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ORIGINAL ARTICLE

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The effect of high altitude on platelet counts, thrombopoietin and erythropoietin levels in young Bolivian airmen visiting the Andes

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Abstract Recognition of thrombosis as a complication of exposure to high altitude has stimulated interest in rheological changes resulting from hypobaric hypoxia. Previous studies of platelet counts at high altitude have yielded conflicting results and have not been studied in conjunction with potential mediating cytokines. We studied the effects of high-altitude exposure on platelet numbers, thrombopoietin (tpo) and erythropoietin (epo) levels in man. A group of 28 volunteers from the Bolivian Airforce stationed at Santa Cruz (600 m altitude) were studied 48 h and 1 week after their ascent to La Paz (3600 m). In addition 105 volunteers based at Santa Cruz for at least 1 year were compared with 175 age- and sex-matched residents at El Alto (4200 m). Platelet counts were measured immediately after sampling and serum samples assayed for tpo and epo. In the ascending group, mean platelet counts were 251×10^9, 367×10^9 and 398×10^9/l at 600 m and following 48 h and 1 week at 3600 m respectively. Mean tpo levels were 132.5, 76 and 92 pg/ml with epo values of 2.98, 11.6 and 7.9 mIU/ml respectively. In the resident populations mean platelet counts were 271×10^9/l in the low- and 471×10^9/l in the high-altitude groups. Mean tpo and epo levels measured 69.3 pg/ml and 4.5 mIU/ml respectively at 600 m and 58.5 pg/ml and 5.1 mIU/ml at 4200 m. In conclusion we have demonstrated a significant and sustained elevation in platelet numbers within 48 h of ascent to high altitude. Our findings do not support a role for tpo as a mediator of the increased platelet count. However, these data do not discount epo as a potential candidate.

Key words Altitude · Erythropoietin · Platelets · Thrombopoietin · Thrombosis

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Introduction

Interest in the effects of a high-altitude environment on platelets and coagulation in man has been stimulated by observations that thrombosis is a recognised complication of such exposure.

Studies involving the effect of altitude on platelet numbers are limited and have yielded conflicting results. Gray et al. (1975) studied 14 men migrating from sea level to 2990 m and demonstrated an overall reduction of 7% in platelet numbers. Further ascent to 5370 m for 2 days resulted in a 25% fall below baseline. An additional 8 days at that altitude produced an increase to 7% below control levels. Similarly Chatterji et al. (1982) found a 12%–26% fall in platelet counts after 48–72 h at 3200 m and 3771 m. Conversely Singh and Chohan (1972a) showed a significant rise in platelet numbers in 32 Indian soldiers ascending to between 3600 m and 5200 m. Sharma (1986) failed to demonstrate a significant difference in platelet numbers when 50 soldiers were moved from low altitude to 3650 m. However, in an earlier study he had reported a slight but significant increase in platelet numbers in well subjects, the reverse being seen in those with symptoms suggestive of acute mountain sickness (AMS) (Sharma 1980). All these studies used manual methods for platelet enumeration. More recently, a study of platelet numbers in 13 individuals ascending to 3500 m showed a highly significant increase of 50%–100% (Simon-Schnass and Korniszewski

1990). Studies in mice and rats have more consistently shown a reduction in platelet numbers during hypobaric hypoxic exposure (Birks et al. 1975).

Platelet function has been evaluated in a limited number of studies. Data from Chohan (1984) suggest an increase in platelet aggregation and adhesiveness on exposure to high altitudes. No consistent change in coagulation factors has so far been demonstrated (Singh and Chohan 1972a, b; Chohan 1984; Andrew et al. 1987). The effect of high altitude on thrombopoietin (tpo) has not been investigated.

It is well documented that ascent to high altitude is associated with expansion of red cell mass. Furthermore, erythropoietin (epo) levels have been shown to increase rapidly on ascent (Faura et al. 1969). Whilst polycythaemia in both primary and secondary settings is known to be related to thrombosis (Pearson 1989) its contribution to altitude-related thrombosis remains unknown (Ward 1975).

