant at .05 level.

terminated on the Behring Coagulation System by the StaClot VIIa-rTF method (Diagnostica Stago, Asnieres-sur-Seine, France). Tissue factor pathway inhibitor activity was measured on the Behring Coagulation System using a method described by Sandset et al.¹² Endogenous thrombin potential and the endogenous thrombin potential–based activated protein C sensitivity ratio were determined as described by Rosing et al.¹³

Fibrinolysis. D-dimer was measured using the VIDAS D-dimer new (DD2) immunoassay and miniVIDAS analyzer (bioMérieux, Marcy l'Etoile, France). In-house ELISAs were used to measure tissue plasminogen activator¹⁴ and plasminogen activator in-

hibitor type-1.¹⁵ Plasmin-antiplasmin complexes were measured by ELISA from Technoclone (Vienna, Austria).

Platelet Activation. Soluble P-selectin (R&D Systems, Oxford, England) and β -thromboglobulin (Roche Diagnostics, Lewes, England) were measured by ELISA as markers of in vivo platelet activation.

Endothelial Activation. ELISAs were used to measure soluble E-selectin (R&D Systems Ltd), von Willebrand factor (antibodies from Dako, Glostrup, Denmark), and soluble thrombomodulin (Diagnostica Stago).

All plasma assays were performed in duplicate after completion of the study by laboratory personnel who were unaware of the participants' identities or

the type of exposure (normobaric or hypobaric). For each marker, all samples were analyzed in the same laboratory using assays and reagents from a single batch.

Statistical Analysis

The sample size was based on published data.⁹ Assuming an SD of 4 ng/mL for TAT, 0.2 nmol/L for prothrombin fragment 1 + 2, and 80 ng/mL for D-dimer, a study with 70 individuals would have 80% power to detect minimum differences of 1.5 ng/mL, 0.1 nmol/L, and 30 ng/mL, respectively, in the mean difference of change from baseline between the normobaric and hypobaric exposures at the .01 level of statistical significance.

Table 3. Effect of Hypobaric Hypoxia on Markers of Coagulation Activation, Fibrinolysis, Platelet Activation, and Endothelial Cell Activation*

	Median (IQR)		Change, Median (95% CI)
	Before Exposure	After Exposure	
Coagulation Activation			
Thrombin-antithrombin complex, ng/mL	1.8 (1.5 to 2.2)	2.0 (1.5 to 2.5)	0.20 (0 to 0.30)†
Prothrombin fragment 1 + 2, nmol/L	0.5 (0.4 to 0.7)	0.5 (0.4 to 0.6)	−0.05 (−0.08 to −0.03)‡
Factor VIIa, mU/mL	74.5 (57.0 to 98.8)	81.5 (61.0 to 114.8)	5.00 (0 to 12.00)†
Factor VIIc, %	108.5 (82.3 to 126.5)	107.0 (83.5 to 127.3)	2.00 (−2.00 to 5.00)
Factor VIIIc, %	96.5 (76.3 to 112.5)	98.0 (80.0 to 116.5)	3.50 (0 to 6.00)§
Tissue factor pathway inhibitor, %	80.0 (67.3 to 97.8)	79.5 (70.0 to 99.3)	2.00 (0 to 5.00)†
Endogenous thrombin potential, %	102.0 (91.0 to 116.0)	102.0 (90.0 to 114.3)	0 (−2.00 to 2.00)
Activated protein C sensitivity ratio	0.8 (0.6 to 1.6)	0.7 (0.5 to 1.4)	−0.11 (−0.19 to −0.01)‡
Prothrombin time, s	12.2 (11.5 to 12.7)	11.7 (11.1 to 12.3)	−0.40 (−0.50 to −0.30)‡
Activated partial thromboplastin time, s	35.9 (32.3 to 38.4)	34.9 (31.7 to 38.2)	−0.80 (−1.40 to −0.30)‡
Fibrinolysis			
Tissue plasminogen activator, ng/mL	7.0 (4.1 to 10.0)	6.1 (4.0 to 8.0)	−1.00 (−1.72 to −0.36)§
Plasminogen activator inhibitor type-1, ng/mL	13.9 (7.0 to 26.1)	3.9 (2.0 to 6.7)	−9.77 (−12.98 to −8.58)‡
Plasmin-antiplasmin complexes, ng/mL	110.0 (60.0 to 150.0)	106.0 (80.0 to 161.0)	0 (−12.50 to 7.50)
D-dimer, ng/mL	151.8 (107.8 to 239.7)	147.8 (110.1 to 217.7)	−8.64 (−14.42 to −0.89)†
Platelet Activation			
β -Thromboglobulin, IU/mL	16.5 (13.4 to 21.1)	16.5 (12.8 to 21.3)	−0.48 (−1.26 to 0.90)
Soluble P-selectin, ng/mL	29.8 (22.7 to 34.3)	28.6 (22.3 to 35.4)	−0.43 (−1.52 to 0.54)
Monocyte-platelet aggregates, %	11.0 (8.9 to 13.7)	12.3 (9.7 to 14.4)	0.95 (0.28 to 1.30)‡
Platelet fibrinogen binding, % positive			
With adenosine diphosphate	71.9 (63.3 to 79.5)	72.6 (64.4 to 77.5)	−1.10 (−3.65 to 2.55)
With thrombin receptor agonist peptide	3.7 (2.5 to 5.0)	3.8 (2.8 to 5.2)	0.34 (−0.20 to 0.72)
Endothelial Cell Activation			
Soluble E-selectin, ng/mL	31.2 (20.0 to 40.0)	33.8 (19.9 to 43.7)	1.14 (−0.53 to 2.45)
von Willebrand factor, %	83.0 (71.0 to 103.0)	83.5 (69.0 to 109.8)	0 (−2.00 to 1.00)
Soluble thrombomodulin, ng/mL	37.1 (29.3 to 47.1)	34.3 (26.5 to 45.7)	−2.78 (−4.63 to −1.60)‡

