Hematological Change in Venous Blood of the Lower Leg during Prolonged Sitting in a Low Humidity and Hypobaric Environment

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Abstract The present study examined the effects of low humidity and hypobaric conditions on hematological change in venous blood of the lower leg during quiet prolonged sitting. Ten healthy male students participated as the subjects after singing a consent form to participate in this study. Their diet and water intake were controlled from 19:00 on the day before the experiments. The subjects sat for 130 min in a climatic chamber. Four experimental conditions in the chamber were designed from a combination of relative humidity (20% or 60%) and air pressure (sea level or equivalent to an altitude of 2,000 m). Ambient temperature was maintained at 24°C in every condition. Venous blood was sampled from the lower leg before and after exposure to the experimental conditions, and was analyzed for blood viscosity and hematological indices. Also, body weight and leg circumference were measured as indices of total water loss and edema, respectively. Regarding the results of ANOVA, significant interactions between humidity and time were observed in blood viscosity, red blood cell count and hematocrit (each $p<0.05$). However, there were no significant differences in these indices among the conditions. Significant increases were observed in leg circumference ($p<0.01$), platelet count ($p<0.05$) and total protein ($p<0.05$) after the exposure compared with those before the exposure. There were no noticeable effects of hypobaric conditions in every measurement. In conclusion, prolonged sitting seems to be a more hazardous factor for thrombogenesis low humidity and hypobaric conditions during a long-distance flight. J Physiol Anthropol Appl Human Sci 24 (6): 611–615, 2005 http://www.jstage.jst.go.jp/browse/jpa [DOI: 10.2114/jpa.24.611]

Introduction Deep vein thrombosis related to long-distance air travel, and particularly that occurring in the leg with subsequent pulmonary embolism, is also known as economy class syndrome (ECS). According to Virchow’s triad (1868), the risk factors of thrombogenesis are venous stasis, vein wall injury and hypercoagulability of the blood. Prolonged sitting during a flight could bring about venous stasis due to constraint of the vein by the seat and immobility. Additionally, a number of researchers have suggested that the environmental characteristics of an airplane, such as low humidity and hypobaric conditions, could be risk factors leading to ECS (Eklof et al., 1996; Bendz et al., 2000). Since low humidity accelerates evaporation from the skin surface and respiratory pathway, such conditions could promote dehydration and subsequent blood condensation. Hypobaric conditions could also promote evaporation from the body, as water diffusion increases under low pressure conditions. Furthermore, there have been several reports indicating that acute exposure to a hypobaric environment causes an increase in serum erythropoietin concentrations in humans (Eckardt et al., 1989; Rodríguez et al., 2000). Since erythropoietin promotes red blood cell production, the red blood cell count may increase during a long-distance flight, potentially leading to an increase in hematocrit and blood viscosity. An increase in blood viscosity may in turn cause a decrease in blood fluidity leading to venous stasis. Furthermore, hypobaric conditions may be a risk factor of thrombogenesis in terms of hypercoagulability, since blood coagulation enzyme is located on red blood cell membranes (Iwata and Kiba, 2004).

Thus, it is held to be quite likely that low humidity and/or hypobaric conditions could be risk factors of thrombogenesis. Nevertheless, there have been few reports to date which discuss the effects of these environmental factors in an airplane on hematological change in the lower leg. In the present study,
we examined the effects of low humidity and hypobaric conditions on hematological changes in the venous blood and its blood viscosity during prolonged sitting.

Methods

Subjects

The present subjects were 10 healthy male students (age: 23.3±1.1 years; BMI: 20.4±1.0). They were well informed about the procedure and possible risks of the experiment, and signed a consent form before participating in the study. Physical examination of each subject was conducted by a medical doctor before the experiment. This study was approved by the ethics committee of Kyushu University. The subjects’ diet and water intake were restricted from 19:00 on the day before the experiment.

Procedure

The subjects arrived at the Research Center for Human Environmental Adaptation at Kyushu University at 10:00 am, and consumed a certain amount of diet and water (280 ml). The subjects then entered a climatic chamber wearing the following clothes: a T-shirt, a long-sleeved knit shirt, a long-sleeved sweatshirt, briefs, thick trousers, ankle socks and shoes. After resting for 10 min, they remained seated on a general chair in the chamber for an additional 130 min. We tested four experimental conditions consisting of different combinations of relative humidity (20% or 60%) and air pressure (sea level or equivalent to an altitude of 2,000 m). Under hypobaric conditions, it took 20 min for the reduction and an additional 20 min for the compression of the air pressure in the chamber. Ambient temperature in the chamber was maintained at 24°C though the experiments in every condition. The subjects were allowed to read a book or watch a movie during the experiment to relieve their boredom and sleepiness. They were instructed not to move their legs during the experiment.

