Effect of Vitamin C and E in Modulating Peripheral Vascular Response to Local Cold Stimulus in Man at High Altitude

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Abstract: At high altitude (HA), cold stress is aggravated by hypoxia, perhaps due to the increased formation of free radicals which trigger oxidative stress. This may be one of the contributing factors for adverse effects including disturbances in microcirculation and capillary permeability resulting in decreased peripheral blood flow. This leads to altered cold-induced-vasodilatation (CIVD) response on exposure to HA. The present study was conducted on 40 male volunteers (4 groups of 10 each) to evaluate the utility of supplementation of vitamin C (500 mg/d) and vitamin E (400 mg/d) singly, as well as in combination, in modulating peripheral vascular response by assessing CIVD response under local cold stimulus both at Delhi (200 m) and at HA (3,700 m). On exposure to 3,700 m, decreased CIVD response was observed in all the groups. The responses were better in vitamin supplemented groups, in general, as compared to the placebo group. The best CIVD response was seen in the vitamin C (singly)–treated group. Administration of vitamin C and E together did not result in any additional benefit. Facilitation of CIVD response due to supplementation of vitamin C may be attributed to its (a) antioxidant effect, and (b) major physiological functions of increased metabolism and thermogenic properties, facilitation of collagen synthesis, restoration of intercellular substances and better maintenance of the rheological status of the blood. Hence, vitamin C is effective for improving peripheral blood flow and thereby reduces the incidence of cold injuries during acclimatization or outdoor duties at HA. [Japanese Journal of Physiology, 49, 159–167, 1999]

Key words: cold-induced-vasodilatation (CIVD), peripheral blood flow, antioxidant, high altitude, cold injury.

Physiological responses in local cold tolerance have often been demonstrated by cold-induced-vasodilatation (CIVD) during hand immersion in cold water, which has been taken as an index for peripheral vascular response. Individuals, who chronically expose their hands in cold water show enhanced CIVD response [1, 2]. Local adaptation of the extremities as well as general cold acclimatization of the individual, performed either in a natural cold environment or under laboratory conditions [2–8] results in manifesting higher local skin temperatures and less pain due to reduced sympathetic nervous system activation [4, 9]. The effect of limited exposure to cold, however, produces a more variable response in CIVD [5, 10]. It is known that, at high terrestrial altitude, the effect of cold is aggravated by hypoxia, which decreases the peripheral blood flow [11, 12] leading to decreased CIVD response [13]. On exposure to HA, there occurs a redistribution of blood from the systemic to pulmonary circuits, and the occurrence of adverse HA effects, such as acute mountain sickness, cerebral and pulmonary oedema, retinal hemorrhage, and crippling cold injuries, are often attributed to disturbances in microcirculation including capillary per-
meability and damage to cell structures. All of these changes might be due to oxidative stress, which is enhanced by hypoxia and cold. Oxygen radicals generated under hypoxic conditions are very reactive, unstable and cytotoxic. The cytotoxic effects of oxygen radicals could result from the peroxidation of lipid component of cell membranes [14] leading to increased vascular permeability. This suggests that HA exposure leads to an increased free radicals burden on the cells [15]. Increased formation of free radicals triggers further oxidative stress resulting in more lipid peroxidation and mitochondrial damage [16]. The body's susceptibility to free radical stress and related damage is associated with the overall balance between the stress level and antioxidant potential of body tissues. The degenerating changes may be one of the contributory factors for decreased blood flow to the extremities, leading to higher incidence of cold-induced injuries on exposure to snow-bound areas at HA.

Vitamin C and vitamin E are well-known natural antioxidants in the biological system and protect the cells through their ability to scavenge free radicals. Vitamin E is reported to be a suitable prophylactic measure in HA mountaineers as it prevents impairment of the blood flow characteristics with beneficial effects on physical performance [17, 18]. Ascorbic acid, present as ascorbate in biological systems, is considered the most important antioxidant [19], and is reported to afford protection against frostbite [20]. Thus, for counteracting the stress of cold and hypoxia, and in particular the peripheral vascular response, the present study was undertaken to evaluate the utility of the supplementation of vitamins C and E singly, as well as in combination, in human subjects by assessing the CIVD response, and by calculating the digital blood flow from the temperature changes under local cold-stress during acclimatization in HA-cold situations, which has not yet been investigated.