Abbreviations: CI, confidence interval; IQR, interquartile range.

*Combined data from all participants.

†Significant at .05 level.

‡Significant at .001 level.

§Significant at .01 level.

The analysis of primary outcomes was based on the difference in the change from baseline between normobaric and hypobaric; the Wilcoxon signed rank test was used. Absolute change from baseline was used as the primary effect measure because correlation between baseline and postexposure values was generally considerable ($r > 0.50$) and the use of absolute change (rather than percentage change) has been shown to be more powerful in such situations.¹⁶ Exposure-period interaction was assessed by comparing the median response (across both the normobaric and hypobaric exposures) between the 2 allocation sequences (ie, normobaric normoxia followed by hypobaric

hypoxia; hypobaric hypoxia followed by normobaric normoxia), using a Mann-Whitney test.¹⁷ As a sensitivity test, a first period analysis was also performed.¹⁷ All analyses were performed using Stata software version 8.2 (Stata-Corp, College Station, Tex). *P* values were interpreted conservatively due to multiple comparisons, for which no adjustment was made.

RESULTS

Seventy-three individuals participated in the study. Demographic and clinical characteristics are shown in Table 1. All individuals completed the study but blood samples were unavailable for 1 individual after the normobaric expo-

sure and for 1 individual before and after the hypobaric exposure. Both of these individuals were in group 3. In some instances, technical problems resulted in missing data for 1 or more assays or time points. However, data were available for all assays on the primary outcome (ie, the difference in change between the 2 conditions) for at least 63 individuals. No clinically evident venous thromboembolism was reported during or within 1 week after the exposures.

Arterial Oxygen Saturation

Arterial oxygen saturation, expressed as the mean of the hourly measurements, was lower in all groups during the hypobaric exposure than during the normobaric exposure (Table 1). The mean differences between the normobaric and hypobaric exposures were -3.2% (95% confidence interval [CI], -2.8% to -3.6%) for group 1; -3.6% (95% CI, -2.7% to -4.5%) for group 2; and -5.1% (95% CI, -4.5% to -5.8%) for group 3 ($P < .001$ for each comparison).

Coagulation Activation and Fibrinolysis

During the normobaric exposure, which provided a control for the combined effects of prolonged sitting and circadian variation, there were significant changes in several markers of coagulation activation and fibrinolysis. There was, however, no significant difference in the overall change for any marker between the normobaric and the hypobaric exposures in either the individual groups (FIGURE 1) or the combined data (TABLE 2, TABLE 3, and TABLE 4). Supplementary tables containing the data for each group are available online at <http://www.le.ac.uk/cv/wrightsuppldata.pdf>.