Plasma volume changes significantly on ascent to altitude, with radionucleotide studies demonstrating a reduction in volume in otherwise well subjects (Heath and Williams 1995).

Studies have shown an increase in the risk of venous thrombosis on ascent to altitudes above 4000 m (Dickinson et al. 1983; Singh 1973; Ward 1975). Post mortem studies have shown an increased incidence of pulmonary thrombosis at an altitude of 1800 m (Presti et al. 1990). Peripheral venous, pulmonary and cerebrovascular thromboses have been reported in healthy subjects at altitudes between 4300 m and 8200 m (Pugh 1962; Ward 1975; Ward et al. 1989). Ascent to high altitude has been associated with fatal acute cerebrovascular events (Evans 1956). Microvasculature thrombosis is a feature of high-altitude pulmonary oedema and also of high-altitude cerebral oedema, although this may be a secondary feature (Dickinson et al. 1983).

We report a study of platelet numbers, epo and tpo levels in a group moving from 600 m to 3600 m (ascending group) and in two groups resident at 600 m and 4200 m respectively (resident populations).

Materials and methods

Location

The study was based in La Paz, Bolivia at the Instituto Boliviano de Biologica de Altura at an altitude of 3600 m. High-altitude sites were the military college at La Paz (3600 m) and the airbase at El Alto (4200 m). Low-altitude samples were collected at the military college in Santa Cruz, Bolivia (600 m).

Subjects

All volunteers were 19- to 23-year-old Bolivian Airforce personnel. Informed consent was obtained from all subjects prior to sampling. All were fit and well at the time of sampling. A total of 26 subjects were tested 48 h and 1 week after their ascent to 3600 m from 600 m. Following this they returned to 600 m for 2 weeks prior to low-altitude sampling. Transit between low- and high-altitude sites was by a 1-h journey in a pressurised aeroplane. A group of 175 volunteers were studied, who had been resident for more than 1 year at 4200 m, and 105 subjects based at 600 m for more than 1 year.

Equipment

Blood was sampled by antecubital fossa venepuncture into EDTA-anticoagulated containers. Blood counts were performed by a QBC Autoread Haematology System (Becton Dickinson, supplied by Gammador, Abingdon, UK). Tests were performed at room temperature at a high and low-altitude sites (18°C and 22°C respectively). Hypobaric testing of the QBC analyser was performed by decompression in a pressure chamber simulating sea level and altitudes of 4200 m and 5300 m with analysis of samples from 8 healthy volunteers. Reproducibility was assessed by performing several measurements on the same QBC microhaematocrit tubes, and also by taking repeated microhaematocrit tubes from the same EDTA samples.

Serum samples for epo and tpo assays were frozen in aliquots in solid CO_2 and flown to the UK for assay. Assays were performed by commercial enzyme-linked immunosorbent assay (ELISA) kits (R & D systems Inc., Europe; Abingdon, UK).

Statistical analysis

Platelet counts and haematocrit values in all groups were approximately normally distributed. Platelet counts at 3600 m and 600 m were compared by paired *t*-tests. In addition, we have taken changes in haematocrit following 48 h and 1 week at high altitude in the ascending group to give an approximate indication of changes in plasma volume. High-altitude platelet counts have therefore been corrected, using the following expression:

$$\text{platelet count} \times (Hct_{600}/Hct_{3600})$$

where the platelet count is the platelet count at high altitude Hct_{600} is the haematocrit value at 600 m and Hct_{3600} is the haematocrit value at 3600 m. Population means were compared, using Student's *t*-test to examine differences between the low- and high-altitude resident populations and between the two low-altitude groups in the study at Santa Cruz.

Comparison of epo and tpo values was by paired *t*-tests in the ascending group. In comparing the low- and high-altitude resident populations, Student's *t*-test was used for tpo and the Kruskal Wallis test for epo, as the latter data were not normally distributed. All available sera in the mobile group were assayed and 14 samples in each of the static populations; 95% confidence limits are shown in parantheses after relevant data.

Results

Hypobaric pressure testing of the QBC blood counter

No significant variation in either platelet count or haematocrit was demonstrated on decompression to pressures equivalent to altitudes of sea level, 4200 m and 5300 m, confirming that the system is not pressure-sensitive. Serial testing of microhaematocrit tubes and microhaematocrit tube sampling from EDTA bottles failed to show significant variation in either platelet count or haematocrite value.

