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Elevated plasma levels of reduced homocysteine in common variable immunodeficiency – a marker of enhanced oxidative stress

P. AUKRUST, R. K. BERGE,* F. MÜLLER, P. M. UELAND,† A. M. SVARDAL† & S. S. FRØLAND Section of Clinical Immunology and Infectious Diseases and Research Institute for Internal Medicine, Medical Department A, University of Oslo, The National Hospital, Rikshospitalet, Oslo, and Divisions of *Clinical Biochemistry and †Pharmacology, Department of Clinical Biology, University of Bergen, Haukeland Hospital, Bergen, Norway

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Abstract. Based on previous studies from our group, we hypothesized that enhanced oxidative stress in association with a persistent immune activation may be important in both the immunopathogenesis and certain clinical manifestations in a subgroup of patients with common variable immunodeficiency (CVI). To explore this hypothesis further, we examined plasma levels of lipid peroxidation, antioxidant vitamins and redox status of various thiol species in 20 CVI patients and 16 healthy control subjects. We found significantly higher malondialdehyde (MDA) levels in plasma from CVI patients than in healthy control subjects. Furthermore, in a subgroup of CVI patients characterized by persistent immune activation *in vivo* (CVI_{Hyper}), we found significantly decreased levels of vitamin E and β-carotene. In the CVI patients, there was a significant inverse correlation between MDA levels and levels of vitamin E and β-carotene. Finally, we found a marked elevation in plasma levels of reduced homocysteine in the CVI group, but no corresponding rise in plasma levels of total homocysteine. In the CVI group, the high plasma levels of reduced homocysteine were significantly correlated with enhanced lipid peroxidation and low levels of vitamin E. The results of the present study further support a role for enhanced oxidative stress in the immunopathogenesis of CVI. Furthermore, our finding of markedly elevated plasma levels of reduced homocysteine in CVI patients without simultaneous elevation of other homocysteine species suggests that this disturbance in homocysteine metabolism may be related to enhanced oxidative stress.

Keywords. β-Carotene, homocysteine, common variable immunodeficiency, lipid peroxidation, oxidative stress, vitamin E.

Introduction

Aerobic life processes are characterized by a steady formation of reactive oxygen species (ROS) balanced by a similar rate of their consumption by antioxidants [1]. To maintain homeostasis, there is a requirement for a continuous regeneration of antioxidant capacity, and if this is not met oxidative stress ensues [1]. Oxidative stress seems to be involved in the pathogenesis of several clinical disorders, including heart and brain ischaemic diseases, and may also be involved in carcinogenesis [1,2]. Increased oxidative stress may also play an important pathogenic role in the development of immunodeficiency in man, including age-related decline in immunological functions [3] and human immunodeficiency virus (HIV) infection [4,5].

Common variable immunodeficiency (CVI) is a heterogeneous group of B-cell deficiency syndromes characterized by defective antibody production and recurrent bacterial infections [6,7]. However, subgroups of these patients are also associated with an increased incidence of lymphoid hyperplasia, autoimmune diseases and malignancies [6,7]. Furthermore, several studies have demonstrated that the immunological abnormalities in CVI are not restricted to B cells but also involve T cells and monocytes/macrophages [8,9].

We have demonstrated the presence of persistent immune activation in CVI, particularly in the tumour necrosis factor system, and also evidence for increased oxidative stress in subgroups of patients [9–11]. We have hypothesized that this increased oxidative stress associated with a sustained immune activation may be important both in the immunopathogenesis and for certain clinical manifestations in subgroups of CVI patients. In the present study, we used different experimental approaches to explore this hypothesis further. We have recently demonstrated abnormalities in glutathione and cysteine metabolism in CVI patients [10], and in the present study we analysed the redox status of two other thiols in plasma, homocysteine and cysteinylglycine. All these thiols are metabolically related [12], and it is of particular interest to analyse homocysteine redox status as this thiol species has been suggested to have important pro-oxidant effects [13]. Furthermore, several antioxidants appear to have co-operative interactions [14]. For example, glutathione has been found to be involved in

Correspondence: P. Aukrust, Section of Clinical Immunology and Infectious Diseases, Medical Department A, Rikshospitalet, N-0027 Oslo, Norway.

recycling of both β-carotene and vitamin E [15]. To study further the antioxidant status in CVI patients, we therefore analysed plasma levels of β-carotene and vitamin E.

Materials and methods

Patients and control subjects

We studied 20 patients with CVI previously described by us [10]. The diagnosis of CVI was defined as described elsewhere [7,8,16]. The clinical and immunological features of the patients are summarized in Table 1. Based on previously defined criteria [16], 11 patients had splenomegaly, seven had chronic rhinosinusitis, three had nodular intestinal lymphoid hyperplasia, three had bronchiectasis and two had autoimmune disorders.

