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Original article

# The relationship between acute changes in the systemic inflammatory response and plasma ascorbic acid, alpha-tocopherol and lipid peroxidation after elective hip arthroplasty☆

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SUMMARY

*Background & aims:* Vitamin C (ascorbic acid, AA) is a water soluble vitamin with many functions including antioxidative properties, haemostasis, hormone synthesis, collagen synthesis, carnitine synthesis, bile salt production and enhancing iron absorption. There is some evidence that there is a negative inverse relationship between plasma vitamin C concentration and the systemic inflammatory response as measured by C-reactive protein (CRP).

The aim of the present study was to examine, in the context of a longitudinal study, the change in plasma concentrations of ascorbic acid (AA) and Vitamin E (α-tocopherol, AT) and their relationship to free radical damage during the evolution of the systemic inflammatory response.

*Methods:* Venous blood samples were obtained pre-operatively and at 1, 2, 3 and 90 days post-operatively from 11 patients undergoing elective hip arthroplasty at Glasgow Royal Infirmary. AA, AT, cholesterol, MDA (marker of free radical damage), CRP and albumin were measured in plasma.

*Results:* Plasma AA fell significantly by 74% ($P < 0.01$), AT fell by 36% ($P < 0.01$), cholesterol by 40% ($P < 0.01$), MDA by 38% ($P < 0.01$), albumin by 29% ($P < 0.01$) and CRP increased significantly by 160 fold ($P < 0.01$) during the systemic inflammatory response. The fall in plasma AA remained significant when adjusted for albumin ($P < 0.01$). Plasma AT adjusted for cholesterol did not change significantly during the study period. The fall in plasma MDA remained significant when adjusted for albumin ($P$ 0.01). At 3 months post-operatively, all measurements (including AA) except albumin had returned to baseline values.

*Conclusions:* Plasma AA levels are unlikely to be a reliable measurement of Vitamin C where there is evidence of a systemic inflammatory response. The decrease in plasma AA concentration is likely to be secondary to increased consumption, increased usage neutralising free radicals, increased utilisation in supporting AT regeneration and increased urinary excretion.



## 1. Introduction

Vitamin C (ascorbic acid, AA) is a six-carbon lactone not synthesized in humans [1,2] and deficiency can result in scurvy. Roles of AA include collagen synthesis, carnitine synthesis, bile salt production, haemostasis, hormone synthesis as well as enhancing iron absorption [1,3]. Humans receive Vitamin C from diet (fruit and vegetables) and supplements.

AA is one of the most effective water soluble antioxidants in biological fluids preventing free radical mediated oxidative damage to biological macromolecules including DNA, lipids and proteins. A second important role includes regenerating other antioxidants including the lipid soluble antioxidant α-tocopherol (Vitamin E).

There is some evidence that plasma concentrations of AA can be lower as part of the systemic inflammatory response and this may confound the interpretation and the need for supplementation of AA. Louw and co-workers (1992) reported that following uncomplicated orthopaedic operations, plasma AA concentration fell by approximately 40% at 48 h postoperatively with CRP concentration

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of approximately >100 mg/l. They postulated that these findings could be attributed to redistribution, overhydration and increased requirements for antioxidants. More recently Duncan and co-workers (2012) reported that in a large cohort of patients, there was a larger fall of 78% in plasma AA for CRP concentrations of >80 mg/l [4].

AA and AT play key roles in neutralising free radicals generated by the inflammatory response syndrome. Malondialdehyde (MDA) is a product of lipid peroxidation and levels reflect oxidative stress. Also, AA is involved in the regeneration of AT.

The aim of the present study was to examine the relationship between acute changes in the systemic inflammatory response and plasma concentrations of the antioxidants AA, AT and oxidative stress during the evolution of the systemic inflammatory response, following an elective hip arthroplasty.

## 2. Materials and methods

### 2.1. Patients and study design

Eleven patients, 6 males and 5 females with age range 36–75 years (median 65 years) who underwent elective primary hip replacement surgery and had no evidence of systemic inflammatory response were recruited into the study. Venous blood samples (EDTA) were obtained immediately pre-operatively and post-operatively on each morning for 3 days for the measurement of plasma AA, AT, cholesterol, MDA (marker of oxidative stress), C-reactive protein (CRP) and albumin. Final samples were collected at 90 days.

Blood samples were centrifuged at 2,500 *g*, 4 °C for 15 min. Plasma was stored at −70 °C prior to analysis. Plasma AA was stabilised within 48 h of collection with metaphosphoric acid to prevent oxidation. All samples for each patient were protected from light and assayed in a single batch for each of the analytes to minimise interbatch analytical variation.

The study was approved by the local Ethics Committee. All patients were informed of the purpose and procedure of the study and gave informed consent.

### 2.2. Analytical methods

Cholesterol, CRP and albumin were measured by routine laboratory procedures using an automated analyser (Architect Abbott Diagnostics, USA).