Ascending group

In the lowlanders ascending to 3600 m, mean platelet counts were 367×10^9, 398×10^9 and 251×10^9/l after 48 h

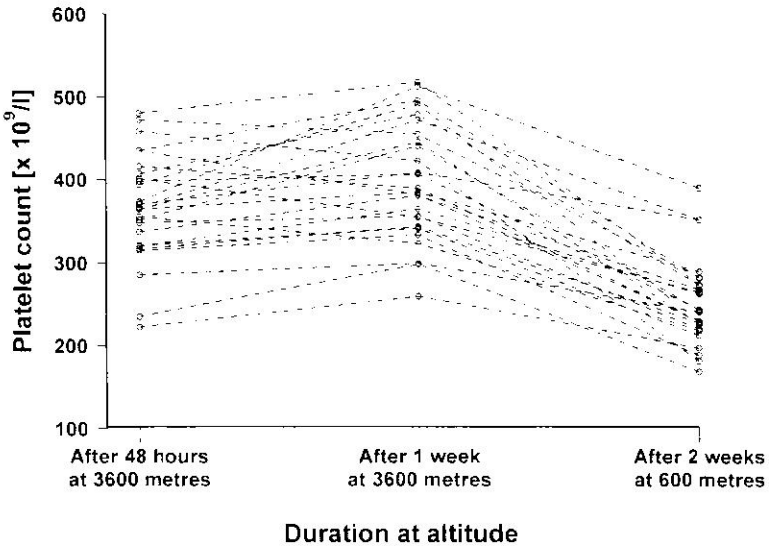

Fig. 1 Platelet counts in the 28 airmen in the ascending group. Mean values are 367(±24.9)×10⁹/l, 398(±27.3)×10⁹/l and 251 (± 20.2)×10⁹/l after 48 h and 1 week at high altitude and 2 weeks after return to 600 m

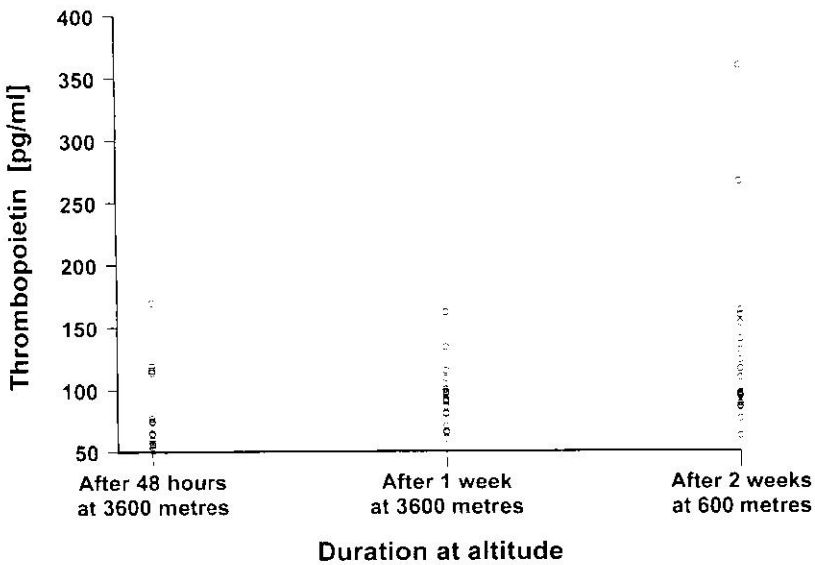

Fig. 2 Thrombopoietin levels in the ascending group. Mean values were 70.6(±12.7), 92(±12) and 132.5(±34.7) pg/ml after 48 h and 1 week at high altitude and 2 weeks after return to 600 m

and 1 week at high altitude and 2 weeks after returning to 600 m respectively (±24.9×10⁹, 27.3×10⁹ and 20.2×10⁹ respectively, Fig. 1). In this group the mean percentage increase in platelet counts, when compared with the value at 600 m after the ascent, was 50% at 48 h (50±12.7%; $P<0.0001$) and 61% after 1 week (61±10.9%; $P<0.0001$) at high altitude. After correcting for changes in haematocrit, mean platelet counts after 48 h and 1 week at 3600 m were 365×10⁹/l (±26.2×10⁹/l; $P<0.0001$) and 384×10⁹/l (±28.4×10⁹/l; $P<0.001$) respectively, giving a rise in mean corrected platelet count of 48% (±12.2%) and 56% (±11.6%) respectively.