We have previously delineated a specific subgroup of CVI patients (CVI_{Hyper}) characterized by persistent immune activation *in vivo*, reduced numbers of $CD4^+$ lymphocytes in peripheral blood and increased occurrence of splenomegaly compared with other CVI patients (CVI_{Norm}) [10,11,16]. In the present study, patients were classified as CVI_{Hyper} if they had $CD4^+$ lymphocyte counts $< 400 \times 10^6\,L^{-1}$, serum neopterin levels > the mean + 4 SD of control subjects ($22{\cdot}0\,nmol\,L^{-1}$) and splenomegaly (Table 1). These characteristics persisted when patients were evaluated at least three times over a minimum of 2 years.

At the time of the study, serum levels of ALAT were $< 50\,U\,L^{-1}$ and serum creatinine levels $< 100\,\mu mol\,L^{-1}$ in all patients. All patients were treated with subcutaneously self-administered immunoglobulin during the last 3 months before blood collection. All patients were without manifestations of acute infection or acute exacerbation of chronic infection at the time of blood collection (3 weeks before to 1 week after), and all had a C-reactive protein (CRP) level in serum below $5\,mg\,L^{-1}$. None was receiving antibiotics.

There was no weight loss, defined as a loss of body weight greater than 10% during 6 months, in any of the patients. None of the patients suffered from diarrhoea, defined as more than two loose stools per day for at least 1 month.

Control subjects in the study were 16 sex- and age-matched healthy blood donors (Table 1). All patients and control subjects were without family history of coronary heart disease or hyperlipidaemia, none were smokers and none were abusing alcohol. None were supplementing their diets with vitamins or minerals and none were vegetarians. Informed consent was obtained from all patients and control subjects.

Blood sampling protocol

Blood samples were drawn between 08.00 and 10.00 h after an overnight fast. For determination of various thiol components in plasma, blood was routinely collected into three evacuated tubes placed in melting ice containing heparin as an anticoagulant and either monobromobimane (mBrB, Molecular Probes, Eugene, OR, USA) or *N*-ethylmaleimide (NEM, Sigma, St Louis, MO, USA) as thiol-derivatizing reagent, or no addition [17]. Plasma from the heparin tube with no thiol-reactive reagent was also used for determination of vitamin E, β-carotene and malondialdehyde (MDA) levels. The blood was centrifuged within 15 min ($400 \times \boldsymbol{g}$ for 10 min at 4 °C). Plasma was then transferred to sterile Eppendorf tubes (Eppendorf, Hamburg, Germany) and further centrifuged at $10\,000 \times \boldsymbol{g}$ for 5 min at 4 °C. To NEM- and mBrB-treated plasma a solution of sulphosalicylic acid (final concentration of 5%; Merck, Darmstadt, Germany) containing dithioerythritol (DTE, final concentration of 50 mmol L^{-1}; Sigma) was added before freezing. Serum and plasma samples were stored at −70 °C until analysis and were frozen and thawed only once.

Determination of redox status of plasma thiols

The amounts of reduced and oxidized thiols were

Table 1. Clinical and immunological characteristics of the study group

	Controls ($n = 16$)	CVI_{Hyper} ($n = 9$)	CVI_{Norm} ($n = 11$)
Median age in years (range)	41 (22–65)	39 (21–63)	44 (25–67)
Men/women	6 (38%)/10 (63%)	4 (44%)/5(56%)	3(27%)/8(73%)
$CD2^+$ lymphocytes ($\times 10^6\,L^{-1}$)	1025 (740–1150)	630* (365–1040)	980 (820–1100)
$CD4^+$ lymphocytes ($\times 10^6\,L^{-1}$)	560 (470–690)	215**† (190–310)	410* (325–500)
$CD8^+$ lymphocytes ($\times 10^6\,L^{-1}$)	350 (220–400)	300 (180–450)	490 (300–690)
$CD19^+$ lymphocytes ($\times 10^6\,L^{-1}$)	150 (120–170)	40** (25–120)	140 (95–210)
Monocytes ($\times 10^6\,L^{-1}$)	260 (210–290)	250 (220–390)	280 (230–365)
Serum neopterin ($nmol\,L^{-1}$)	9·1**† (5·9–10·1)	38·7**† (27·1–55·3)	23·2* (10·1–32·4)
Serum cobalamin ($pmol\,L^{-1}$)	380 (280–410)	340 (250–490)	300 (280–450)
Serum folate ($nmol\,L^{-1}$)	11·5 (9·0–13·8)	10·9 (8·6–15·3)	10·2 (8·1–14·2)
Erythrocyte folate ($nmol\,L^{-1}$)	820 (590–910)	780 (550–1080)	790 (525–1005)