Two individuals in group 1 had elevated levels of TAT (>10 ng/mL) before the hypobaric exposure (18.9 ng/mL and 11.8 ng/mL). These may be attributable to artifact, although no problem was reported with venepuncture in either case. The data from these individuals were included in the primary analysis but a sensitivity analysis excluding them

Table 4. Difference in Change of Markers of Coagulation Activation, Fibrinolysis, Platelet Activation, and Endothelial Cell Activation Between the Hypobaric and the Normobaric Exposures

	No. of Individuals*	Difference in Change Between HH and NN, Median (95% CI)	<i>P</i> Value
Coagulation Activation			
Thrombin-antithrombin complex, ng/mL	69	0 (−0.30 to 0.30)	.62
Prothrombin fragment 1 + 2, nmol/L	69	−0.02 (−0.03 to 0.01)	.38
Factor VIIa, mU/mL	71	0 (−8.00 to 8.00)	.97
Factor VIIc, %	68	−3.50 (−10.00 to 4.00)	.17
Factor VIIIc, %	69	1.00 (−3.00 to 4.00)	.58
Tissue factor pathway inhibitor, %	68	2.00 (−2.00 to 4.00)	.19
Endogenous thrombin potential, %	66	−2.00 (−4.00 to 1.00)	.37
Activated protein C sensitivity ratio	65	−0.01 (−0.90 to 0.10)	.69
Prothrombin time, s	69	0 (−0.20 to 0.20)	.38
Activated partial thromboplastin time, s	69	0.30 (−0.40 to 0.80)	.35
Fibrinolysis			
Tissue plasminogen activator, ng/mL	63	0.34 (−0.39 to 1.24)	.18
Plasminogen activator inhibitor type-1, ng/mL	68	−0.12 (−2.91 to 2.59)	.96
Plasmin-antiplasmin complexes, ng/mL	68	5.00 (0 to 25.00)	.22
D-dimer, ng/mL	68	1.38 (−3.63 to 9.72)	.55
Platelet Activation			
β-Thromboglobulin, IU/mL	68	−0.08 (−1.36 to 2.03)	.86
Soluble P-selectin, ng/mL	71	−0.54 (−1.71 to 2.18)	.76
Monocyte-platelet aggregates, %	65	0 (−0.60 to 0.75)	.47
Platelet fibrinogen binding, % positive			
With adenosine diphosphate	66	−0.25 (−3.30 to 3.05)	.83
With thrombin receptor agonist peptide	64	−0.10 (−0.80 to 0.70)	.58
Endothelial Cell Activation			
Soluble E-selectin, ng/mL	68	1.87 (0.76 to 3.69)	.01
von Willebrand factor, %	69	1.00 (−4.00 to 2.00)	.95
Soluble thrombomodulin, ng/mL	64	−0.80 (−3.60 to 1.90)	.45

Abbreviations: CI, confidence interval; HH, hypobaric hypoxia; NN, normobaric normoxia.

*Number of individuals for whom data were available before and after both the normobaric and hypobaric exposures.

showed little effect on the median difference in change from baseline between the hypobaric and normobaric exposures for either the pooled results (0.10 ng/mL [95% CI, -0.20 to 0.30 ng/mL]; $P=.35$) or those from group 1 to which they belonged (0 ng/mL [95% CI, -0.30 to 0.30 ng/mL]; $P=.76$).

In the absence of an overall effect of hypoxia, an exploratory analysis was performed to assess whether some individuals might be hyperresponsive. The number of individuals for whom the absolute change in TAT, prothrombin fragment 1 + 2, and D-dimer exceeded a threshold (defined as the 95th percentile in the normobaric exposure) was compared for the hypobaric and the normobaric exposures. For each marker, the number of high responders was greater in hypobaria than in normobaria (TAT, 7 vs 3 high responders; prothrombin fragment 1 + 2, 7 vs 3 high responders; D-dimer, 8 vs 3 high responders; respectively). However, comparing the response of each individual to hypobaria and to normobaria, these differences were less apparent. FIGURE 2 shows the difference between the responses in hypobaria and in normobaria for each participant, plotted in rank order. Comparing the tails of the distribution for each marker, the number of high responders in hypobaria (defined as a difference in change exceeding the 95th percentile) was not significantly different from the number of high responders in normobaria (TAT, 4 vs 5 high responders; prothrombin fragment 1 + 2, 4 vs 3 high responders; D-dimer, 4 vs 2 high responders; respectively) using the same cut-off values (TAT, 2.32 ng/mL; prothrombin fragment 1 + 2, 0.26 nmol/L; D-dimer, 90 ng/mL). Neither was there any significant difference between the magnitude of hyperresponsiveness in the outliers at either end (difference between medians for those above the 95th percentile and below the 5th percentile for TAT, -2.6 ng/mL [95% CI, -11.9 to 14.3 ng/mL]; prothrombin fragment 1 + 2, 0.55 nmol/L [95% CI, -0.05 to 0.66 nmol/L]; and D-dimer, 277 ng/mL [95% CI, -42 to 445 ng/mL]). The findings were simi-

