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4-Hydroxynonenal is Markedly Higher in Patients on a Standard Long-term Home Parenteral Nutrition

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Parenteral nutrition, a commonly used procedure in patients with gastrointestinal disorders, may lead with time to liver steatosis and fibrosis, whose pathogenesis has yet to be elucidated. Oxidative stress and particularly lipid peroxidation likely contribute to the expression of such hepatobiliary complications, by means of their recognized proinflammatory and profibrogenic effects. To evaluate the adequacy against oxidative insult of a standard micronutrient supplementation in patients under long term parenteral nutrition, a comprehensive patterns of redox indices has been determined on peripheral blood samples from forty one adults in comparison to fifty eight blood donors taken as controls. A sustained oxidative stress in peripheral blood of home parenteral patients was observed. Of the two lipid peroxidation markers found to be markedly increased, namely fluorescent plasma protein adducts with malondialdehyde and 4-hydroxynonenal, respectively, only the second was statistically correlated with all the antioxidant-related changes consistently detected in the patients, namely decreased plasma α-tocopherol and selenium intake and higher erythrocyte oxidized glutathione.

Plasma level of 4-hydroxynonenal-protein adducts appears to be a reliable and easily measurable marker of oxidative status, particularly indicated to monitor the adequacy of dietary regimen during parenteral nutrition.

Keywords: 4-Hydroxynonenal; Oxidative stress; Lipid peroxidation; Parenteral nutrition; Antioxidants

INTRODUCTION

Parenteral nutrition is a commonly-used procedure in patients with gastrointestinal disorders in order to prevent malnutrition-associated diseases and death.

The most common indications for parenteral nutrition are mesenteric vascular disease, Crohn's disease, radiation enteritis and gastrointestinal cancer.[1,2] Perfusion of nutritional solutions has over time been better standardized and technologically improved, thus significantly reducing spectrum and frequency of complications and increasing the number of patients maintained at home. However, the hepatobiliary complications occurring in patients undergoing long-term home parenteral nutrition (HPN) are still relatively frequent, in particular steatosis, steatohepatitis, cholestasis and gall bladder disease.[3]

While it is certainly difficult to identify the pathomechanisms of such complications in a nutritional regimen often lasting more than 5 years, these disturbances have generally been related essentially both to a decreased bile flow[3] and to unbalanced i.v. intake of nutrients (high energy amounts and dextrose or lipid overfeeding).[4,5]

With regard to hepatobiliary complications of HPN patients, oxidative changes of cell redox equilibrium have been increasingly associated to the pathogenesis of several chronic liver diseases, mainly because of the known pro-inflammatory and pro-fibrogenic effects that certain lipid peroxidation products may exert.[6,7]

Some recent human studies have provided evidence of oxidative stress in patients undergoing parenteral nutrition. Serum malondialdehyde (MDA) was

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the marker of lipid peroxidation almost constantly considered,[8–10] being in very few reports pentane exhalation measured;[11] MDA as well as pentane production showed a significant increase in HPN patients compared to healthy donors. In parallel, a net decrease of plasma α-tocopherol was consistently observed in these patients. The biochemical redox imbalance observed in the blood of HPN is potentially depending upon different causes: (i) inadequate intake of molecules involved in activity and/or synthesis of antioxidant compounds, such as metallic micronutrients (zinc, selenium, copper, manganese) or amino acid precursors of glutathione; (ii) deficiency of vitamins with antioxidant properties (A, C, E); (iii) overload of *n*-6 polyunsaturated fatty acids (PUFA); (iv) intake of significant amounts of oxidative species generated within the parenteral solutions.[8–12]

Of note, of the aldehydic products of lipid peroxidation, MDA shows a biochemical reactivity which is at least one order of magnitude lower than that of another quantitatively important aldehyde, i.e. 4-hydroxynonenal (HNE). Like MDA, HNE derives from the oxidation of *n*-6 PUFAs, among which are the two most represented fatty acids in biomembranes, i.e. arachidonic and linoleic acids. HNE has been shown to possess a large number of biochemical effects, some with demonstrated biological impact.[13–15] In addition, both biochemical and histochemical analyses have proved the consistent association of increased HNE steady-state levels with chronic liver diseases characterized by steatosis and/or fibrosis.[16,17]

Thus, we deemed of interest to analyze the biochemical redox balance of a relatively large group of adult patients with intestinal failure on standard long-term HPN, looking, in particular, at the HNE plasma level as a marker of high toxicologic and pathophysiologic significance. By this way, the problem of the actual adequacy of standard HPN regimen in preventing oxidative damage was also addressed and revisited.