CVI_{Hyper} represents patients with $CD4^+$ lymphocyte counts less than $400 \times 10^6\,L^{-1}$, serum neopterin levels greater than $22{\cdot}0\,nmol\,L^{-1}$ and splenomegaly (see Materials and methods). CVI_{Norm} represents the other CVI patients. Lymphocyte subsets and monocytes are analysed in peripheral blood. Data are given as medians and 25th–75th percentiles if not otherwise stated. *$P < 0{\cdot}01$ vs. controls, **$P < 0{\cdot}001$ vs. controls, $^\dagger P < 0{\cdot}05$ vs. CVI_{Norm}.

obtained from blood collected into solutions containing mBrB and NEM, respectively, as described previously [17]. The total amount of thiol components (oxidized + reduced + protein-bound forms) was assayed in non-treated plasma in which a solution of sulphosalicylic acid (final concentration of 5%) containing DTE (final concentration of 50 mmol L^{-1}) was added before thawing. The protein-bound fraction was determined by subtracting the reduced and free oxidized species from the total amount. For each of the thiols, the ratio of reduced to total species was determined as a measure of the redox status of that thiol.

Measurement of β-carotene, vitamin E and MDA

Plasma was analysed for β-carotene and vitamin E by the high-performance liquid chromatography (HPLC) method as described elsewhere [18]. MDA as its thiobarbituric acid complex was measured by the HPLC method described by Li & Chow [19].

Other biochemical analyses

Serum levels of creatinine, ALAT, CRP, albumin and cholesterol were analysed in a Hitachi-705 autoanalyser (Hitachi, Tokyo, Japan). Serum level of prealbumin was quantified by nephelometry calibrated by commercial standards (Boehringer Mannheim, Mannheim, Germany). Serum cobalamin and folate in serum and erythrocytes were quantified by standard methods using radioimmunoassays (Diagnostic Product, Los Angeles, CA, USA).

Immunological analyses

The numbers of $CD2^+$, $CD4^+$, $CD8^+$, $CD19^+$ lymphocytes and monocytes were determined by immunomagnetic quantification [16]. Neopterin levels were determined by radioimmunoassay (IMMUtest Neopterin, Henning Berlin, Berlin, Germany).

Statistical analysis

For comparison of two groups of individuals, the two-tailed Mann–Whitney *U*-test was employed. When more than two groups were compared, the Kruskal–Wallis test was used as described elsewhere [11].Coefficients of correlation (*r*) were calculated by the Spearman rank test. Data are given as medians and 25th–75th percentiles if not otherwise stated. *P*-values are two-sided and considered significant when $< 0{\cdot}05$.

Results

Plasma levels of MDA

We first measured plasma levels of MDA, as a parameter of lipid peroxidation [1], in both CVI patients and control subjects. As shown in Fig. 1, CVI patients had significantly higher MDA levels than healthy blood donors with the highest levels in the CVI_{Hyper} group (approximately 50% increase in CVI_{Hyper} patients compared with control subjects).