METHODS

Subjects. Forty young healthy male subjects (age 20–28 years, body wt. 59.2–60.9 kg), hailing from the plain regions of northern India (lowlanders) and without prior exposure to cold or HA areas, acted as volunteers. Prior to experimental studies, a clinical examination was conducted to rule out any systemic illness. The subjects were briefed about the details of the experimental protocol, which was approved by the Ethics Committee of the institute. The volunteers gave informed consent to act as subjects and had the option to withdraw from the experiments at any time. The subjects were divided into 4 groups of 10 each. Utmost care was taken to match the groups with respect to age, height and body weight, so that the physical characteristics of all the groups were comparable. Their average energy intake at Delhi and at HA were, respectively, 3,967 and 4,516 kcal. On an average, the distribution of calories between protein, carbohydrate and fat, respectively, was 11.25, 64.23 and 24.52% for Delhi, and 10.37, 62.16% and 27.47% for HA (3,700 m), which was quite balanced. All of the groups consumed the same food cooked through a common kitchen to ensure adequate nutrition. The same practice continued at HA, except that the quantities of raw food issued to the kitchen were higher in energy, correspondingly, in other nutrients. Special care was taken for palatability and variety in the menu. The dietary regimes were ad libitum, and the subjects consumed adequate food and fluid at altitude and did not complain of any loss of appetite.

Experimental protocol. Five sets of experiments were carried out in two phases: Phase-I included two sets (D1 and D2) at Delhi (200 m), and Phase-II included three sets (HA1, HA2 and HA3) at HA (3,700 m).

Phase-I. The first set (D1) of the initial study was conducted at Delhi (200 m) after the volunteers were stabilized for 15 d on the authorized ration (3,967 kcal). Subjects of group I served as the control and received calcium gluconate as the placebo. Group II received 500 mg of vitamin C (Glaxo) daily. Group III was administered vitamin E at 400 mg/d (Evion, E. Merck), and group IV received a combination of vitamins C and E (500 mg/d and 400 mg/d, respectively) for the entire duration of the study. After 15 d of supplementation of the therapy (oral), the second set (D2) of recordings was monitored again at Delhi. The ambient temperature during the study period at Delhi ranged between 19.5 and 30.0°C. On completion of the baseline studies, all the subjects were transported to an altitude of 3,700 m in the Eastern Himalayas by motorable road in batches of 10, with a gap of 2 d in between. The road journey from foothills to the 3,700 m spot took about 5 h.

Phase-II. Three sets of experiments were performed in this phase at HA (3,700 m); (1) on the third day after arrival (HA1), and (2) on the tenth day (HA2) and (3) 21st day of acclimatization (HA3). At HA, all of the subjects stayed in billets made of metal sheets. They used protective winter clothing throughout the stay at HA. The ambient temperature during the study period at altitude ranged from 8 to 11°C (maximum) and −4 to 0°C (minimum) with occasional snowfall and rain. The studies at Delhi and HA
were both conducted in the same way, by the same observers and using the same equipment under similar conditions of room temperature (20–24°C). The subjects stayed under similar environmental conditions both at Delhi and HA, without any variation in their daily activities and exposure schedule. The experimental room temperature and the time of experiments were kept similar and no undue stress or exertion was imposed on them. The various factors affecting the CIVD responses had no influence in our experiments, as all 4 groups of subjects belonged to the same geographical region (low landers) of India and had no previous exposure to HA or cold areas. They were all medically fit, had comparable physical characteristics in respect to age, height, body weight and sex including nutritional status, which are some of the well documented general factors attributed to induce variations in the responses to local cold exposure [8, 9, 21, 22].