PATIENTS AND METHODS

Patients Recruitment

All the patients currently on HPN were included in this restrospective study under the Clinical Nutrition Center of San Giovanni Battista Hospital of Turin (Italy). Forty one patients were enrolled (25 women, 16 men) with an average age of 61 ± 15 years, affected by chronic non-malignant intestinal failure (14 mesenteric vascular disease; 9 radiation enteritis; 5 Crohn's disease; 3 fibroadhesive peritonitis; 2 hypogammaglobulinemia; 3 surgical sequelae; 2 chronic pseudo-obstruction; 1 small bowel diverticulosis; 1 intestinal lymphoma; 1 Whipple disease). The indications for HPN were: short bowel syndrome in 36 subjects, chronic subocclusion in 2 subjects, malabsorption in 3 subjects. Median HPN duration was 2636 days (172–5499) and the number of i.v. infusions/week ranged from 3 to 7.

The HPN regimen was: glucose 2.55 ± 1.27 g/kg/day; amino acids 0.81 ± 0.34 g/kg/day; total lipids 26.7 ± 16.2 g/day; PUFA 5.39 ± 2.47 g/day; vitamin E 0.55 ± 0.32 mg/g of PUFA/day; selenium 41.25 ± 19.53 μg/day; vitamin C 252 ± 138 mg/day; vitamin A 4905 ± 8167 U/day.

Fifty eight blood donors (29 women, 29 men), 42 ± 10 years old, were recruited as control group; the exclusion criteria were active smoking, recent physical stress, micronutrient supplementation, hepatic and/or renal failure. Written informed consent was obtained from each patient enrolled. Both nutritional and biochemical data obtained from blood donor group were plotted to obtain a reference range.

Collection of Blood Samples

Fast venous blood samples were collected in metal-free Vacutainer tubes (Becton-Dickinson, Milan, Italy) containing ethylendiaminetetraacetic acid (EDTA), kept in ice in the dark and the plasma was divided into aliquots within 1 h from withdrawal. Two aliquots of plasma were treated with 3% metaphosphoric acid (MPA) (v/v) to determine ascorbic, following the method described by Schorah;[18] samples were stored at +4°C until analysis, which was performed within 5 days. The remaining plasma samples were stored at −20°C for other biochemical evaluations. Red blood cells (RBC) were separated by centrifugation of blood samples at 250 rpm for 5 min. Collected RBC were immediately hemolyzed by adding distilled water (1:20 v/v), processed and stored at −20°C as described by Cereser[19] to determine reduced and total glutathione, within 10 days from blood withdrawal. For the evaluation of glutathione peroxidase a further RBC aliquot was washed with isotonic saline solution then assayed as described by Paglia and Valentine.[20]

Nutritional and Inflammatory Status, Hepatic and Renal Function Tests in HPN Patients

Serum tyroxine binding pre-albumin, hemoglobin and total cholesterol were taken as routine markers of nutritional status. Possible inflammatory status was evaluated in terms of total blood leukocytes (WBC), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP).

To detect irreversible liver damage and/or cholestasis, serum levels of aspartate and alanine

TABLE I Nutritional and inflammatory status, renal and hepatic function tests in HPN patients