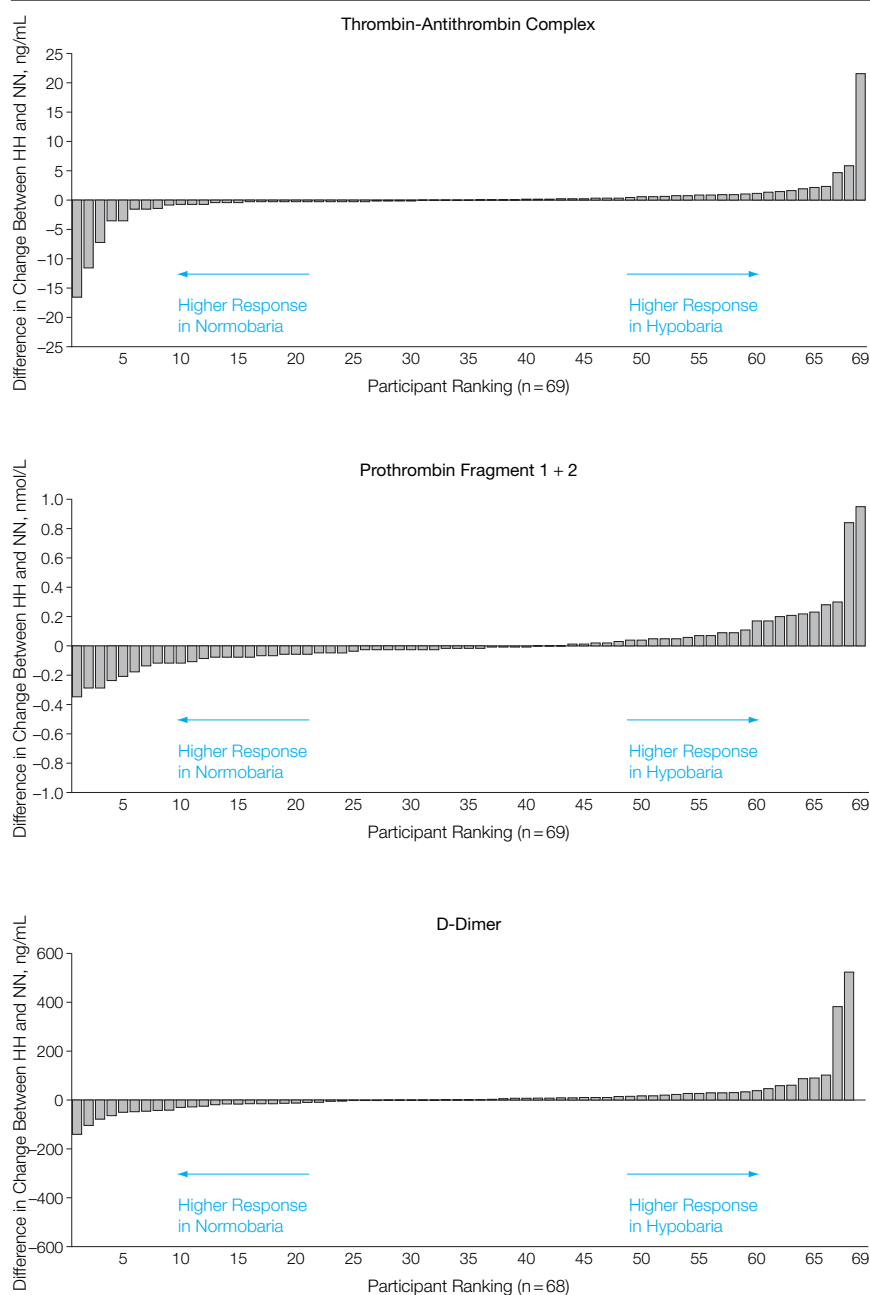
lar using the 90th and 10th percentiles and when the 2 participants with high baseline TAT were excluded.

Platelet and Endothelial Activation

During the normobaric exposure, there was a small increase in monocyte-

platelet aggregates. There was, however, no significant difference between the responses to the normobaric and hypobaric exposures. There was no other evidence of platelet activation or responsiveness, or endothelial activation (Table 2, Table 3, and Table 4 and

Figure 2. Differences in Change Between the Hypobaric and the Normobaric Exposures in Thrombin-Antithrombin Complex, Prothrombin Fragment 1 + 2, and D-Dimer for Each Individual, in Ascending Rank Order for Each Marker



HH indicates hypobaric hypoxia; NN, normobaric normoxia.

