Influence of exercise on ascorbic acid status in man

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SUMMARY

1. The response of circulating leucocytes with regard to changes in number, proportion of granulocytes and lymphocytes, as well as changes in the ascorbic acid (AA) concentration of plasma and isolated lymphocytes, were studied in nine men who ran a 21 km race. A marked leucocytosis was noted 5 min after the race, the predominant increase being in granulocytes ($P < 0.001$) with smaller relative increases in circulating lymphocytes ($P < 0.01$) and platelets ($P < 0.001$). Numbers of leucocytes and platelets returned to pre-exercise levels within 24 h after the race.

2. The concentration of AA in plasma increased from 52.7 ± 4.1 μmol/l before the race to 67.0 ± 5.3 μmol/l within 5 min after the race ($P < 0.001$). This increase in plasma AA concentration was positively correlated with the rise in plasma cortisol concentration during the race ($r = 0.89 ; P < 0.01$). However, within 24 h after the race the plasma concentration of AA fell 20 ± 4% below pre-exercise values ($P < 0.01$) and remained low for at least the next 2 days ($P < 0.05$).

3. Lymphocyte AA concentration increased from 15.6 ± 0.6 to 19.7 ± 0.9 μmol/g of lymphocyte protein during the race ($P < 0.01$) but returned to normal levels within 2 days after the race.

4. It is suggested that the adrenal gland may be the major source of AA efflux into the circulation during exercise.

Key words: ascorbic acid, exercise, lymphocytes, plasma.

Abbreviations: AA, ascorbic acid; HPLC, high-performance liquid chromatography; UA, uric acid.

INTRODUCTION

Physiological stress, such as myocardial infarction, in man is associated with a rapid and profound reduction in plasma and leucocyte (buffy layer) ascorbic acid (AA) concentration, approaching the deficiency level [1]. Other forms of stress, including emotional stress, trauma, surgery and acute infection, also appear to reduce the blood concentration of AA in man (see [2]). The reason for such marked falls in AA levels during stress situations in man is unclear and contrasts with reports of experiments performed on rats and guinea pigs in which the stress of haemorrhage [3, 4] or exposure to hypoxic gas [5] produced large elevations of plasma AA concentration.

Exercise may be considered to be an acute non-traumatic stressor and as such could be a useful model for the investigation of changes in AA metabolism during stressful situations in man. A 2 h football training session involving vigorous exercise has been reported [6] to decrease leucocyte (buffy layer) AA concentration. J. Chen (unpublished work) noted that exercise consumes certain amounts of AA in the body, and Karnaukh [7] reported an increased urinary excretion of AA after light exercise in the heat. We recently reported [8] an increased concentration of AA in plasma and lymphocytes after a marathon race, and Fishbaine & Butterfield [9] noted a positive relationship between physical activity level and serum AA concentration. These findings suggest that exercise induces a change in the distribution, metabolism or excretion of AA.

In order to clarify the effects of exercise stress on AA status in man we have measured the concentration of AA in plasma and isolated lymphocytes before and up to 3 days after a 21 km road race. Blood cell counts and plasma cortisol levels were also determined.

MATERIALS AND METHODS

Subjects

The nine subjects were healthy men aged 26 ± 1 years (mean ± sem), 181 ± 2 cm tall and weighing 74.1 ± 2.7 kg. All subjects were physically active on a recreational basis, but only two had undertaken regular running (> 25 miles/week) in the preceding 6 months. Only one subject had taken vitamin supplements (30 mg of AA per day) in the
preceding 6 months. Approval for the study was obtained from the Joint Ethical Committee of the Grampian Health Board and the University of Aberdeen. The purpose of the study and the procedures involved were explained to the subjects before their written consent was obtained.