	Reference range	HPN patients
Nutritional status:		
Hb (g/dl) (M/F)	13–17/12–16	11.9 ± 1.9/11.0 ± 1.4
Cholesterol (mg/dl)	100–200	121 ± 37
TBPA (mg/dl)	20–40	24 ± 9
Inflammatory status:		
WBC (10^6 cells/l)	4000–9000	5211 ± 1266
ESR (mm/h)	1–20	35 ± 26
CRP (mg/l)	<5.0	5.4 ± 11.0
Renal function test:		
BCrC (ml/min) (M/F)	70–135/70–120	89 ± 38/55 ± 22
Hepatic function test:		
AST (U/l) (M/F)	8–45/8–30	34 ± 9/33 ± 20
ALT (U/l) (M/F)	8–45/5–35	41 ± 19/26 ± 14
γGT (U/l) (M/F)	10–50/8–35	63 ± 84/49 ± 53
Total Bilirubin (mg/dl)	0.20–1.00	0.92 ± 0.74

Values are means ± SD. Reference range: data obtained from healthy donor group (controls). M/F: male/female; Hb: hemoglobin; TBPA: tyroxine binding pre-albumin; WBC: white blood cells; ESR: erythrosedimentation rate; CRP: C-reactive protein; BCrC: creatinine clearance; AST: aspartate amino transferase; ALT: alanine amino transferase; γ GT: γ-glutamyl transpeptidase.

amino transferase (AST, ALT), γ-glutamyl transpeptidase (γ-GT) and total bilirubin were evaluated. The degree of liver steatosis was determined by echography.

As regards the evaluation of kidney function, chronic renal disease diagnosis was formulated in the presence of creatinine clearance below 40 ml/h and diuresis above 1000 ml (see Table I for details).[21]

Biochemical Indexes of Antioxidant Status

Selenium, ascorbic acid, retinol and α-tocopherol in plasma samples, selenium dependent glutathione-peroxidase (GSH-PxSe), reduced and total glutathione (GSH and GSHt) in RBC were considered as biochemical indexes of antioxidant status.

Briefly, selenium (P-Se) was detected by SpectrAA 20 Graphite Furnace Atomic Absorption Spectroscope Varian as described in the analytical method supplied by the manufactures (Varian Techtron Pty. Limited, Mulgrave, Victoria Australia); ascorbic acid (P-AA) was detected by HPLC (Solvent Delivery System 9012 with an UV-Visible Detector 9050, Varian) using the method described by Harapanalli:[22] plasma samples treated with DTT were used to evaluate total ascorbic acid. Retinol and α-tocopherol were simultaneously evaluated by HPLC using a kit supplied by Chromsystems (Chromsystems Instruments and Chemical GmbH, Martinsried/München, Germany). Selenium-dependent-glutathione peroxidase (GSH-PxSe) activity in RBC was evaluated by the direct spectrophotometric method described by Paglia and Valentine.[20] Total and reduced glutathione were analyzed by HPLC after derivatization of hemolyzed samples with ammonium 7-fluorobenzo-2-oxa-1,3-diazole-4-sulphonate (SBD-F), using a fluorescence detector (Varian 9070) with excitation at 385 nm and emission at 515 nm.[23] Oxidized glutathione (GSSG) was calculated as the difference between total and reduced glutathione.

Indexes of Oxidative Stress

Plasma indexes of oxidative status were estimated in terms of 2,4-dinitrophenyl-hydrazine (DNPH) (Fluka from Sigma-Aldrich, Milan, Italy) derivatives of carbonyl compounds and in terms of adducts between protein amino functions and malondialdehyde (MDA-adducts) or 4-hydroxynonenal (HNE-adducts).

Aldehyde compounds were evaluated in the plasma through the reaction of carbonyls with DNPH; the hydrazone derivatives were extracted and separated by thin layer chromatography into the principal classes of aldehydes. The total carbonyl content of each fraction was estimated spectrophotometrically (absorption coefficient 25,000 l/mol/cm) and the individual hydrazone fraction of 4-hydroxyalkenals were recovered at 365 nm by an HPLC isocratic separation (chromatography system from Waters S.p.A., Vimodrone, Milan, Italy): a RP18 column (Merck, VWR International s.r.l., Milan, Italy) and a methanol/water eluent mixture (70/30, v/v) were used following the procedure described elsewhere.[24]

Steady-state concentration of free HNE in the plasma was measured at 220 nm by HPLC using a direct method of aldehyde extraction as described by Esterbauer and Cheeseman.[25]

Plasma fluorescent adducts formed between peroxidation derived aldehydes (MDA and HNE) and plasma protein were monitored by spectrofluorimetry as described by Tsuchida *et al.*:[26] fluorescence emission was at 460 nm, excitation was at 390 nm for MDA-adducts and at 355 nm for HNE-adducts.