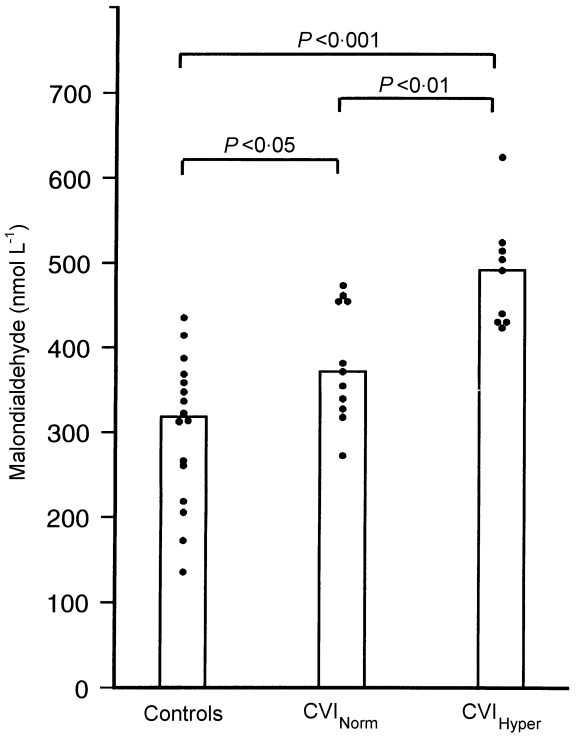
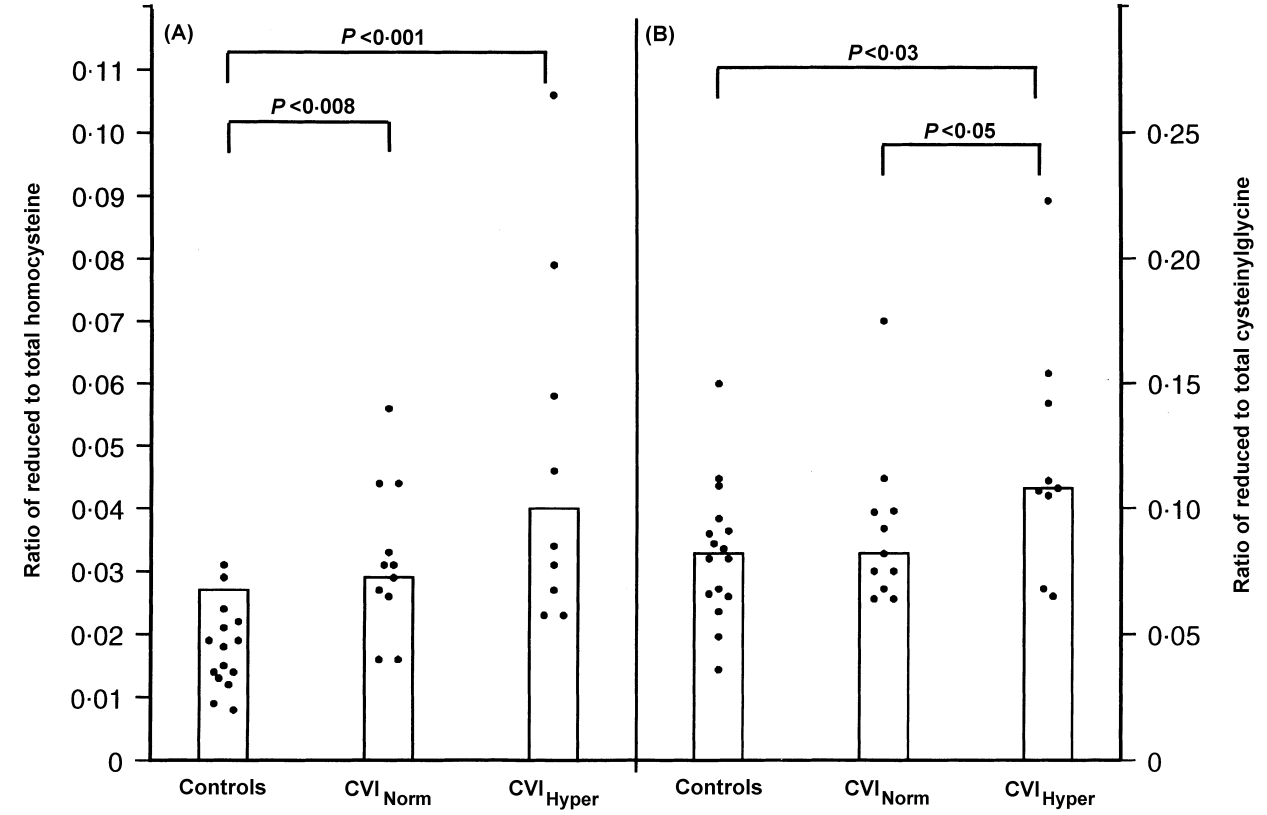


Figure 1. Plasma levels of MDA in two different subgroups of CVI patients and in healthy control subjects ($n = 16$). CVI_{Hyper} ($n = 9$) represents patients with $CD4^+$ lymphocyte counts in peripheral blood less than 400×10^6 L^{-1}, serum neopterin levels greater than 22·0 nmol L^{-1} and splenomegaly (see Materials and methods). CVI_{Norm} ($n = 11$) represents the other CVI patients. The bars represent median values.

Redox status of homocysteine, cysteine and cysteinylglycine

We found a marked increase in plasma levels of reduced homocysteine in CVI patients compared with levels in healthy control subjects (approximately 120% increase, Table 2). In fact, only four of the CVI patients had plasma concentration of reduced homocysteine within the range of the control subjects. Also, plasma levels of reduced cysteinylglycine were significantly increased in CVI (approximately 40% increase, Table 2). Some of the cysteine parameters have previously been described in this study population [10], and are summarized in Table 2. As for homocysteine and cysteinylglycine, there was a significant increase in plasma level of the reduced form also of cysteine in the CVI group. In contrast, no significant differences in levels of either total, oxidized or protein-bound forms of these aminothiols were found between CVI patients and control subjects (Table 2).

Thus, whereas we have previously demonstrated low plasma levels of total glutathione in these CVI patients [10], we found a significant increase in plasma levels of the reduced form of the other aminothiols with the most marked increase in levels of reduced homocysteine.