Mean haematocrit values were 47.3%, 48.7% and 46.9% after 48 h and 1 week at high altitude and 2 weeks after return to 600 m (±1.0%, 0.9% and 1.0%) respectively. The haematocrit value rose by a mean of 0.95% (±1.6%; not significant) above the 600-m value at 48 h. After 1 week there was a significant increase in haematocrit value of 3.9% (±1.9%; $P<0.001$).

Mean tpo values were 70.6 (±12.7), 92 (±12) and 132.5 (±34.7) pg/ml after 48 h and 1 week at high altitude and 2 weeks after return to 600 m ($P=0.02$, $P<0.001$; Fig. 2) respectively. Mean epo values differed significantly, being 11.6 (±2.2), 7.9 (±1.9) and 2.98 (±0.92) mIU/ml after 48 h and 1 week at high altitude and 2 weeks after return to 600 m ($P<0.00001$; $P<0.00005$; Fig. 3) respectively.

Resident populations

Amongst the resident populations studied there was a significant difference in platelet counts between the low-altitude [mean (271±10.7×10⁹/l] and high-altitude groups [mean (471±14.6)×10⁹/l; $P<0.0001$] (Fig. 4). The haematocrit was significantly higher in long-term residents at 4200 m (51.3±0.4%) when compared with the population at 600 m (42.2±0.6%; $P<0.0001$). There was

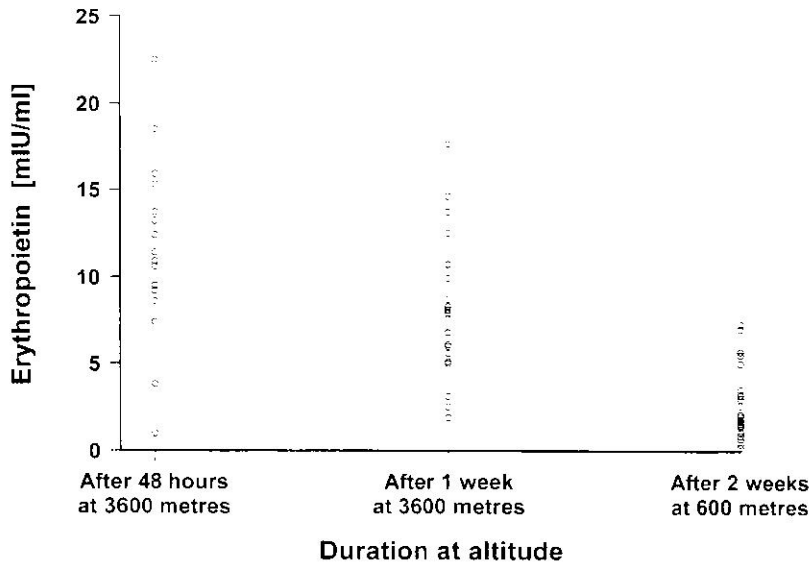

Fig. 3 Erythropoietin levels in the ascending group. Mean values were 11.6(±2.2), 7.9(±1.9) and 2.98(±0.92) mIU/ml after 48 h and 1 week at high altitude and 2 weeks after return to 600 m respectively