Statistical Analyses

The following clinical, nutritional and biochemical variables were considered for their potential influence on redox balance:

age, gender, primary disease, HPN duration and HPN regimen (number of infusions/week, glucose, amino acids, total lipids, PUFA, α-tocopherol, α-tocopherol/PUFA ratio and selenium);
blood inflammatory indexes (ESR, CRP and WBC);
biochemical indexes of antioxidant status (Se, AA, α-tocopherol and retinol in plasma, GSH-PxSe, GSH and GSSG in RBC).

Linear regression test (univariate) and stepwise multiple linear regression (multivariate) analyses were used to evaluate the independent effect of each factor on the production of plasma MDA- and HNE-protein adducts. When univariate analysis showed a significant difference ($p < 0.05$), the multivariate analysis was performed (cut off level of significance: $p < 0.01$).

Two-tailed unpaired *t*-test was used to evaluate the statistical significance of MDA- or HNE-protein adducts between HPN patients and controls.

Linear regression was also used to analyze the correlation between MDA- and HNE-protein adducts in the plasma of the single patients. The relation between the two variables was expressed by Pearson's correlation coefficient.

All the statistical analyses were performed with SAS software package (SAS Institute Inc. Cary, NC, 1998).

RESULTS

Nutritional and Inflammatory Status, Hepatic and Renal Function Tests in HPN Patients

On the basis of the considered parameters (Table I), the nutritional status of the HPN patients recruited in the study resulted to be relatively satisfactory. A modest inflammatory trend in HPN was suggested by the slight average increase of ESR and CRP which was not accompanied by leukocytosis (Table I). All but two patients showed normal AST and ALT levels. In the two abnormal cases, ALT was below 80 U/l. Only six patients showed a modest increase in laboratory indexes for cholestasis, and 11 patients showed echographic evidence of moderate liver steatosis, but not fibrosis. Moreover, modest renal dysfunction was recorded in 10 out of 41 patients with an average creatinine clearance (BCrC) slightly below the normal range.

Higher Plasma Levels of MDA- and HNE-protein Adducts in HPN Patients Compared to Healthy Controls

The level of the relatively stable Schiff-base adducts[15] formed by both MDA and 4-hydroxynonenal with plasma proteins was measured both in patients and in a group of adult blood donors with no smoking habit, absence of micronutrient supplementation and evident disease-free state; the data obtained are plotted in Fig. 1. A marked rise of both lipid peroxidation markers was evident in the HPN group versus controls. The mean elevation of MDA- and that of HNE-fluorescent adducts were similar, i.e. 58 and 59%, respectively. Of note, only 6 patients out of 41 showed a level of HNE adducts equal to or lower than the mean control value. In the case of MDA-protein adducts, only 3 patients out of 41 showed values below the control mean.

To support the reliability and specificity of HNE-protein adduct as plasma marker of lipid peroxidation, we also quantified 4-hydroxyalkenals, the carbonyl class which HNE belongs to, and measured directly this aldehyde in plasma samples from a small group of randomly-selected HPN and healthy individuals by means of suitable HPLC analytical procedures. The HPLC analysis of the complex pattern of aldehyde-dinitrophenyl hydrazone derivatives confirmed the rise of total 4-hydroxyalkenals in HPN as to control individuals (Fig. 2A,B). The direct demonstration of higher HNE steady-state level in the plasma of HPN patients was also achieved (Fig. 2C,D).