Malondialdehyde in plasma was measured using reverse–phase HPLC with fluorometric detection as previously described [5]. For total MDA measurements, 50 μL of EDTA plasma sample or standard (tetramethoxypropane) was hydrolysed with orthophosphoric acid (100 °C). The MDA released from plasma proteins and the unbound MDA reacted with the thiobarbituric acid (TBA) to form the MDA-TBA adduct and this was measured using reverse-phase HPLC using fluorometric detection to increase sensitivity and specificity [5]. The 95% reference interval for malondialdehyde as established in our laboratory was 0.30–1.00 μmol/l.

Plasma ascorbic acid and α-tocopherol concentrations were measured by high-performance liquid chromatography (HPLC) method [6,7]. Analysis of plasma AA was carried out in brief by, following initial centrifugation and separation, an aliquot of the supernatant was injected on to a C18 reverse-phase chromatographic column (5 μm C18; 3.2 × 250 mm, Nucleosil, Phenomenex, Macclesfield, UK) and the AA concentration assayed using an electrochemical detector. The limit of sensitivity for plasma AA was <10 μmol/l. Plasma AT was deproteinised with alcohol containing tocopherol acetate as an internal standard and extraction was performed using hexane. HPLC analysis was carried out using a reverse-phase analytical column (5 μm C18; 3.2 × 250 mm, Nucleosil, Phenomenex, Macclesfield, UK) with UV monitoring at 295 nm. The limit of sensitivity for plasma α-tocopherol was 3.0 μmol/l.

Within- and between- assay precision was below 10% for all analytes measured.

### 2.3. Statistical analysis

Data are presented as median and range. Data from different time periods were tested for statistical significance using non-parametric ANOVA (Friedman test). Where appropriate, comparisons of data from different time periods was carried out using the Wilcoxon Signed Rank Test. Analysis was performed using SPSS software (SPSS Inc., Chicago, IL, USA).

## 3. Results

The baseline characteristics of the patients who underwent elective hip arthroplasty surgery are presented in Table 1. Pre-operatively, the baseline vitamins and malondialdehyde (MDA) measurements were within our laboratory derived population reference ranges; AA (15–90 μmol/L), AT (12–46 μmol/L) and MDA (0.3–1.00 μmol/L). The results are shown in Table 2.

On postoperative day 2, there was a significant fall in plasma AA of 74% (median 16, range 9–47 μmol/l, $P < 0.01$, see Fig. 1). Plasma AT, cholesterol, MDA and albumin also fell significantly by 36%, 40%, 38% and 29% respectively ($P < 0.01$, see Fig. 2) whereas there was a significant increase in circulating CRP concentrations (median 169, range 92–273 mg/l, $P$ 0.003).

The changes seen in each of the above measurements remained significant throughout the study period before returning to their baseline at 90 days. Albumin, however, remained significantly lower than baseline by 4% ($P < 0.05$). Interestingly, median plasma AA had only returned to 55% of its baseline concentration at 3 months but this result was not found to be significant ($P = 0.306$) .

Since plasma AA is recognised to bind to albumin (approximately 20–30%) and albumin (similar to the other binding proteins) is readily redistributed during the systemic inflammatory response, it was used to adjust for the acute effect of the systemic inflammatory response on plasma AA concentrations. Plasma AA, when adjusted for albumin, still fell significantly by 62% in the first 48 h ($P < 0.01$, see Fig. 3). By 3 months, it remained 26% lower than baseline but again, this was not found to be significant ($P = 0.328$).

Plasma AT is recognised to bind to cholesterol (approximately 70–80%) and cholesterol (similar to the other binding lipids) is readily redistributed during the systemic inflammatory response, it was used to adjust for the acute effect of the systemic inflammatory response on plasma AT concentrations. Plasma AT, when adjusted

**Table 1**
Characteristics and baseline measurements.

| | Reference interval | Operative cohort ($n = 11$) |
|---|---|---|
| Age (Years) | N/A | 65 (36–75) |
| Sex (Male/female) | N/A | 6 (55%)/5 (45%) |
| C-reactive protein (mg/l) | <6 | 6 (6–6) |
| Albumin (g/l) | 35–55 | 45 (39–48) |
| Cholesterol (mmol/l) | 2.8–5.7 | 5.8 (4.20–8.10) |
| Malondialdehyde (μmol/l) | 0.3–1.00 | 0.79 (0.50–1.08) |
| Ascorbic acid (μmol/l) | 15–90 | 61 (23–127) |
| α-Tocopherol (μmol/l) | 12–46 | 21 (25–35) |
| Ascorbic acid/albumin | N/A | 1.36 (0.52–2.82) |
| α-Tocopherol/cholesterol (μmol/mmol) | 3.5–9.5 | 4.31 (3.29–7.29) |