**Test procedure.** Subjects were made to rest in the temperature-maintained (20–24°C) room for about an hour. They were not allowed to smoke or engage in any form of exercise during this period. The oral temperature (T_b) was taken by a clinical thermometer kept sublingually for 5 min. The subjects sat on a comfortable seat and their CIVD response (index for peripheral vascular response) was recorded by asking them to immerse the right hand, up to the level of styloid process, in a constantly stirred water bath maintained at 4 ± 0.2°C by adding ice water [21]. The temperature of the water bath (T_w) as well as the changes in skin temperature (T_s) of the immersed hand index finger were measured at the beginning and every minute thereafter for the 30-min period of immersion with the help of a YSI telethermometer (model 46TUC) with appropriate probes. The skin probe was attached to the pad of the index finger (ventral side, at a site 1 cm proximal to the finger tip) by double layers of adhesive tape. A thin layer of grease was also applied above the tape to ensure that no water entered through the tape. T_b was again measured at the end of the immersion period. From the T_s recordings of the right-hand index finger the values of the T_b before immersion, the minimum T_w, the highest T_s, the time to reach these values during immersion, and the average T_s during the last 25 min of the 30-min immersion were determined. The peripheral blood flow [23] was calculated by using the formula: \( F(\text{ml/cm}^2 \text{ per min}) = 14.6 \times \frac{(T_s-T_w)}{(T_b-T_s)} \), where T_s skin temperature; T_w water bath temperature; and T_b oral temperature. For this, the average T_s of the index finger for the last 25 min and the average T_b before and after immersion were used. The CIVD index proposed by Takano and Kotani [22] was also evaluated as per the method described below. The CIVD response curve of the subject was drawn by putting the time of exposure (min) on the X-axis and the changes in index finger skin temperature (°C) on the Y-axis, and using the same unit scale on both of the axes (i.e., 1 div of 1 cm=1 min for X-axis and 1 div of 1 cm=1°C for Y-axis). From the graph, the CIVD index was evaluated by considering the “total area” covered by the above graph, which is the section between temperature curve and the baseline of 4°C over the last 25 min of immersion. This was measured by a planimeter. The area thus obtained was designated as the “CIVD index” of the subject. It can be indicated that the greater the area, the earlier and greater the rise in finger temperature due to improved CIVD response and increased peripheral blood flow, resulting in a reduced chance of cold injury.

Statistical analysis was done by two way classification of ANOVA using Newman–Keul’s multiple range test for comparison of responses at different locations for each group. \( p<0.05 \) was used to set the level of significance.

**RESULTS**

The body weight of all the groups under different situations did not show any significant change, thereby pointing to adequate nutrition throughout. The responses from the tip of the index finger, T_s, during local cold immersion showed a characteristic pattern of CIVD in all of the groups. Four different types of responses were observed both at Delhi and HA, which were in accordance with our earlier observations in men and animals [13, 21, 24]. The pattern of mean ± SE CIVD response of all the groups under 5 different situations for comparison of various treatments are depicted respectively in Figs. 1–5. To compare and figure out the differences in the responses on different days at Delhi and during exposure at high altitude with single treatment may be difficult with these graphs, hence, the above responses of the control (placebo) and vitamin C (singly)–treated groups are also shown separately in Figs. 6 and 7, respectively. The changes in the index finger T_s induced by cold immersion including the peripheral blood flow and CIVD index of all the groups are given in Tables 1–3 for comparison of various treatments.