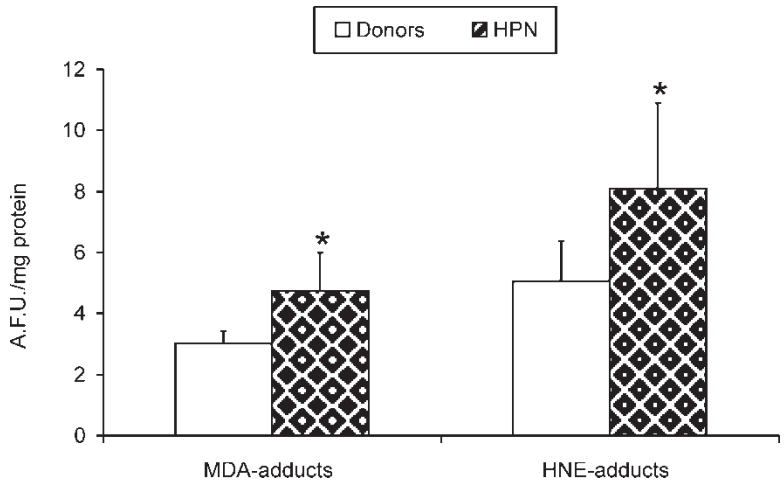

FIGURE 1 Plasma levels of aldehyde-(MDA or HNE) protein adducts in controls and HPN patients. Fluorescent adducts were recorded using wavelengths of 390/460 nm em/ex for MDA and of 355/460 nm em/ex for HNE. Data are mean ± SD. *Significantly different versus controls ($p < 0.0001$). Controls: healthy donor group.