Experimental design

The volunteer runners participated in a 21 km road race in Aberdeen held on a cool (16-18°C), calm, dry day (Sunday 31 August 1986). The race started at 10.00 hours. Venous blood samples were obtained, with minimal stasis, by venepuncture from a superficial forearm vein approximately 1 h before and within 5 min after the race. In addition, resting blood samples were obtained, after an overnight fast, in the morning (08.00-09.00 hours) 2 days before the race, and 1, 2 and 3 days after the race. All blood samples were taken with the subjects in a sitting position. The subjects did not undertake any special dietary regimen (e.g. carbohydrate loading) before or after the race. They exercised little in the 2 days before the race and in the 3 days after the race. On the day of the race the subjects consumed a light breakfast 2-3 h before the race and 1-2 h before the pre-exercise resting blood sample. Only water was allowed during the race and alcohol-containing drinks were not allowed during the experimental period. Nude body weight was recorded before and after the race.

Analytical procedures

Immediately upon collection, one aliquot of blood (4 ml) was added to ethylenediaminetetra-acetate (potassium salt) (1.5 mg/ml). This blood was used for measurement of haemoglobin (by a cyanmethaemoglobin method) and packed cell volume. Plasma volume changes were calculated from changes in haemoglobin and packed cell volume relative to the pre-exercise sample, as described by Dill & Costill [10]. Total leucocyte and platelet counts were performed on a Coulter Counter S Plus I, and white cell differential counts were made by counting 200 cells of a Romanowsky-stained blood film.

An aliquot of blood (10 ml) was added to lithium-heparin-coated tubes; part of this was immediately centrifuged at 13 000 g for 60 s to obtain plasma. An aliquot of the plasma was frozen in liquid nitrogen for subsequent measurement of cortisol by a solid-phase enzyme immunoassay method (Diagnostic Products UK Ltd, Wallingford, Oxon., U.K.). Duplicate 600 µl aliquots of plasma were deproteinized in an equal volume of ice-cooled 0.3 mol/l perchloric acid and centrifuged; the supernatant was used for measurement of AA and uric acid (UA) by high-performance liquid chromatography (HPLC) within 12 h of collection. From the remaining blood, lymphocytes were separated on a Ficoll-Hypaque gradient (Histopaque-1077, Sigma Chemical Company Ltd, Poole, Dorset, U.K.) by methods described elsewhere [11]. Using 5 ml of phosphate-buffered saline each time, the cells were washed twice by centrifugation to obtain lymphocyte cell pellets. To extract AA, 1.0 ml of ice-cooled perchloric acid (0.3 mol/l) was added to the washed cell buttons. The contents of the tube were vortex-mixed and allowed to stand on ice for 10 min, followed by centrifugation. The supernatant was used for AA determination by HPLC within 12 h of collection. The cell protein content of the precipitate was determined by the Biuret procedure of Albanese et al. [12]. The AA concentration of the lymphocytes was expressed per g of cell protein.

The isocratic HPLC system consisted of a Gilson Model 302 pump with 5SC pump head, a Gilson Model 231 auto-injector and a 150 mm×4.1 mm Hamilton reversed phase PRP-1 (10 µm particle size) column. For spectrophotometric detection we used a Gilson HM Holochrome u.v./visible detector at 254 nm set at a range of 0.1 AUFS (Gilson Medical Electronics, Inc., WI, U.S.A.). The chromatogram was recorded and peak areas measured on a Trio computing integrator (Trivet Systems International Ltd, Sandy, Beds., U.K.). The mobile phase was 10 mmol/l perchloric acid at a flow rate of 0.7 ml/min. Retention times of AA and UA were 3.0 and 3.9 min respectively. Pretreatment of specimens or standards with ascorbate oxidase (EC 1.10.3.3) completely eliminated the AA peak without introducing other changes in the chromatogram. The relationship between the area of the AA peak and the concentration of aqueous standards taken through the procedure was found to be linear within the range 1-200 µmol/l. A similar linear relationship was observed for UA in the range 50-1000 µmol/l. Plasma or lymphocyte AA could be reproducibly detected and quantified in concentrations as low as 1.0 µmol/l of plasma or cell acid extract. The coefficient of variation was 2.8% at a mean AA concentration of 47.3 µmol/l (n=40). For a mean UA concentration of 340 µmol/l the coefficient of variation was 2.5% (n=40).