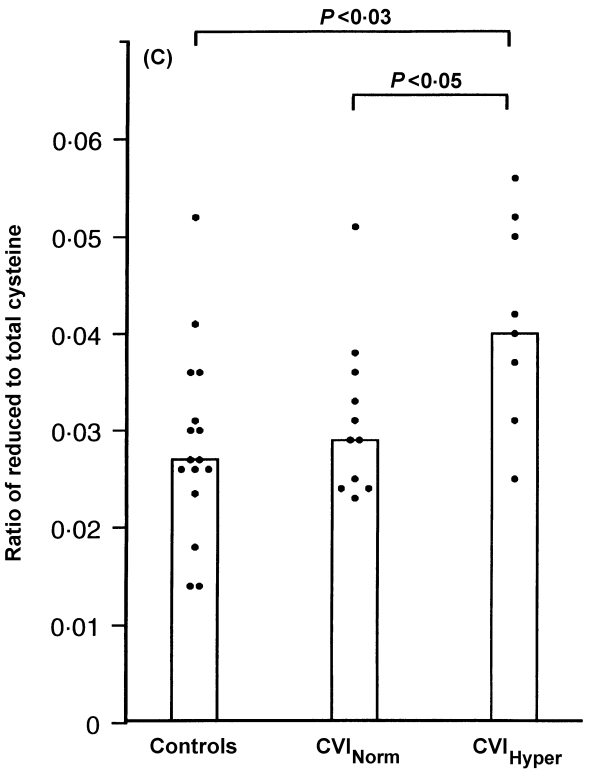


Figure 2. Ratio of reduced to total homocysteine (A), cysteinylglycine (B) and cysteine (C) in healthy control subjects ($n = 16$) and in two different subgroups of CVI patients. The definitions of CVI_{Hyper} ($n = 9$) and CVI_{Norm} ($n = 11$) are given in the legend to Fig. 1. The bars represent median values.

These disturbances resulted in altered redox status with a high ratio of reduced to total form for homocysteine, cysteinylglycine and cysteine in the CVI group (Table 2).

As can be seen in Fig. 2, CVI_{Hyper} patients had higher ratios of reduced to total plasma thiols for cysteine, cysteinylglycine and homocysteine than other CVI patients (Fig. 2). Also, the reduced form of these three thiol species tended to be higher in the CVI_{Hyper} group, although the differences did not reach statistical significance (data not shown).

Plasma levels of vitamin E and β-carotene

The CVI patients as a group tended to have lower levels of vitamin E and β-carotene than healthy control subjects, although these differences did not reach statistical significance [vitamin E 21·3 μmol L^{-1} (16·3–28·4 μmol L^{-1}) vs. 26·5 μmol L^{-1} (23·4–35·1 μmol L^{-1}) and β-carotene 0·131 μmol L^{-1} (0·089–0·232 μmol L^{-1}) vs. 0·257 μmol L^{-1} (0·141–0·378 μmol L^{-1}), CVI patients and control subjects respectively]. However, the CVI_{Hyper} subgroup had significantly lower plasma levels of vitamin E as well as of β-carotene compared both with other CVI patients and with healthy control subjects (Fig. 3).

Vitamin E and β-carotene are transported in blood lipoproteins, particularly in low-density lipoprotein [20], and plasma levels of these carrier proteins will influence levels of lipid-soluble micronutrients. However, we

Table 2. Plasma levels of total, reduced, oxidized and protein-bound thiol species in 20 CVI patients and 16 healthy control subjects

	CVI	Control subjects
Homocysteine (μmol L^{-1})		
Total	11·80 (9·44–13·79)	11·25 (8·87–12·30)
Reduced	0·38 (0·28–0·51)*	0·17 (0·15–0·24)
Oxidized	2·02 (1·73–2·32)	1·69 (1·52–2·53)
Protein-bound	9·18 (7·34–10·92)	8·80 (7·08–10·07)
Ratio of reduced to total	0·031 (0·025–0·045)*	0·018 (0·013–0·022)
Cysteinylglycine (μmol L^{-1})		
Total	31·6 (27·7–36·9)	29·1 (26·1–32·9)
Reduced	3·0 (2·4–3·8)†	2·1 (1·9–2·7)
Oxidized	11·1 (8·6–12·8)	9·9 (8·5–11·0)
Protein-bound	18·5 (13·4–21·2)	16·8 (14·4–19·6)
Ratio of reduced to total	0·098 (0·072–0·112)	0·082 (0·066–0·093)
Cysteine (μmol L^{-1})		
Total	316·4 (288·6–350·2)	306·6 (291·5–355·0)
Reduced	9·9 (9·2–12·2)†	8·4 (7·5–10·4)
Oxidized	119·4 (103·5–130·0)	119·4 (109·0–130·1)
Protein-bound	191·2 (177·8–210·7)	186·4 (169·4–216·5)
Ratio of reduced to total	0·032 (0·027–0·041)	0·027 (0·025–0·035)

