

IS MILD NORMOBARIC HYPOXIA A RISK FACTOR
FOR DEEP VEIN THROMBOSIS?

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Abstract

Modern air travel entails a cabin altitude between 1520-2440m (5000-8000ft) and thus exposure to mild hypoxia. There is debate as to whether hypoxia is causally related to deep vein thrombosis (DVT) occurring during or after travel. One study suggested that a short period of hypobaric hypoxia causes activation of coagulation.

The aim of this study was to test the hypothesis that hypoxia alone (normobaric hypoxia) causes activation of coagulation, possibly through endothelial cell activation.

Method: 6 healthy male volunteers were exposed for three hours, whilst seated, on two separate occasions to i) dry air (control) and ii) hypoxic gas mixture (12.8% O₂ in N₂, equivalent to breathing air at 3660m (12000ft)).

Results: There were no differences in haemostatic or endothelial markers between control and hypoxic groups, but platelet and leucocyte counts increased and were significantly higher in the hypoxic group. There were increases in fibrinogen and von Willebrand factor, as well as rheological changes, but these were not significantly different between control and hypoxic groups.

Conclusion: The results of this small study do not support the previous suggestion that hypoxia causes activation of coagulation and suggests that immobility-induced rheological changes may be more significant in the aetiology of DVT occurring during or after travel.

Introduction

It has been suggested that prolonged travel (>3-5 hours duration) is associated with an increased risk of venous thromboembolism (VTE), a clinical spectrum including deep-vein thrombosis (DVT) and/or pulmonary embolism (PE) (1, 7). However there is some debate regarding this subject (10).

A recent UK government report by the House of Lords Select Committee on Science and Technology found a paucity of data on the occurrence of VTE related to prolonged travel and concluded that although there may be an increased risk of VTE associated with prolonged travel, such a risk has yet to be proven (8).

Venous stasis in the legs due to prolonged sitting is likely to be the key to any such association (13) – one of three factors key to the pathophysiology of DVT described as Virchow's triad: i) reduced blood flow (venous stasis); ii) increased coagulability of blood; and iii) damage or abnormality of blood vessel wall (14, cited in 12).

However, in addition to the immobility of long-haul travel in general it has been suggested that factors specific to air travel (reduced air pressure, hypoxia, low humidity) may further increase any risk of VTE (4, 5).

Studies examining the relationship between hypobaric hypoxia and blood coagulability have yielded conflicting results (2, 3). This may be due to differences in experimental time course, altitude investigated, or other features of the methodology. The chamber study by Bendz et al found that mild hypobaric hypoxia induced a transient activation of coagulation (indicated by raised concentration of thrombin-antithrombin complex and prothrombin fragments 1 and 2) peaking 2 hours into the hypoxic exposure and returning to normal by

24 hours (3), but the study did not include a control group. To resolve this, a controlled study was designed to investigate the effects of hypoxia alone on haemostatic, endothelial and haematological parameters in healthy individuals.

Methods

Local Ethical Committee approval was obtained for six healthy male volunteers, with no risk factors for venous thromboembolism, to take part (age range 22-32). They breathed either dry air (control) or a dry hypoxic gas mixture for three hours, during which time they were asked to remain seated and immobile. They attended on two separate occasions, separated by one week, with each experiment starting between 9.00am and 10.00am, and run order was randomised. The subjects wore a well-fitting silicone, oronasal mask with the breathing gas supplied through a demand regulator from a compressed gas cylinder (BOC Ltd, Guildford, UK). These supplied either dry air or the dry hypoxic gas mixture (12.8% O₂ in N₂, equivalent to breathing air at 3660m altitude, an exaggerated degree of hypoxia relative to the maximum cabin altitude in passenger carrying aircraft) for the control or experimental runs respectively. Validation of this method was confirmed by half hourly pulse oximetry, which demonstrated appropriate hypoxia (saturation 82-91%). Blood was taken using uncuffed, flawless venepuncture. Blood samples were taken before, immediately after and 24 hours after each run, centrifuged at 2,000g and stored at -80°C. The scientist performing all the assays was blinded to the experimental conditions. Full blood counts and plasma viscosity were performed using standard methods. Endothelial markers were measured using ELISA. These were e-selectin, soluble-ICAM, soluble-VCAM (all R&D Systems Europe Ltd., Abingdon, UK), Plasminogen activator inhibitor-1 (PAI-1) (Bio-Stat Diagnostics Ltd., Stockport, UK), soluble-Tissue Factor (Axis-Shield Diagnostics Ltd., Dundee, UK), von Willebrand Factor (in house) and vascular endothelial growth factor (VEGF) (R&D Systems Europe LTD., Abingdon, UK).