Statistical analysis

The significance of any changes observed was assessed using Student's t-test for paired data where appropriate. The accepted level of significance was P<0.05. All values in the text, Tables and Figures are means±SEM.

RESULTS

The average time to complete the race by these nine subjects was 95±4 min (range 75-111 min). Body weight loss during the race was 1.5±0.1 kg. There was only a small decrease of 1.7±0.7% (P<0.01) in plasma volume after the race compared with the pre-race sample. A significant haemodilution was noted in the days after the race compared with the pre-race sample. Applying the formula of Dill & Costill [10], the changes represent an increase in mean plasma volume of 5.6±2.1% (P<0.05) 1 day after the race, 7.9±1.5% (P<0.01) 2 days after the race and 8.7±2.0% (P<0.01) 3 days after the race when compared with the pre-race value on Sunday.

Changes in the measured variables after the race, compared with the pre-race values, are shown in Table 1. A marked leucocytosis was observed after the race with the
Table 1. Mean circulatory values in the participants 1 h before and 5 min after the race

<table>
<thead>
<tr>
<th></th>
<th>Pre-race</th>
<th>Post-race</th>
<th>Statistical significance (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Packed cell volume (%)</td>
<td>42.9 ± 0.5</td>
<td>43.3 ± 0.4</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>14.6 ± 0.2</td>
<td>15.2 ± 0.2</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Plasma volume (%)</td>
<td>100.0</td>
<td>98.3 ± 0.7</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Total platelets (10⁶ cells/l)</td>
<td>281 ± 20</td>
<td>380 ± 27</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Total leucocytes (10⁶ cells/l)</td>
<td>6.18 ± 0.34</td>
<td>13.47 ± 1.62</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Total granulocytes (10⁶ cells/l)</td>
<td>3.83 ± 0.57</td>
<td>9.57 ± 1.34</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Total lymphocytes (10⁶ cells/l)</td>
<td>1.97 ± 0.22</td>
<td>3.30 ± 0.27</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Lymphocyte AA (μmol/g of protein)</td>
<td>15.6 ± 0.6</td>
<td>19.7 ± 0.9</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Plasma AA (μmol/l)</td>
<td>52.7 ± 4.1</td>
<td>67.0 ± 5.3</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Plasma cortisol (nmol/l)</td>
<td>563 ± 29</td>
<td>968 ± 47</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Plasma UA (μmol/l)</td>
<td>420 ± 32</td>
<td>530 ± 33</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

The predominant increase being granulocytes, with smaller relative increases in circulating lymphocytes and platelets. The total leucocyte, granulocyte, lymphocyte and platelet counts returned to normal (pre-exercise) levels within 24 h after the race.

The concentration of AA in plasma increased markedly during the course of the race (Table 1 and Fig. 1). This increase in plasma AA concentration was positively correlated with the rise in plasma cortisol concentration during the race (r = 0.89; P < 0.01; Fig. 2). Plasma cortisol concentration returned to normal levels within 24 h after the race, but by this time plasma AA concentration had fallen significantly below pre-exercise levels (P < 0.01; Fig. 1) and remained lower than control values 2 and 3 days after the race (P < 0.05; Fig. 1). The post-race haemodilution accounts for the lower plasma AA values 2 and 3 days after the race, but cannot wholly account for the lower plasma AA concentration 1 day after the race. After correction for the changes in plasma volume that occurred, there was still a 15 ± 3% (P < 0.01) fall in plasma AA concentration 1 day after the race compared with the pre-race sample.

Lymphocyte AA concentration was also increased after the race (P < 0.01; Table 1 and Fig. 3). Lymphocyte AA concentration was still significantly elevated 1 day after the race (P < 0.05; Fig. 3), but returned to control (pre-exercise) values within 2 days after the race.