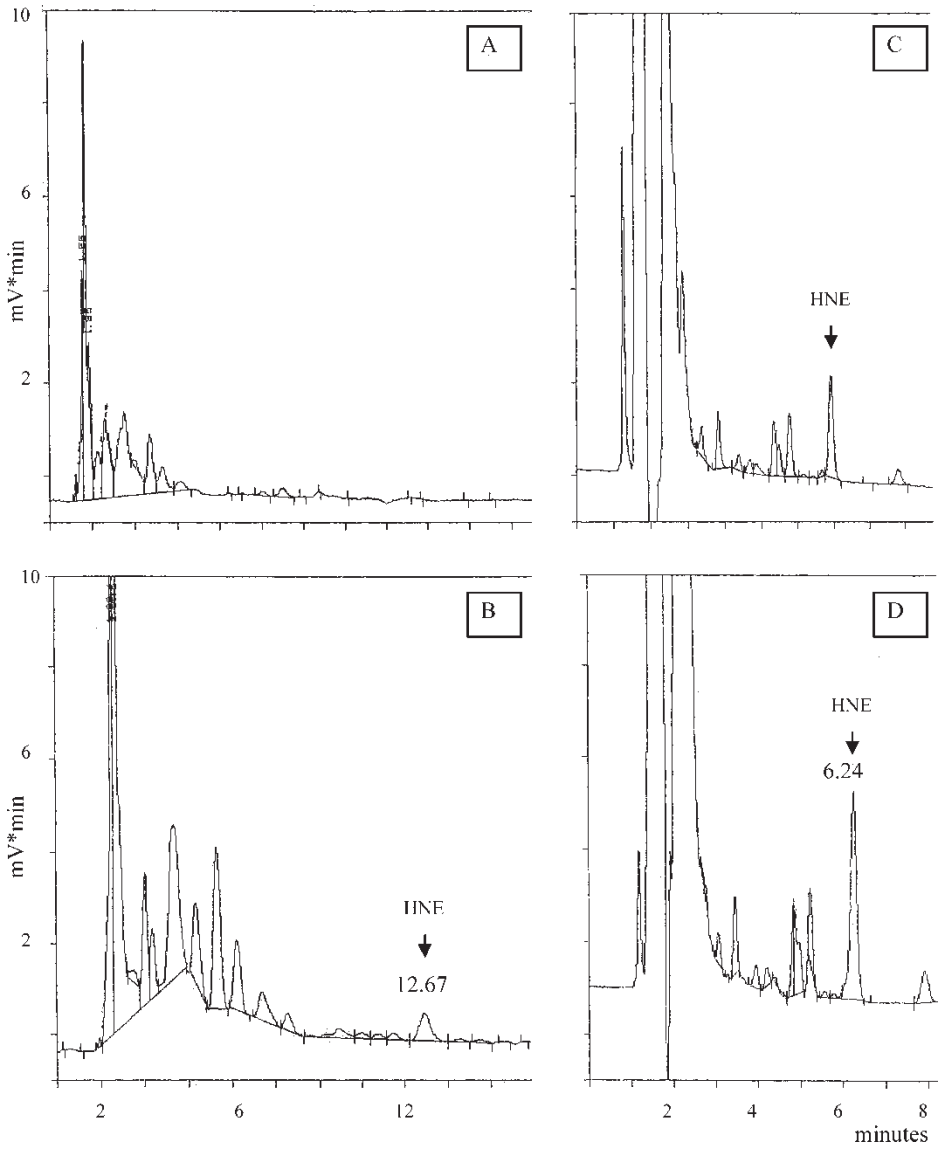

FIGURE 2 Representative HPLC analysis of 4-hydroxyalkenals and free HNE content in plasma of a randomly selected HPN patient and a control subject. Dinitrophenylhydrazone-4-hydroxyalkenal derivatives were evaluated in plasma from a control subject (A) and a HPN patient (B) by isocratic HPLC separation. Panels C and D indicate the corresponding HPLC analyses of free HNE in control and HPN patients, respectively.

Retinol, Ascorbic Acid and α-Tocopherol Plasma Levels in HPN Patients

While hematic retinol (vitamin A) did not differ between patients and controls, the two other vitamins showed significant changes in plasma from HPN group (Table II).

TABLE II Plasma levels of different vitamins in patients submitted to HPN and controls

	Vitamin A (μg/ml)	α-Tocopherol (μg/ml)	Ascorbic acid (mg/ml)
Controls	0.7 ± 0.2	11.8 ± 3.3	8.4 ± 2.1
HPN	0.7 ± 0.4	8.4 ± 3.8*	7.2 ± 3.9**
Normal values	0.3–1.2	5–20	3.2–12.5

Data are means ± SD. Significantly different versus controls (*$p < 0.001$; **$p < 0.05$). Controls: healthy donor group.

Reduced ascorbate was found as modestly but significantly decreased in HPN patients. The difference between patients and controls with regard to α-tocopherol content was definitely that more evident: in fact, the vitamin E stereoisomer with the highest antioxidant activity showed a 40% average decrease in HPN versus the control group.

Plasma Selenium, Erythrocyte GSH/GSSG Levels and GSH Peroxidase Activity in HPN Patients

As reported in Table III, total (reduced + oxidized) glutathione content (GSHt) was significantly decreased in red cells from HPN patients in comparison to the control group. Average plasma selenium in HPN patients appeared reduced as to control, but the value did not reach statistical

TABLE III Status of total glutathione (GSHt), selenium dependent glutathione peroxidase (GSH-PxSe) in red blood cells (RBC) and plasma levels of selenium (p-Se) in HPN patients and controls

	RBC-GSHt (mmol/gHb)	RBC-GSPxSe (Ul/gHb)	p-Se (μg/l)
Controls	5.50 ± 1.04 (41)	9.42 ± 2.47 (58)	81.12 ± 14.01 (57)
HPN	3.83 ± 0.81* (36)	9.78 ± 2.46 (34)	66.19 ± 16.14 (40)
Normal values	2.3–6.0	4.5–18.2	51–110

The number of subjects is in brackets. Data are means ± SD. *Significantly different versus controls ($p < 0.0001$). Controls: healthy donor group.

significance. Despite the latter evidence, glutathione peroxidase in HPN RBC was shown to maintain a normal activity.

Statistical Relationship between HNE- and MDA-protein Adducts and Different Blood Parameters

A positive correlation between the two lipid peroxidation markers (MDA- and HNE-protein adducts) was demonstrated, with a linear regression of $r = 0.70$ coefficient and a high level of significance ($p < 0.0001$) (Fig. 3).

As reported in details in the "Methods" section (statistical analyses), the influence of a defined variety of factors on the levels of plasma aldehyde-adducts was determined.

In the univariate analysis, MDA-protein adducts were inversely related only to selenium i.v. intake ($p = 0.01$). In this case the multivariate analysis was not applicable.

On the contrary, 4-hydroxynonenal-protein adducts were inversely related to both selenium i.v. intake and plasma α-tocopherol, and positively related to oxidized glutathione (RBC-GSSG). These statistical findings were confirmed in the multivariate analysis (Table IV).