FIGURE 3 and FIGURE 4), apart from a small increase in soluble E-selectin after the hypobaric exposure. This contrasted with a small decrease after the normobaric exposure, the median difference in change in the pooled data was 1.87 ng/mL (95% CI, 0.76-3.69 ng/mL; $P=.01$). A similar pattern was seen in all subgroups but the median difference in change was greatest in group 3

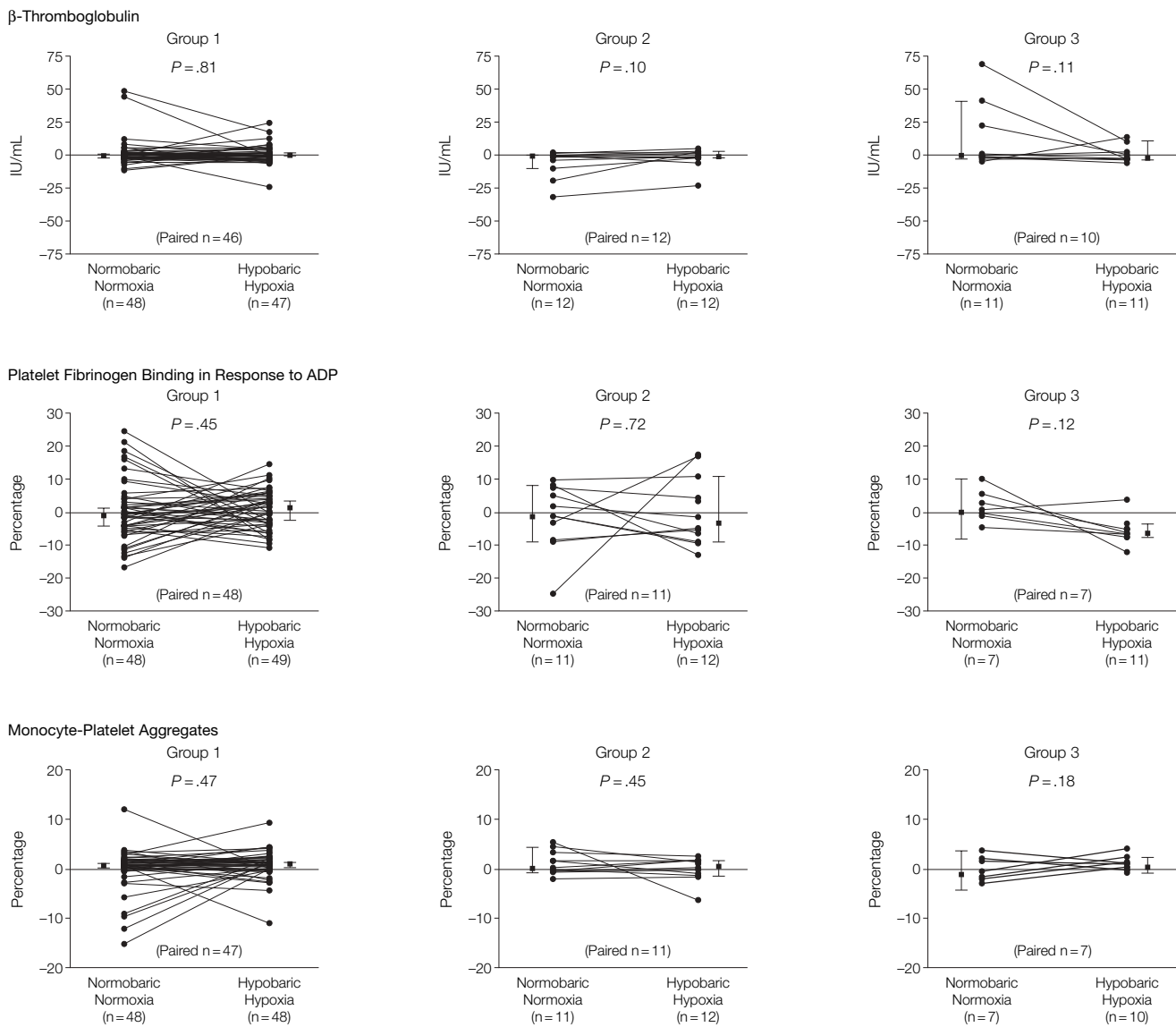
(3.93 ng/mL [95% CI, 1.70-7.68 ng/mL; $P=.02$]; see Figure 4 and the supplementary tables at <http://www.le.ac.uk/cv/wrightsuppldata.pdf>).