Plasma UA concentration was significantly higher after the race (P < 0.001, Table 1) and returned to the pre-exercise value within 2 days after the race. Prolonged exercise is known to increase plasma UA concentration [13], the magnitude of the increase being related to the duration and intensity of the exercise performed.

**DISCUSSION**

This report confirms that prolonged running produces a more marked granulocytosis and less lymphocytosis than short bouts of exercise, as described by Moorthy & Zimmerman [14], despite the shorter distance run by our subjects (21 km compared with 32 km in the study of Moorthy & Zimmerman [14]). The marked increase in plasma cortisol concentration during the run suggests that the level of stress was significant. While it is known that corticosteroids can induce a decrease in peripheral blood lymphocytes [15], the absolute number of lymphocytes...
increased during the run. However, the increase of 67% was small compared with the 150% increase in granulocytes. Platelet numbers increased by only 35% and hence the proportion of both lymphocytes and of platelets to granulocytes in the blood decreased. This observation, which has also been noted after surgical operations [16], has important implications for the assessment of leucocyte AA concentration which is most often considered to be the best indicator of tissue stores of the vitamin.

Unfortunately, the standard methods of estimating the leucocyte (actually the buffy layer) AA concentration give an overestimate because platelet AA is also measured and attributed to the leucocytes. The platelets contribute 50% of the buffy layer AA in normal subjects [17, 18]. Under stressful conditions (e.g. surgical trauma or prolonged exercise) during which the platelet/leucocyte ratio decreases, an apparently lower AA concentration in the leucocytes would be measured because the newly released cells would artificially dilute the platelet contribution to the leucocyte AA measurement as shown experimentally by Evans et al. [19]. Furthermore, the concentration of AA in essentially pure preparations of lymphocytes has been shown to be more than double that found in granulocytes [20–22]. Thus any change in the relative distribution of granulocytes and lymphocytes in the blood would also result in an apparent change in the leucocyte AA content regardless of any actual change in the AA concentration of the cells. In the present exercise stress situation which induced a larger increase in granulocytes relative to both platelets and lymphocytes, an apparent fall in leucocyte (buffy layer) AA concentration would result. A recent report [16] attributes the observed fall in buffy layer AA concentration in the days after surgical operations entirely to this artifact. Indeed the reported falls in leucocyte (buffy layer) AA concentration after vigorous exercise [6], myocardial infarction, trauma, emotional disturbance and acute infection (reviewed by Schorah [2]) could all be artifactual because of associated granulocytosis. Clearly, AA measurements in separated individual cell fractions should be made where platelet, granulocyte and lymphocyte numbers may vary greatly, such as in the seriously ill patient or during acutely stressful conditions. Even these measurements may not necessarily reflect changes in whole-body reserves, but may be of use in contributing to our understanding of how AA metabolism changes during stress and disease.

Our data on the AA content of isolated lymphocytes indicate that there was an increase in cellular AA concentration by the end of the race, possibly as a result of increased AA uptake from the plasma in which the AA concentration was markedly elevated at this time. The increased plasma AA concentration immediately after the race appears similar to findings of a greatly elevated plasma AA concentration in rats during hypoxia [5] or after non-fatal haemorrhage [3]. The highest concentration of AA in the mammalian body is in the adrenal gland (more than 150 times that found in plasma) and there is an inverse relationship between the amount of AA in the gland and the increased secretory activity of the gland in states of stress [3, 4]. In addition, profound decreases in rat adrenal AA content (together with large rises in plasma AA concentration) have been observed [3] after administration of adrenocorticotropic hormone. We observed a significant positive correlation between the increase in plasma cortisol and AA levels during exercise. The adrenal gland is probably the major source of AA efflux into the circulation during exercise and the rapid rise in plasma AA may be associated with the release of cortisol itself. Adrenocorticotropic hormone, levels of
which may be elevated under stress, has been shown under conditions in vitro to inhibit the uptake of AA into the adrenal cortex (23) and this could also account for an increment in plasma AA concentration.

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REFERENCES