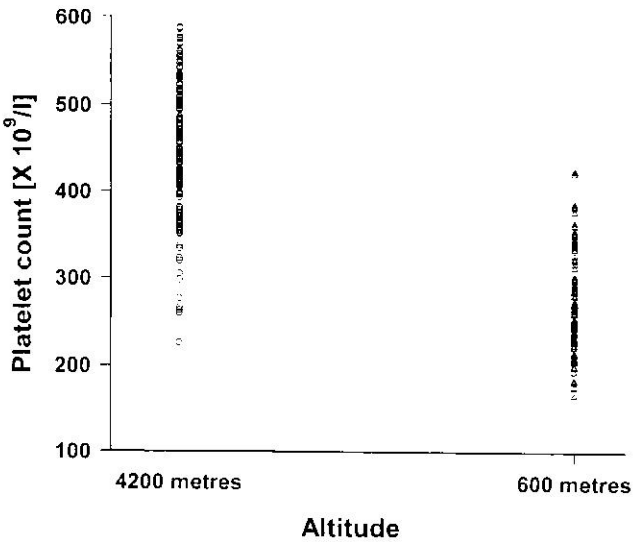

Fig. 4 Platelet counts in the low- and high-altitude resident populations. There was a significant difference in mean values between low- (271±10.7×10⁹/l) and high-altitude groups (471±14.6×10⁹/l)

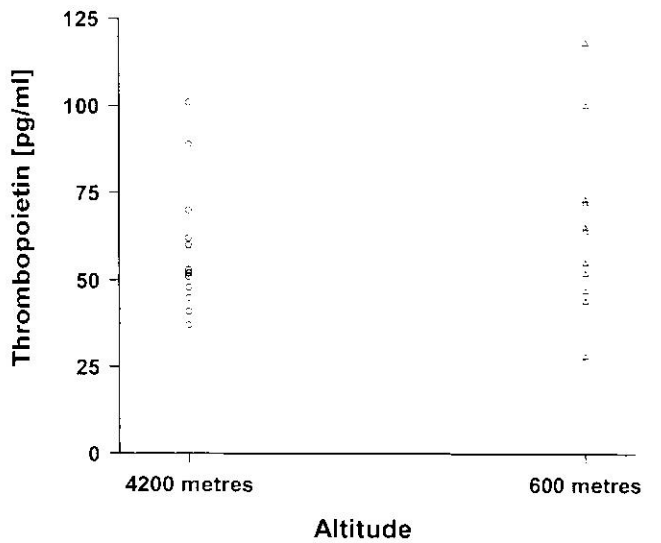

Fig. 5 Thrombopoietin levels in the resident populations. There was no significant difference in mean thrombopoietin levels between the populations at 4200 m (58.5±10 pg/ml) or 600 m (69.3±17 pg/ml)

no significant difference in tpo levels (Fig. 5) in long-term residents at 4200 m (58.5±10 pg/ml) when compared with the population at 600 m (69.3±17 pg/ml; P=0.29). Similarly, mean epo concentrations in long-term residents at 4200 m (5.1±2.94 mIU/ml) showed no significant difference when compared with values for the population at 600 m (4.5±1.2 mIU/ml; P=0.2) (Fig. 6).

Discussion

This study has demonstrated, in a group of 26 young healthy Bolivian airmen ascending from low altitude (600 m), that acute exposure for only 48 h to an altitude of 3600 m leads to an increase in mean platelet count to 367×10⁹/l. Sustained exposure to this high altitude for a week leads to a further rise to 398×10⁹/l. Descent to low altitude effected a fall in mean platelet count to 251×10⁹/l, a value comparable with that seen in long-term low-altitude residents.

It has long been held that there is an acute fall in plasma volume on exposure to high altitude (Pugh 1964). However, haematocrit data presented here do not suggest a reduction in plasma volume during the first 48 h of ascent, and more is probably little change for 1 week. Symptoms of AMS are probably associated with a rise in plasma volume, which might counter the usual haemocrit concentration occurring on acute exposure to altitude. However, all our subjects were fit and well throughout the study. Use of haematocrit data to correct platelet