Measurements

Venous blood was sampled from the foot or an adjacent site before and after exposure to the experimental conditions. Blood analysis was performed regarding blood viscosity, red blood cell (RBC) count, leukocyte count (LC), platelet count (PC), hematocrit (Ht), total protein (TP), albumin concentration (Alb), prothrombin time (PT), thrombin-antithrombin III complex (TAT) and fibrinogen concentration (FC). Body weight (BW) and leg circumference were also measured as indices of total water loss and edema, respectively. Blood viscosity was analyzed with a digital viscosity analyzer (LVDV-I+, BrookField Co., Ltd., Massachusetts, U.S.A.) using a spindle (CPE-40) to analyze a 0.5-ml sample. BW was measured with a sensitive balance (ID2, Mettler Toledo, Melbourne, Australia) with an accuracy of ±1 g. Leg circumference was measured before and after the experiment at the one-third and two-thirds heights between the head of the fibula and the lateral malleolus using a nonextensible tape.

Statistics

All statistical analyses were performed using STATISTICA for Windows ver. 5.1J (StatSoft, Inc., Tulsa, OK, U.S.A.). Since there were several cases in which venous blood could not be sampled, analysis of variance (ANOVA) was performed using the data of the subjects from whom venous blood could be sampled under every experimental condition. Significant differences were established at the $p<0.05$ level. Each figure is shown as the mean value and the standard deviation.

Results

Table 1 shows the results of every measurement before and after the experiment. ANOVA revealed a significant main effect of time in BW, leg circumference (in both the upper and lower sites), LC ($p<0.01$ each), PC, TP and Alb ($p<0.05$ each), each of which showed a significant decrease in BW and increase in leg circumferences, LC, PC, TP and Alb after the experiment compared with the measurements taken before the experiment ($p<0.01$ each). Furthermore, ANOVA revealed a significant interaction between humidity and time in blood viscosity, RBC count, Ht and Alb ($p<0.05$ each). However, there were no significant differences in the indices among the conditions. No significant differences were observed in PT, TAT or FC.

Figure 1 shows the relationship between blood viscosity and red blood cell (RBC) count (a), hematocrit (Ht) (b), total protein (TP) (c).

![Fig. 1 Relationship between blood viscosity and red blood cell (RBC) count (a), hematocrit (Ht) (b), total protein (TP) (c).](image-url)
and RBC count (Fig. 1a), Ht (Fig. 1b), TP (Fig. 1c). Blood viscosity was found to have a significant relationship to RBC count \( (r = 0.792, p < 0.01) \), Ht \( (r = 0.665, p < 0.01) \) and TP \( (r = 0.515, p < 0.01) \).

**Discussion**

Although an interaction between humidity and time was observed in blood viscosity, RBC count, Ht and Alb (Table 1), there were no statistically significant differences among the conditions in each measurement. Since evaporation from the body surface and respiratory pathway is known to be accelerated in low humidity environments, it was expected that blood condensation and a subsequent increase in blood viscosity would occur under low humidity conditions. However, no definitive results were observed in the present study. This discrepancy may have arisen due to the short exposure time (130 min) to the experimental conditions; it must be kept in mind that in long-distance flight, people often have to stay in an airplane for a much longer time. In addition, the relative humidity in an airplane is often as low as approximately 10% (Maehara et al., 1991). Such low humidity

| Table 1 Results of every measurement before and after exposure to the experimental conditions |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
|                                | Relative humidity | Sea level 20% | Sea level 60% | Equivalent to an altitude of 2,000 m |
|                                |                  | Before      | After        | Before      | After        | Before      | After        |
| Blood viscosity (cP, n=5)       |                  | 3.7         | 3.9          | 3.6         | 3.6          | 3.7         | 3.8          |
| (kg, n=10)                      |                  | 60.8        | 60.7         | 60.8        | 60.6         | 60.8        | 60.7         |
| Leg circumference (upper site)  |                  | 37.2        | 38.0         | 37.2        | 38.0         | 37.2        | 37.8         |
| (cm, n=10)                      |                  | 1.7         | 1.7          | 1.6         | 1.3          | 1.7         | 1.4          |
| Leg circumference (lower site)  |                  | 25.6        | 25.9         | 25.9        | 26.1         | 25.6        | 26.1         |
| (cm, n=10)                      |                  | 1.1         | 1.0          | 1.2         | 1.3          | 1.7         | 1.4          |
| RBC count \( (×10^4/\mu l, n=7) |                  | 484.0       | 492.1        | 477.4       | 479.4        | 485.6       | 492.7        |
| LC \( (/\text{mm}^3, n=7) \)    |                  | 4657.1      | 4957.1       | 4300.0      | 5100.0       | 4457.1      | 5157.1       |
| PC \( (\times10^5/\mu l, n=7) \)|                  | 22.2        | 23.2         | 21.2        | 22.2         | 21.5        | 21.7         |
| Ht \( (\%, n=7) \)              |                  | 45.3        | 46.2         | 44.7        | 44.8         | 45.2        | 45.8         |
| TP \( (g/dl, n=5) \)            |                  | 7.3         | 7.6          | 7.2         | 7.3          | 7.3         | 7.6          |
| Alb \( (g/dl, n=5) \)           |                  | 4.7         | 4.9          | 4.7         | 4.8          | 4.8         | 4.9          |
| PT \( (sec, n=5) \)             |                  | 11.5        | 11.3         | 11.5        | 11.6         | 11.4        | 11.3         |
| TAT \( (ng/ml, n=5) \)          |                  | 1.6         | 1.5          | 1.5         | 2.2          | 1.6         | 3.3          |
| FC \( (mg/dl, n=5) \)           |                  | 246.8       | 228.6        | 211.2       | 216.0        | 220.4       | 225.4        |