The initial CIVD responses of all 4 groups monitored at Delhi (D1) before supplementation were almost similar (Fig. 1). In the 2nd set (D2) of experiments, the pattern and magnitude of CIVD response were found to be better in the vitamin-supplemented
groups as compared to the placebo group (control), whose responses remained similar to that of the initial recordings (set D1) with minor fluctuations (Figs. 2, 6). Supplemented groups also showed some improvement in the values of average \( T_0 \) (Table 1), minimum and maximum \( T_0 \) (Table 2), finger blood flow and CIVD index (Table 3) as compared to the placebo group, even though the values were not statistically significant. On early exposure to HA (HA1), a change in the pattern and marked reduction in the magnitude of CIVD response were seen in all of the groups (Fig. 3). For the control group, the response was of the lowest magnitude during the second set (HA2) of recordings at HA (Figs. 4, 6), which showed no recognizable change by way of improvement for the control group by the 3rd (HA3) week (Figs. 5, 6). Reduction in the initial index finger \( T_0 \) (Table 1) and the minimum and maximum \( T_0 \) of the index finger reached during local cold immersion at altitude (Table 2) were less com-
Fig. 5. Mean(±SE) CIVD response of all 4 groups monitored during the HA3 set of experiments, for comparison among various treatments on the 21st day of acclimatization at 3,700 m.

Fig. 6. Mean(±SE) CIVD response of group I (placebo) on different days at Delhi (D1, D2) and during acclimatization at HA (HA1, HA2, HA3), for comparison of the responses without any supplementation of vitamins.

Fig. 7. Mean(±SE) CIVD response of group II (vitamin C supplemented) on different days at Delhi (D1, D2) and during acclimatization at HA (HA1, HA2, HA3), for comparison of various days at Delhi and HA with one treatment of vitamin C only.

day of acclimatization (HA2), and thereafter an improvement was observed. All of the supplemented groups showed definite improvement by the 3rd week of acclimatization at HA (HA3). Group II, supplemented with vitamin C (singly), showed the maximum beneficial effect and elicited the best CIVD response on the 21st day (HA3) of altitude acclimatization (Fig. 7). This was followed by the combination group (group IV). The vitamin E-supplemented group (group III) was better than the control (placebo) group, who manifested the lowest response. By the 3rd week of acclimatization at HA (HA3), the mean CIVD response curve of the combination (vitamin C and E) group was almost similar to that of the response pattern of their own initial values (set D1) at Delhi. But the response for the vitamin C (singly)–supplemented group overshot the response of the initial values (set D1) and became closer to that of set D2 monitored at Delhi (Figs. 5, 7), even though the minimum $T_s$ reached during cold immersion at HA was less (Table 2, Fig. 7).

The blood flow also showed a similar response pattern to that of the CIVD response (Table 3). Identical responses of CIVD and peripheral blood flow were evident because the peripheral blood flow was calculated from $T_s$, $T_w$, and $T_b$, of which $T_s$ was the main variable parameter, while $T_w$ was kept constant (4±0.2°C) and $T_b$ showed very negligible change. Hence, the peripheral blood flow during immersion was proportional to the changes in the magnitude of CIVD response. This
Table 1. Comparison of initial index finger $T_s$ and average index finger $T_s$ of all 4 groups under 5 different situations and among various treatments.

<table>
<thead>
<tr>
<th>Group</th>
<th>$T_s$ Locations</th>
<th>Statistical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$T_s$</td>
<td>D1 (A)</td>
</tr>
<tr>
<td>Control</td>
<td>Initial</td>
<td>33.0</td>
</tr>
<tr>
<td>(group I)</td>
<td>Average</td>
<td>7.10</td>
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<tr>
<td>Vit C</td>
<td>Initial</td>
<td>33.5</td>
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<tr>
<td>(group II)</td>
<td>Average</td>
<td>7.09</td>
</tr>
<tr>
<td>Vit E</td>
<td>Initial</td>
<td>32.2</td>
</tr>
<tr>
<td>(group III)</td>
<td>Average</td>
<td>6.93</td>
</tr>
<tr>
<td>Vit (C+E)</td>
<td>Initial</td>
<td>32.4</td>
</tr>
<tr>
<td>(group IV)</td>
<td>Average</td>
<td>6.92</td>
</tr>
</tbody>
</table>

Values are mean±SEM. *= p<0.05, **= p<0.01, NS: not significant.