Blood Cell Count and Hematocrit

Red blood cell count, white blood cell count, platelet count, and hematocrit increased after both the normobaric and hypobaric exposures in all groups but

the combined data showed no significant differences between normobaric normoxia and hypobaric hypoxia. The most marked changes were in white blood cell count, which increased by 14.9% of the preexposure value after the normobaric exposure and 8.9% after the hypobaric exposure. The corresponding figures for platelet count were 7.5% and 6.6%. The increases in red blood

Figure 3. Paired Changes in β -Thromboglobulin, Platelet Fibrinogen Binding in Response to Adenosine Diphosphate (ADP), and Monocyte-Platelet Aggregates After the Normobaric and Hypobaric Exposures



Unpaired data are included for individuals in whom data were only available for 1 of the 2 conditions. Squares indicate medians (95% confidence intervals) for each group.

cell count and hematocrit were all less than 2%.

Exposure-Period Interaction

None of the tests for an exposure-period effect was statistically significant at the .01 level. The first period analysis also was concordant with the overall results. For all analytes, the median difference in change from baseline between the hypobaric and normobaric exposures was not statistically significant at the .01 level.

COMMENT

In this large controlled study with measurement of a wide range of markers of coagulation activation, fibrinolysis, platelet activation, and endothelial cell activation, we found no procoagulant changes attributable to hypobaric hypoxia. Although changes in some parameters were observed after sitting for 8 hours in a hypobaric and hypoxic environment, there were no significant differences compared with the changes observed after sitting for the same period

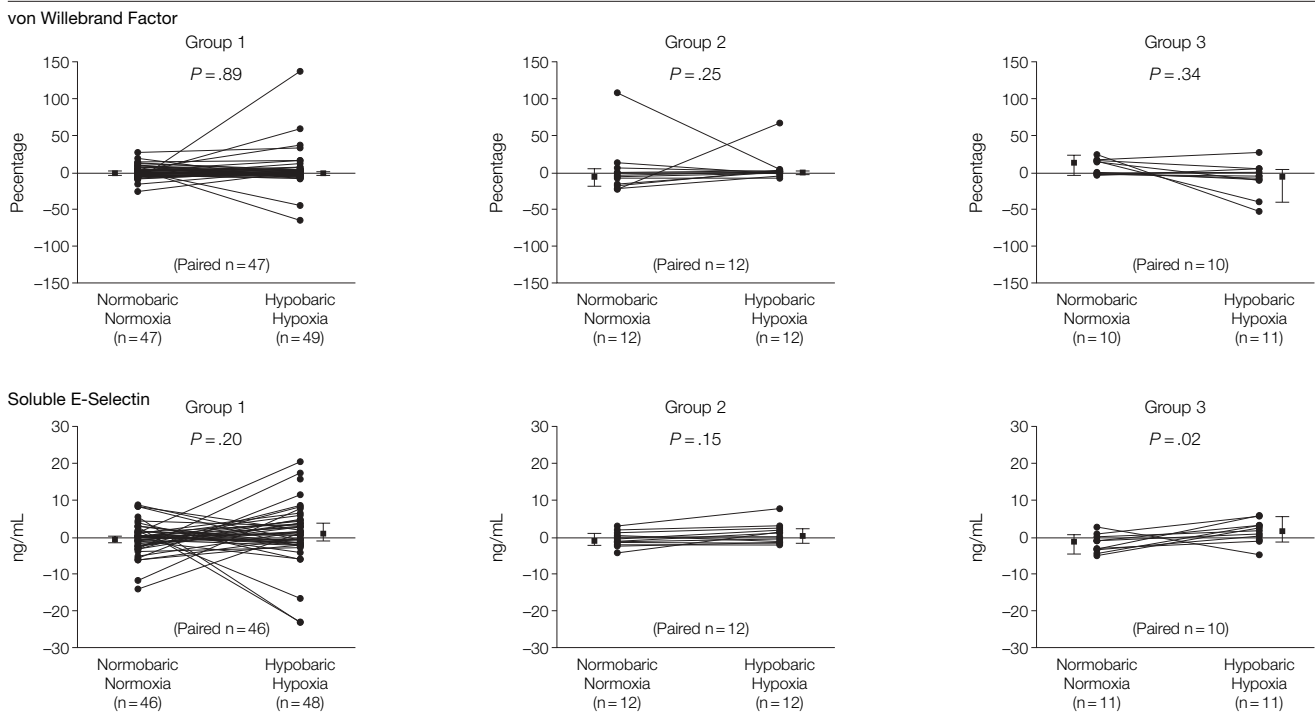
in a normobaric and normoxic environment, apart from the small increase in soluble E-selectin, which was discordant with other markers of endothelial activation and most likely due to chance.