Data are given as medians and 25th–75th percentiles. *$P < 0{\cdot}001$ vs. control subjects; $^{\dagger}P < 0{\cdot}03$ vs. control subjects.

found no significant difference in cholesterol levels between CVI patients and control subjects [5·2 mmol L^{-1} (4·3–6·3 mmol L^{-1} vs. 5·5 mmol L^{-1} (4·5–6·5 mmol L^{-1}), CVI patients and control subjects, respectively], or between the CVI_{Hyper} subgroup and other CVI patients (data not shown). This indicates that the low plasma levels of vitamin E and β-carotene in the CVI_{Hyper} subgroup do not merely reflect low levels of carrier proteins in these patients.

In CVI patients, there was a significant correlation between β-carotene and vitamin E levels ($r = 0{\cdot}67$, $P < 0{\cdot}002$). Furthermore, in the CVI group, but not in healthy control subjects (data not shown), vitamin E and β-carotene levels in plasma were significantly inversely correlated with MDA levels ($r = -0{\cdot}57$, $P < 0{\cdot}009$ and $r = -0{\cdot}55$, $P < 0{\cdot}02$, β-carotene and vitamin E respectively).

Clinical manifestations and plasma levels of MDA, thiols and vitamins

Acute-phase response and infections may influence plasma levels of various vitamins and may result in increased oxidative stress [21]. However, none of the CVI patients had any clinical manifestations of acute infection at the time of the study, and all had serum levels of CRP within normal limits. Furthermore, patients with chronic rhinosinusitis or bronchiectasis did not have levels of MDA, thiols or vitamins that were significantly different from those of other CVI patients (data not shown).

Malabsorption and malnutrition may influence plasma levels of vitamins and various thiol species [22]. However, there were no signs of either weight loss or diarrhoea in the CVI patients, and all had serum levels of albumin and prealbumin within normal limits (data not shown). Furthermore, the three CVI_{Hyper} patients with nodular intestinal lymphoid hyperplasia were not significantly different from other CVI_{Hyper} patients with regard to levels of MDA, thiols and vitamins (data not shown).

Blood concentration of cobalamin and folate

Decreased levels of folate and cobalamin may markedly influence homocysteine metabolism [23]. However, all CVI patients, except for one with a slight decrease in concentration of erythrocyte folate (410 nmol L^{-1}), had cobalamin and erythrocyte and serum folate levels within normal limits and equal to control subjects (Table 1). The one patient with a low folate level had similar concentrations of the various homocysteine species, including level of reduced homocysteine, to the other CVI patients (data not shown).

Levels of reduced homocysteine in relation to levels of antioxidants and lipid peroxidation

In CVI patients plasma levels of reduced homocysteine, but not any of the other thiol parameters, were positively correlated with plasma levels of MDA ($r = 0{\cdot}46$, $P < 0{\cdot}05$) and negatively correlated with plasma levels of vitamin E ($r = -0{\cdot}59$, $P < 0{\cdot}009$; Fig. 4). Also, plasma levels of β-carotene tended to be inversely correlated with reduced homocysteine levels among CVI patients ($r = -0{\cdot}41$, $P = 0{\cdot}06$). Thus, the marked increase in plasma levels of reduced homocysteine in the CVI group without any simultaneous increase in the corresponding levels of total homocysteine appears to be