Table 2. Comparison of minimum and maximum index finger $T_s$ reached during immersion of all 4 groups under 5 different situations and among various treatments.

<table>
<thead>
<tr>
<th>Group</th>
<th>$T_s$ Locations</th>
<th>Statistical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$T_s$</td>
<td>D1 (A)</td>
</tr>
<tr>
<td>Control</td>
<td>Minimum</td>
<td>5.6</td>
</tr>
<tr>
<td>(group I)</td>
<td>Maximum</td>
<td>8.7</td>
</tr>
<tr>
<td>Vit C</td>
<td>Minimum</td>
<td>5.5</td>
</tr>
<tr>
<td>(group II)</td>
<td>Maximum</td>
<td>8.7</td>
</tr>
<tr>
<td>Vit E</td>
<td>Minimum</td>
<td>5.4</td>
</tr>
<tr>
<td>(group III)</td>
<td>Maximum</td>
<td>9.3</td>
</tr>
<tr>
<td>Vit (C+E)</td>
<td>Minimum</td>
<td>5.3</td>
</tr>
<tr>
<td>(group IV)</td>
<td>Maximum</td>
<td>8.6</td>
</tr>
</tbody>
</table>

Values are mean±SEM. *= p<0.05, **= p<0.01, NS: not significant.

was also the case with CIVD index (Table 3).

**DISCUSSION**

There was no significant difference in the baseline values (D1) of mean CIVD response in all 4 groups. The variation in the responses of CIVD observed in all of the experimental groups as compared to the placebo group under different situations, both at Delhi and HA, could only be attributed to the supplementation of vitamins, since it was assured that no other factor could affect the CIVD response in the present experiments; most of the documented factors [8, 9, 21, 22] attributed to induce variations in the response.
Vit C and E in CIVD Response at Altitude

<table>
<thead>
<tr>
<th>Group</th>
<th>Locations</th>
<th>Statistical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D1 (A) vs. D2 (B)</td>
<td>HA1 (C) vs. HA2 (D)</td>
</tr>
<tr>
<td>Control (group I)</td>
<td>1.54 1.61 0.61 0.55 0.82</td>
<td>0.21 0.20 0.08 0.09 0.02</td>
</tr>
<tr>
<td>CIVD index</td>
<td>78.0 80.0 34.0 30.0 46.0</td>
<td>4.38 4.16 1.08 1.11 3.52</td>
</tr>
<tr>
<td>Vit C (group II)</td>
<td>1.53 1.89 0.98 1.20 1.67</td>
<td>0.22 0.25 0.20 0.12 0.18</td>
</tr>
<tr>
<td>CIVD index</td>
<td>76.0 92.0 54.0 62.0 84.0</td>
<td>4.65 5.45 3.64 2.34 3.81</td>
</tr>
<tr>
<td>Vit E (group III)</td>
<td>1.48 1.69 0.88 0.98 1.24</td>
<td>0.31 0.21 0.26 0.10 0.23</td>
</tr>
<tr>
<td>CIVD index</td>
<td>72.0 87.0 48.0 51.0 64.0</td>
<td>5.47 4.52 3.83 1.78 4.44</td>
</tr>
<tr>
<td>Vit (C+E) (group IV)</td>
<td>1.46 1.78 0.96 1.11 1.40</td>
<td>0.28 0.21 0.14 0.19 0.26</td>
</tr>
<tr>
<td>CIVD index</td>
<td>72.0 88.0 52.0 58.0 70.0</td>
<td>5.30 4.37 2.48 3.44 5.23</td>
</tr>
</tbody>
</table>

Values are mean±SEM. *= p<0.05, **= p<0.01, NS: not significant.

to local cold exposure were kept similar for all of the groups. The test results of the control (placebo) group are in confirmation with our previous observations in man at altitude [13]. The reduction seen in the CIVD response during altitude exposure is due to the decreased blood supply to the extremities, brought about by the combined effect of hypoxia and cold. It has also been shown by various observers that there is a reduction in blood flow to the extremities at HA [11, 12]. This could be attributed to peripheral vasoconstriction of the cutaneous vessels arising out of sympathetic over-activity at HA [25].