The changes observed during both the hypobaric and normobaric exposures most likely reflect the combined effects of prolonged sitting and circadian variation in clotting parameters, which is thought to account for a relatively hypercoagulable state with decreased fibrinolysis during the morning.¹⁸ The observed decreases in prothrombin fragment 1 + 2, plasminogen activator inhibitor type-1, and D-dimer are consistent with known diurnal changes.^{18,19} However, the increase in factor VIIa and factor VIIIc, and the decrease in tissue plasminogen activator, prothrombin time, and activated partial thromboplastin time would not be expected from the usual circadian fluctuation^{20,21} and were presumably due to prolonged sitting. This also may explain the small increase in monocyte-platelet aggregates, which is at vari-

ance with the reported diurnal pattern of platelet activation,²² and the decrease in soluble thrombomodulin, which is not believed to show significant diurnal variation.²³ The relevance of these minor and disparate changes after prolonged sitting in either normobaric normoxia or hypobaric hypoxia is uncertain but it is noteworthy that there was no significant change in endogenous thrombin potential, a global marker of coagulation activation.

The absence of an overall effect of hypobaric hypoxia on coagulation activation in our study contrasts with the findings of Bendz et al,⁹ who reported an increase in TAT, prothrombin fragment 1 + 2, and factor VIIa, and a decrease in factor VII antigen and tissue factor pathway inhibitor, in a group of 20 healthy men exposed for 8 hours to hypobaric hypoxia equivalent to an altitude of 2400 m. In that study, the individuals' activity was not restricted during the exposure. The study has been criticized because there was no

Figure 4. Paired Changes in von Willebrand Factor and Soluble E-Selectin After the Normobaric and Hypobaric Exposures



Unpaired data are included for individuals in whom data were only available for 1 of the 2 conditions. Squares indicate medians (95% confidence intervals) for each group.

normobaric control and baseline levels of TAT and prothrombin fragment 1 + 2 were inexplicably high, raising the possibility of artifact associated with blood sampling or processing.²⁴ The changes in TAT and prothrombin fragment 1 + 2 were also transient, with peak levels after 2 hours, prompting the investigators to suggest that the rapid transition to hypobaria might account for their findings.²⁵ We chose not to perform interim blood sampling to avoid the risk of coagulation activation by repeated venepuncture. Although we might have missed transient changes, the study by Bendz et al²⁵ did show persistent albeit submaximal elevation of TAT and prothrombin fragment 1 + 2 at 8 hours, which was not found in our larger study.

Three subsequent studies found no overall effect of normobaric hypoxia. Hodkinson et al²⁶ studied 6 healthy men who breathed a hypoxic gas mixture (equivalent to air at 3660 m) or dry air while seated for 3 hours. Arterial oxygen saturations during the hypoxic exposure were 82% to 91%. Despite this, there was no significant effect on a range of markers of hemostatic and endothelial function. Crosby et al²⁷ exposed 8 healthy individuals alternately to normobaric hypoxia (equivalent to breathing air at 3600 m) and air while seated in a small chamber for 8 hours. They found no significant effect of hypoxia on TAT, prothrombin fragment 1 + 2, factor VIIa, or D-dimer. Dick et al²⁸ compared the effects of hypoxia, achieved by breathing 16% oxygen, and normoxia in 6 volunteers and also found no significant effect of hypoxia on TAT, prothrombin fragment 1 + 2, or D-dimer at 3 hours.

We are aware of only 2 previous controlled studies of hypobaric hypoxia. A group from Japan reported no effect on activated partial thromboplastin time, thrombomodulin, von Willebrand factor, platelet factor 4, or β -thromboglobulin in 8 volunteers after a 1-hour exposure to hypobaric hypoxia (0.7 atm) compared with a normobaric control.²⁹ Similar results were subsequently reported after a 6-hour study.³⁰

Although we found no overall effect of hypoxia on hemostasis, it is possible that some individuals might respond differently. Our exploratory analysis did show greater dispersion with more outliers in the distribution of changes in coagulation activation in hypobaria than in normobaria. While this might reflect a true biological diversity of response, the similar frequency and magnitude of relative hyperresponsiveness in hypobaria and in normobaria suggest that it may be due to chance.