Haemostatic markers assayed were Prothrombin Fragment 1+2 (PF1+2) (Dade-Behring Ltd., Milton Keynes, UK), p-selectin (R&D Systems Europe Ltd., Abingdon, UK) and D-dimer (Dade-Behring Ltd., Milton Keynes, UK) (all ELISA). Fibrinogen concentration was measured using the IL Test TM Fibrinogen-C clotting assay (Instrumentation Laboratory (UK) Ltd., Warrington, UK). Statistical analysis was performed using the Mann-Whitney U-test.

Results

Results are summarised in Tables 1 and 2. Pre-control values did not differ significantly from pre-hypoxic values for any variable and, with the exception of sP-Selectin concentration, all pre-run values were within expected normal physiological ranges.

Table 1	Control Runs			Hypoxic Runs		
	Pre-run	Immed. after	24hr after	Pre-run	Immed. after	24hr after
<i>Haemostatic markers</i>	Value	% of pre	% of pre	Value	% of pre	% of pre
Fibrinogen (g/l)	2.6(0.16)	*112(5.6)	110(5.8)	2.5(0.22)	*105(2.3)	99(4.2)
Prothrombin fragments 1+2 (nmol/l)	0.65(0.07)	*87(7.1)	96(6.0)	0.66(0.05)	90(5.8)	95(3.4)
Soluble P-Selectin (ng/ml)	205(11)	**62(2.8)	**56(2.6)	197(9.6)	**66(5.2)	**54(3.9)
<i>Endothelial markers</i>						
Soluble E-Selectin (ng/ml)	47(8.9)	97(2.2)	**92(2.5)	44(10.8)	101(1.5)	**94(2.1)
Soluble ICAM (ng/ml)	217(13.4)	100(2.7)	97(2.4)	22(14.0)	**104(0.5)	98(1.78)
Plasminogen activator inhibitor-1 (ng/ml)	20(7.1)	**65(4.9)	*83(27.0)	15(1.0)	**62(3.7)	**62(5.2)
Vascular endothelial growth factor (pg/ml)	82(19)	107(15)	150(28)	124(34)	96(22)	86(23)
Tissue Factor (pg/ml)	168(25)	**94(1.6)	99(3.3)	187(47.2)	94(6.0)	90(10)
von Willebrand Factor (iu/dl)	111(16)	104(4.7)	104(6.9)	105(12.0)	*115(7.9)	104(5.0)

*Table 1: The effect of sitting immobile for 3hr whilst breathing dry air (control) or dry hypoxic gas mixture on haemostatic and ECA markers in venous blood from the arm. Data are presented as mean (+/- S.E.), n=6. Immed.=immediately. Significant (p<0.05) and highly significant (p<0.01) differences from pre-run values indicated by * and ** respectively. There were no significant (p<0.05) difference between control and hypoxic groups.*

There were significant changes in most haemostatic and endothelial markers (see Table 1) with increases in both fibrinogen and vWF in both groups, but there were no significant differences between these changes on comparison of hypoxic and control groups for any marker. There were significant rheological changes with increases in plasma viscosity ($p<0.05$) and red-cell count ($p<0.01$) in both groups immediately after the run with a slight trend for this to be exaggerated in the hypoxic group (see table 2).