BW: Body weight, RBC count: Red blood cell count, LC: Leukocyte count, PC: Platelet count, Ht: Hematocrit, TP: Total protein, Alb: Albumin concentration, PT: Prothrombin time, TAT: Thrombin-antithrombin III complex, FC: Fibrinogen concentration. Values are means (above) and standard deviation (below). *: p<0.05, **: p<0.01
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conditions (only 20% and 60% were tested in the present study) may bring about more noticeable water loss effects or a more dramatic increase in blood viscosity in actual situations.

Since previous studies have reported that serum erythropoietin concentrations increase in humans under hypoxic conditions (Eckardt et al., 1989; Rodríguez et al., 2000), we hypothesized that the RBC count would increase under hypobaric conditions. However, no remarkable effects of exposure to hypobaric conditions were observed in any measurement. This discrepancy may depend on differences in altitude conditions and/or exposure time. Specifically, an increase in erythropoietin (Eckardt et al., 1989: 5.5 h at 3,000 m or 4,000 m; Rodríguez et al., 2000: 1.5 h at 5,500 m) was observed under severe hypobaric conditions compared with the conditions of the present study (130 min at 2,000 m). Here, the air pressure in the simulated airplane was equivalent to altitudes from 1,500 m to 2,400 m when actual altitude generally ranges from 10,000 m to 13,000 m (Brundrett, 2001). Unfortunately, there have been few reports which discuss hematological changes in response to acute exposure to moderate hypobaric conditions (Bendz et al. 2000: 8 h at 2,400 m). Therefore, it is not possible to conclude at present whether or not hypobaric conditions in an airplane could cause a risk of thrombogenesis.

It appears that low humidity conditions could be a more hazardous contributing factor to thrombogenesis than hypobaric conditions based on the present results. However, the problem in the present experimental design has already been pointed out regarding differences in the exposure time to the low humidity and hypobaric conditions. Specifically, the subjects were exposed to low humidity conditions immediately after entering the climatic chamber, while it took 20 min each time for the reduction and compression of the air pressure to and from the hypobaric conditions. Thus, the exposure times to the low humidity (130 min or more) and to the hypobaric conditions (90 min) were different, and it cannot therefore be definitively determined which environmental factor causes a higher risk of ECS.

Leg circumference showed a significant increase after exposure to test conditions compared with that before exposure. LC, PC, TP and Alb also increased significantly after exposure. These results indicate edema and subsequent blood condensation in the leg veins during prolonged sitting. Hitosugi et al. (2000) found that TP and Ht levels did not change in arm venous blood but increased significantly in foot venous blood after 2 hours of quiet sitting, indicating that the sitting position could cause local venous stasis in the lower leg. Platelets are known to release blood coagulation factors (Bode et al., 1981; Walsh and Griffin, 1981). Additionally, an increase in TP could decrease blood fluidity, since TP has a significant relationship to blood viscosity, as shown in Fig. 1c. Therefore, prolonged sitting could promote thrombogenesis due to hypercoagulability and venous stasis in the legs.

In the present study, no obvious effects of low humidity and hypobaric conditions were observed in most measurements. On the other hand, edema and blood condensation were found to be induced by sitting for 130 min; thus, quite prolonged sitting may be a more notable risk factor for ECS. Furthermore, there were no significant changes in indices of blood coagulation (PT, TAT and FC) and exposure to the present experimental conditions did not significantly accelerate blood coagulation in the present study. Bendz et al. (2000) reported that concentration of prothrombin fragments 1 and 2, TAT and activity of factor VIIa increased during exposure to hypobaric conditions (equivalent to an altitude of 2,400 m). On the other hand, Maher et al. (1976) and Crosby et al. (2003) demonstrated that there were not any noticeable changes in indices of blood coagulation even during exposure to severe hypobaric conditions (Maher et al., 1976: 48 h at 4,400 m; Crosby et al., 2003: 8 h at 3,600 m or 4,600 m). Also, Schoberberger et al. (2003) reported that PC, FC, PT and TAT remained unchanged even during an actual flight that lasted more than 8 hours. Therefore, the environmental factors in an airplane, particularly the hypobaric conditions, may not present a serious risk of thrombogenesis with regard to hypercoagulability. However, the acceleration of evaporation under low humidity and hypobaric conditions is not necessarily inevitable. Additionally, the diuretic effect of hypoxia (Swenson et al., 1995; Hildebrandt et al., 2000) may promote water loss from the body during a long-distance flight. Thus, the environmental characteristics in an airplane could accelerate water loss from the body, although this effect may not pose a serious problem.

In conclusion, we were unable to clarify definitively whether low humidity or hypobaric conditions in an airplane presents the greater risk for ECS. Prolonged sitting seems to be a more hazardous factor for thrombogenesis; however, environmental characteristics in an airplane may promote this risk due to the acceleration of water loss from the body during a long-distance flight.

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