It is also possible that more profound hypoxemia resulting from cardiopulmonary disease or genetic factors might have some effect, although it is noteworthy that Hodkinson et al²⁶ observed no coagulation activation at arterial oxygen saturations as low as 82%. Finally, while we found no evident interaction in those at modestly increased risk due to oral contraceptive pill use or older age, these subgroups were relatively small and we cannot exclude the possibility that genetically determined thrombophilia or other risk factors for thrombosis might interact with hypoxia in individuals at higher risk. In a recently reported study,³¹ which in contrast to our study included a high proportion of individuals who were oral contraceptive pill users, carriers of the factor V Leiden mutation, or both, median TAT levels were higher after an 8-hour flight in a Boeing 757 airplane than after similar periods while watching movies or performing usual daily activities. There was also a greater prevalence of high responders after the flight, particularly among those who were both oral contraceptive pill users and carriers of the factor V Leiden mutation, raising the possibility that preexisting risk factors might interact with some flight-related factor other than immobility. While our data do not support a causative role for hypobaric hypoxia in that regard, neither do they rule it out.

In conclusion, our findings do not support the hypothesis that hypobaric hypoxia of the degree that might be encountered during long-haul air travel

is associated with prothrombotic alterations in the hemostatic system in healthy individuals at low risk of venous thromboembolism.

Author Contributions: Drs Toff and Abrams had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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Drafting of the manuscript: Toff, Jones, Abrams.

Critical revision of the manuscript for important intellectual content: Ford, Pearce, Watson, Watt, Ross, Gradwell, Batchelor, Meijers, Goodall, Greaves.

Statistical analysis: Toff, Jones, Abrams, Goodall.

Obtained funding: Toff, Goodall, Greaves.

Administrative, technical, or material support: Toff, Jones, Ford, Pearce, Watson, Watt, Ross, Gradwell, Batchelor, Meijers, Goodall, Greaves.

Study supervision: Toff, Ford, Watt, Abrams, Meijers, Goodall, Greaves.

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The question of whether surveillance reduces bladder cancer mortality is important but outside the scope of our study. The answer to this question can only be resolved with a very large study and long-term follow-up. Nevertheless, the finding that a T2 tumor was initially missed, then detected on short-term follow-up, suggests that vigilant surveillance will improve the survival of some patients. Neither cytology nor the NMP22 test are perfect. False-negative and false-positive tests do occur. Both tests can be used as an adjunct to cystoscopy to increase the detection of bladder cancer.

Cytology is highly user-dependent, has low sensitivity, and is moderately expensive. In response to Drs Eggener and Herr, most published sensitivity rates for voided cytology are from single sites. Our study included 23 clinical sites across the United States, with urine cytology analyzed according to standard practice at the participating institutions. The sensitivity in this study reflects the variability and subjectivity of cytology. The NMP22 test is objective, point-of-care, less expensive, and has higher sensitivity but slightly lower specificity. All tests vary in performance. Using urine-based markers effectively requires knowing their test characteristics and defining the type of information to be obtained from them.^{3,4}

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CORRECTIONS

Incorrect Data Order: In the Original Contribution entitled "Long-term Efficacy of Bivalirudin and Provisional Glycoprotein IIb/IIIa Blockade vs Heparin and Planned Glycoprotein IIb/IIIa Blockade During Percutaneous Coronary Revascularization: REPLACE-2 Randomized Trial" published in the August 11, 2004, issue of *JAMA* (2004;292:696-703), the data were presented out of order. In the sentence, in the third column on page 699, that read "Among patients at high risk, however, death occurred significantly less frequently with bivalirudin than with heparin plus Gp IIb/IIIa blockade (6.0% vs 3.9%, respectively, $P=.047$)," the parenthetical data should have been presented in reverse order to read "(3.9% vs 6.0%, respectively, $P=.047$)."

Incorrect Median Difference: In the Original Contribution entitled "Effect of Hypobaric Hypoxia, Simulating Conditions During Long-Haul Air Travel, on Coagulation, Fibrinolysis, Platelet Function, and Endothelial Activation" published in the May 17, 2006, issue of *JAMA* (2006;295:2251-2261), the following median difference should be corrected. On page 2251, in the abstract under "Results," line 6, the correct median difference in change between the hypobaric and normobaric exposure is " -0.02 nmol/L" for prothrombin fragment 1 + 2. This correction was made previously to the online version of this article.

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