The significant finding in this study is that, under normoxia and hypoxic conditions, the administration of vitamin C (500 mg/d) is highly beneficial for best CIVD response with increased digital blood flow. The vitamin E–supplemented group also showed a better CIVD response compared to the control (placebo) group, but the supplementation of vitamin E together with vitamin C (group IV) did not show any additional benefit. The possible reason for the absence of synergistic action due to the combination of vitamins E and C in peripheral vascular response is not very clear. But, at the same time, this finding is in good agreement with the observation of Burton et al. [26], who reported that vitamin C does not spare vitamin E antioxidant action in vivo. However, a recent study on rats has suggested that, in vivo, vitamins E and C interact and each can exert sparing effects in the absence of the other [27]. Chow [28], in his review, has suggested that vitamin E supplementation is beneficial to certain groups of a population, but supplementing vitamin E in experimental subjects maintained on a nutritionally adequate diet does not always provide an additional benefit. Our subjects, too, consumed an adequate diet, as evidenced from their body weight, and possibly this resulted in not having an added advantage from the combined supplementation of vitamins C and E. In regards to the assessment of oxidative stress at HA, contemporary literature [17, 18, 29] has reported a beneficial influence of vitamin E on physical performance and some rheological parameters in mountaineers, indicating an increased oxidative stress at high altitude. It has been stated that vitamin E promotes an economical energy metabolism on one side, and on the other it acts as a stabilizing antioxidant against lipid peroxidation in membranes [17]. An animal study at simulated altitude is also suggestive of an increased level of free radicals on intermittent exposure to 4,000 m [15].

Vitamin C is an excellent water soluble antioxidant with a strong reducing potential. It is capable of scavenging singlet oxygen and free radicals [30]. Because of its antioxidant properties, it is expected to help in providing antioxidant defence by counteracting stress of cold and hypoxia during exposure to high altitude. The facilitation of better CIVD response on the administration of vitamin C can also be attributed to its effects in accelerating thermogenesis [2, 31, 32], peripheral vasodilatation and, to an extent, attenuating
sympathetic hyper-reactivity [33]. Further, vitamin C, due to its labile nature, rapidly enters from the plasma into leucocytes and might help in restoring the intracellular substances. The physiological functions of ascorbic acid also include the formation of collagen and intercellular cement substances. Without ascorbic acid collagen formation is defective and weak [34]. Moreover, vitamin C interacts with membrane-bound vitamin E by reducing the tocopherol radical back to tocopherol [35]. Involvement of vitamin C in the regeneration and restoration of antioxidant properties of vitamin E has also been indicated by others in vitro [26, 36, 37]. The protective thermogenic effect of vitamin C against cold and cold injuries has also been lucidly demonstrated by many workers [2, 38-40]. These results suggest the possibility of least alteration in the rheological properties of the blood in the vitamin C (singly)-supplemented group and, hence, support of the elicitation of best CIVD response in this group during acclimatization to HA.

Thus, the efficacy of vitamin C in potentiating a peripheral vascular response during altitude acclimatization may be attributed to (a) its antioxidant properties, and (b) major physiological functions including metabolic and thermogenic properties, collagen synthesis, anti-stress activity, and restoration of intercellular substances as well as better maintenance of the rheological status of the blood. Thus, on the basis of our findings, the beneficial effects of vitamin C supplementation on CIVD response during HA acclimatization are recommendable for improving peripheral blood flow at high altitude and thereby reduce the chance of cold injuries. This conclusion further emanates from our earlier experiments on monkeys, wherein it was confirmed that animals with higher CIVD response are better protected against cold injuries [24].

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Vit C and E in CIVD Response at Altitude


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