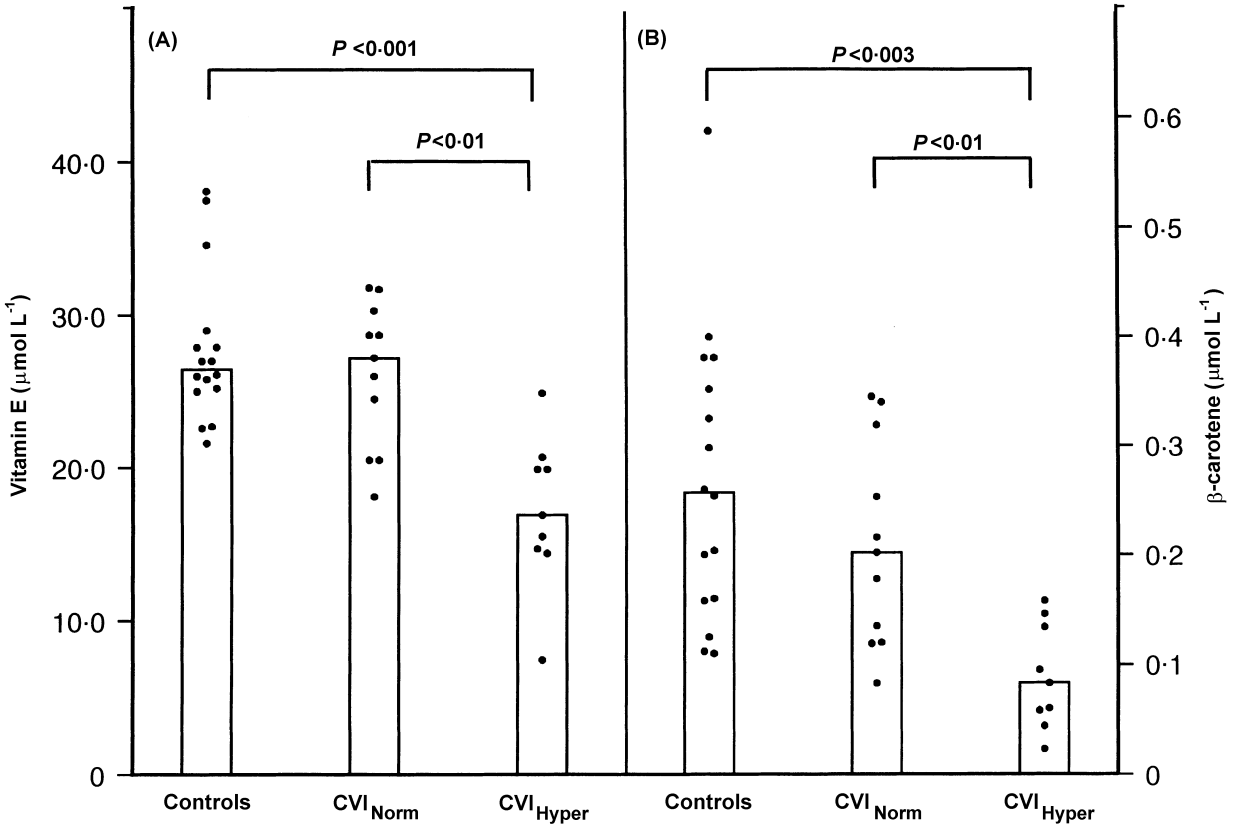


Figure 3. Levels of vitamin E (A) and β-carotene (B) in healthy control subjects ($n = 16$) and in two different subgroups of CVI patients. The definitions of CVI_{Hyper} ($n = 9$) and CVI_{Norm} ($n = 11$) are given in the legend to Fig. 1. The bars represent median values.

correlated with enhanced lipid peroxidation and decreased antioxidant levels in plasma in these patients.

Discussion

The results of the present study support a role for increased oxidative stress in the immunopathogenesis of CVI. We found that CVI patients are characterized by enhanced lipid peroxidation in plasma, and in a particular subgroup of CVI patients (CVI_{Hyper}) we found markedly decreased levels of the lipid-soluble antioxidants β-carotene and vitamin E. These findings support the notion that enhanced oxidative stress is an important characteristic in this subgroup of CVI.

Another striking finding in the present study was the markedly elevated plasma level of reduced homocysteine in the CVI group without simultaneously elevated levels of the other homocysteine species. Elevated plasma levels of reduced homocysteine have been found in various other clinical conditions such as homocystinuria, cobalamin deficiency and in patients with early-onset peripheral vascular disease [24]. However, in contrast to the present study, in all these conditions, the elevation of reduced homocysteine has been associated with increased plasma concentrations of total homocysteine. We have recently reported markedly raised plasma levels of reduced homocysteine in combination with normal plasma levels of total homocysteine in HIV-infected individuals [25]. Interestingly, there are several immunological similarities between HIV-infected individuals and subgroups of CVI patients, including persistent immune activation *in vivo* [10,11]. Furthermore, as in HIV-infected individuals [25], we could not relate the elevated levels of reduced homocysteine to either folate or cobalamin deficiency. We suggest that, whereas elevated levels of both reduced and total homocysteine may be related to deficiency of

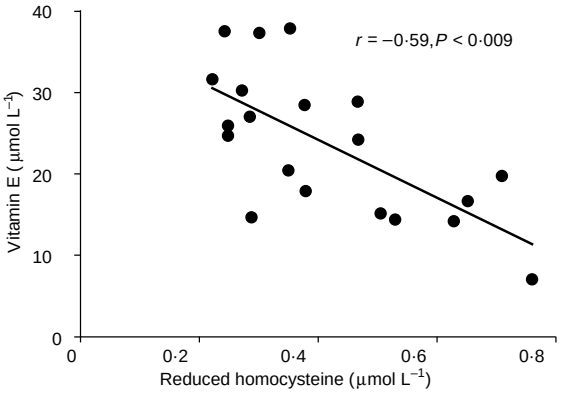


Figure 4. Correlation between vitamin E and reduced homocysteine levels in plasma in 20 CVI patients.