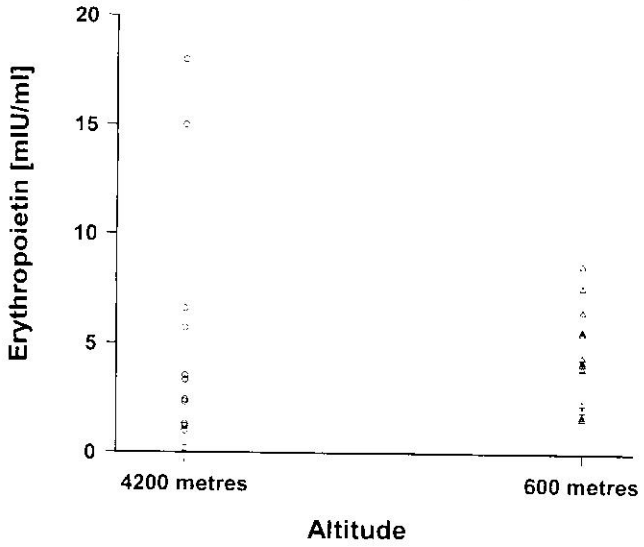

Fig. 6 Erythropoietin values in the resident populations. Mean concentrations in long-term residents at 4200 m (5.1±2.94 mIU/ml) showed no significant difference when compared with values for the population at 600 m (4.5±1.2 mIU/ml)

counts for possible changes in plasma volume confirms that, after 48 h and 1 week at high altitude, a true increase in platelet numbers occurs.

These data differ from findings in a number of previous studies. Gray (1975) and others have suggested that a fall in the peripheral platelet count seen on acute exposure to high altitude occurs as a result of platelet sequestration in the pulmonary vasculature. However, other studies have noted a small increase in platelet numbers at high altitude in asymptomatic subjects, with a fall or very slight increase in platelet numbers in subjects with symptoms and signs suggesting high-altitude pathology (Singh and Chohan 1972a, b). This latter observation could be explained by the fluid retention thought to occur in those subjects suffering AMS. All our subjects remained fit and well throughout the study. Our findings are similar to those of the only comparable study using an automated platelet counter (Simon-Schnass and Korniszewski 1990).

Thrombopoietin is known to stimulate megakaryocytopoiesis and hence platelet production (Kaunshansky 1995). Furthermore, recent data suggest that reactive thrombocytosis may, in association with certain conditions, be stimulated by tpo (McCarty 1997). Outside the setting of severe thrombocytopenia tpo levels have generally shown a poor correlation with platelet numbers. This may be explained by megakaryocyte and, to a lesser extent, platelet tpo receptor binding free ligand (Emmons et al. 1996). The similar tpo values (69.3 and 58.5 pg/ml) in the resident populations at low and high altitude accord with this. However, rapidly induced experimental thrombocytopenia in animals has suggested a reciprocal relationship between tpo levels and platelet numbers (Kuter and Rosenberg 1995). The tpo data in the ascending group show this phenomenon with an increased level at the lower platelet count seen on return to low altitude. Despite demonstrating a rapid increase in platelet count in the week following ascent we found no evidence that, following 48 h at altitude, tpo might be responsible for this. It is possible to speculate that analysis of tpo levels at an earlier time might have demonstrated elevated levels. However, our data do not suggest that the thrombocytosis of altitude is tpo-driven.

Might other cytokines be responsible? It is well documented that epo levels increase on ascent to high altitude (Faura et al. 1969). Data from the ascending group in which epo levels are significantly elevated following 48 h at altitude suggest a potential role for epo as a mediator of the thrombocytosis. However, in vitro studies investigating a thrombopoietic role for epo have been discordant (Dessypris et al. 1987; Hill and Leven 1989). Furthermore, despite extensive use of recombinant human epo in renal failure, there is no evidence suggesting it might cause thrombocytosis (Eschbach et al. 1987). These in vitro and observational data make it difficult to support a role for epo as the mediator of the thrombocytosis of altitude.

On exposure to high altitude, the rise in platelet numbers and increased haematocrit described in this paper, combined with increased coagulation factors plus elevated platelet adhesiveness and aggregation (Singh and Chohan 1972a, b; Chohan 1984) may all contribute to a greater risk of thrombosis.

We have demonstrated a significant elevation in platelet numbers within 48 h of ascent to high altitude, which is sustained as a permanent feature in long-term residents. Our findings do not support a role for tpo as a mediator of the thrombocytosis associated with altitude. However, although there are few historical supporting data, epo remains a candidate cytokine.

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