Table 2	Control Runs			Hypoxic Runs		
	Pre-run	Immed. after	24hr after	Pre-run	Immed. after	24hr after
	Value	% of pre	% of pre	Value	% of pre	% of pre
Platelet count ($\times 10^9/l$)	194.7(15.6)	100.0(2.8)	109.3(8.3)	189.0(10.8)	**+107.3(2.2)	**103.5(1.8)
Leucocyte count ($\times 10^9/l$)	5.6(0.2)	*111.6(4.8)	107.5(4.8)	5.3(0.1)	**+129.2(4.7)	107.0(9.1)
Neutrophils ($\times 10^9/l$)	3.0(0.2)	*117.5(7.7)	112.1(6.9)	2.8(0.1)	**135.8(9.6)	*105.8(13.7)
Lymphocytes ($\times 10^9/l$)	1.9(0.2)	108.9(4.9)	107.0(3.5)	1.9(0.1)	**124.1(9.6)	108.7(5.4)
Monocytes ($\times 10^9/l$)	0.5(0.0)	101.4(8.2)	93.9(7.2)	0.5(0.0)	*124.7(8.5)	103.3(10.3)
Red-cell count ($\times 10^{12/l}$)	4.86(0.18)	**102.0(0.7)	99.4(1.9)	4.80(0.19)	**103.3(0.9)	98.7(0.8)
Haemoglobin (g/100ml)	14.8(0.4)	**101.9(0.7)	98.9(1.8)	14.7(0.5)	**103.0(0.8)	98.2(0.7)
Plasma viscosity (mPa at 37°C)	1.22(0.01)	*101.7(0.7)	101.0(1.9)	1.21(0.01)	**102.8(0.6)	98.9(0.7)

*Table 2: The effect of sitting immobile for 3hr whilst breathing dry air (control) or a dry hypoxic gas mixture on haematological and rheological variables in venous blood from the arm. Data are presented as mean (+/- S.E.), n=6. Immed.=immediately. Significant ($p<0.05$) and highly significant ($p<0.01$) differences from pre-run values indicated by * and ** respectively. Significant ($p<0.05$) difference between control and hypoxic groups indicated by +.*

Platelet ($p<0.04$) and white cell counts ($p<0.04$) were significantly increased in the hypoxic group immediately post-run compared to the control group, the increase in white cell count was primarily attributed to a neutrophilia ($p<0.04$) (see Table 2).

Discussion

Modern air travel entails a cabin altitude of between 1,520-2,440m and thus exposure to mild hypobaric hypoxia. This small study suggests that three hours of normobaric hypoxia greater than that of a standard aircraft cabin is not associated with activation of coagulation or endothelial activation. Our haemostatic findings are in contrast to the prothrombotic changes described by Bendz et al, who showed increases in coagulation markers, including PF1+2 within two hours of exposure to a lesser degree of hypoxia (76kPa, equivalent to atmospheric pressure at 2,400m) (3). The key differences between the two studies are that our study involved normobaric hypoxia whereas Bendz et al induced hypobaric hypoxia, and the latter study did not include a control group (3). If both sets of findings were reproduced, this would support a novel hypothesis that changes in environmental pressure rather than changes in oxygen partial pressure are responsible for the prothrombotic changes reported by Bendz et al (3).

In vitro hypoxia is known to affect endothelial cell function in a manner that would promote local thrombosis (6). To our knowledge, this is first time that the effect of mild hypoxia on endothelial markers has been studied *in vivo*. It is reassuring that changes in endothelial markers were not detectable with a level of hypoxia associated with modern air travel.

Stasis is a major risk factor for thrombosis (13). In our study, sitting immobile for 3 hours resulted in significant prothrombotic rheological changes and increases in fibrinogen, as well as increases in von Willebrand factor. The increases in leucocytes and platelet count were significantly exaggerated during hypoxia, and such changes are conducive to thrombus formation (11). Similar increases in platelet count have been described in volunteers climbing to 3,600m (9).

Conclusion

Our findings support the idea that seated immobility is likely to be the key to any association between VTE and long-haul travel, but suggest that further studies are warranted to assess: i) whether changes in environmental pressure activate haemostasis; and ii) whether the effects of hypoxia on cellular elements in the blood is related to the VTE risk (if any) associated with air travel.

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