vitamin co-factors required for homocysteine metabolism [13,23], an exclusive elevation of reduced homocysteine may be caused by other factors. We cannot exclude the possibility that the measured reduced homocysteine in plasma in CVI is trapped in a form that reacts with derivatizing reagents, e.g. S-nitroso-homocysteine [13]. However, the results of the present study suggest that such an elevation of reduced homocysteine, possibly representing a redistribution within the pool of various homocysteine species, may be a consequence of increased oxidative stress. Another non-mutually exclusive possibility is that the decreased glutathione levels in these CVI patients [10] may at least partly be caused by decreased synthesis, which in turn might lead to elevated concentrations of its precursors such as cysteine and homocysteine. However, further studies are needed to clarify the mechanism leading to this disturbance in homocysteine metabolism.

Homocysteine has recently received considerable attention, especially because hyperhomocysteinaemia is a risk factor for early onset of cardiovascular disease [23]. Although the mechanisms are somewhat unclear, it is believed that homocysteine exerts its effects in the pathogenesis of atherosclerosis through mechanisms involving oxidative stress-induced damage [13]. The determination of redox thiol status in plasma is particularly challenging because redox conditions change rapidly (within seconds) after blood sampling [26], and in most studies the association between increased levels of homocysteine and atherosclerosis is based on the demonstration of increased levels of total homocysteine [23]. However, the results of the present study, demonstrating significant correlations between high plasma levels of reduced homocysteine and enhanced levels of lipid peroxidation and low plasma levels of antioxidants, suggest that reduced homocysteine rather than total homocysteine may be involved in the generation of increased oxidative stress. However, analyses based on correlations should be interpreted with caution, and additional functional studies are needed to clarify further the biological significance of this exclusive elevation of reduced homocysteine. Nevertheless, our findings of markedly elevated levels of reduced homocysteine without simultaneous elevation of other homocysteine species, representing a previously unrecognized disturbance in homocysteine metabolism, may possibly be a 'new' marker of enhanced oxidative stress.

In addition to raised levels of reduced homocysteine, the present study demonstrates for the first time high plasma levels of MDA and low plasma levels of β-carotene and vitamin E in CVI, with the most pronounced disturbances in the CVI_{Hyper} group. Although a moderate elevation of MDA levels may be caused by factors other than enhanced lipid peroxidation, these findings support the notion of enhanced oxidative stress in CVI patients. Several factors may result in low plasma levels of β-carotene and vitamin E such as low intake, malabsorption, inadequate release from the liver, acute-phase response, inadequate availability of its carrier molecule and/or rapid metabolism [27]. In the CVI patients, several of the above-mentioned factors are unlikely explanations for the low plasma levels of vitamins (see Results). However, the previous demonstration of increased ROS production and increased oxidative stress in CVI [9,10], combined with the correlation between high levels of MDA and low levels of vitamin E and β-carotene in CVI patients found in the present study, suggest that enhanced consumption as a result of increased oxidative stress may at least partly explain the low vitamin levels [1,28].

The pro-oxidant–antioxidant balance is an important determinant of immune cell function [4,28]. Even a marginal deficiency of several antioxidants including vitamin E and glutathione may adversely affect the immune response [28,29]. Notably, water-soluble antioxidants, such as glutathione, and the lipid-soluble vitamin E may act synergistically to protect cells from oxidative stress-induced damage [14,15,29]. Thus, the combined deficiency of glutathione [10], β-carotene and vitamin E in CVI patients may markedly increase the oxidative stress in these patients with important immunological as well as clinical consequences. Deficiency of these vitamins has been found to impair several immune functions with relevance to CVI, e.g. lymphocyte proliferation and B-cell functions including antibody production and mucosal immunity [30,31]. Furthermore, the demonstrated vitamin deficiency may contribute to the increased incidence of cancer found in CVI patients [1,7,8].

In conclusion, although the fundamental cause of CVI is not known, and most probably aetiological and immunopathogenic factors may differ between different subgroups of patients, the demonstration of enhanced lipid peroxidation and decreased levels of antioxidant vitamins in a subgroup of CVI patients further supports a role for enhanced oxidative stress in the pathogenesis of CVI. Furthermore, our finding of markedly elevated plasma levels of reduced homocysteine in CVI patients, without simultaneous elevation of the other homocysteine species, correlated with low levels of vitamin E and enhanced lipid peroxidation, suggest that this disturbance in homocysteine metabolism may be related to enhanced oxidative stress.

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