DISCUSSION

Analyses of the biochemical redox balance of patients under long-term HPN showed that the worldwide recommended dietary regimen was not adequate to avoid the onset of a biochemical condition of oxidative stress in various tissues, as mirrored by the marked hematic rise of the lipid peroxidation products MDA and HNE (Figs. 1 and 2C,D).

Increased serum MDA during HPN has recently been reported by two studies, respectively on 12 and 22 adult patients with benign intestinal failure.[8,10] Actually, the measurement of free MDA in the serum is not considered a very reliable marker of lipid peroxidation, due to the many disadvantages of this method.[25] Moreover, the serum MDA increase observed in the study performed on 12 HPN patients was not statistical significant versus a first control group of 25 healthy subjects, becoming significant only when the number of controls was increased to 40.[8] Thus, the presently available reports of oxidative stress in HPN patients which are simply based on serum MDA measurement do not appear reliable and indicative enough. The detection of F2-isoprostanes as an index of lipid peroxidation,

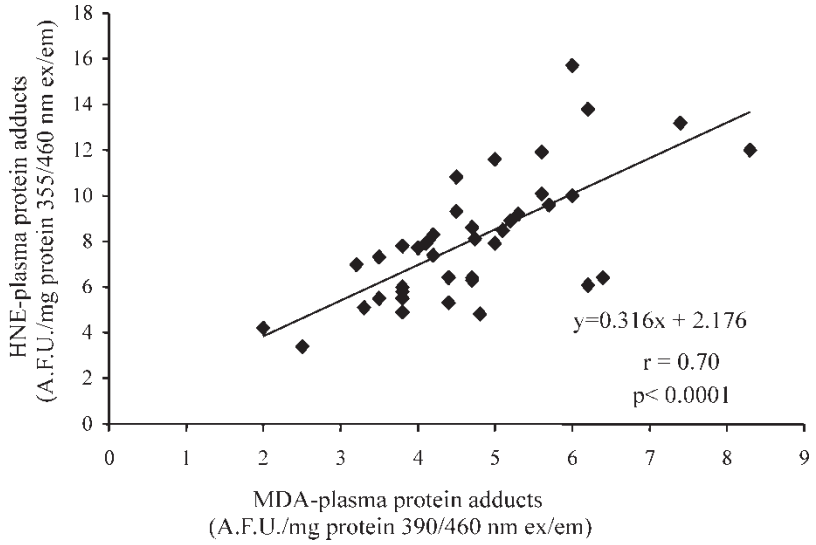

FIGURE 3 Relationship between HNE-protein adducts and MDA-protein adducts in plasma of HPN patients. Linear regression was used to analyse the correlation between MDA- and HNE-protein adducts in the plasma of the single patients. The relation between the two variables was expressed by Pearson's correlation coefficient (r) with a chance (p) to obtain a correlation coefficient with a slope as far as from zero.

TABLE IV Probability levels in univariate and multivariate analysis of factors (as independent variables) contributing to the formation of HNE-protein adducts (as dependent variable) in HPN patients

Independent variables	Univariate analysis (probability level)	Multivariate analysis (probability level)
Selenium intake	0.02	0.001
Plasma-selenium	NS	–
α-Tocopherol intake	NS	–
Plasma α-tocopherol	0.03	0.058
RBC-GSSG	0.04	0.006

RBC-GSSG: oxidized glutathione.

applied to a group of infants under total parenteral nutrition, resulted to be much more sensitive but technologically sophisticated and time consuming.[9]

As regards the present report, we deemed more reliable to measure serum levels of both MDA and HNE reversibly, but steadily bound to protein NH_2-residues through Schiff-base formation; in a way, the indication given by these two markers is comparable to that provided by glycated hemoglobin in monitoring plasma glucose level. Indeed, the observed higher levels of HNE and MDA-protein adducts in HPN patients as to control group showed to be highly consistent and quite marked, independently from the cause of malabsorption (Fig. 1); further, the two markers of lipid peroxidation positively correlated each other (Fig. 3).

The detection of higher levels of HNE-protein adducts in the blood is undoubtedly of much greater pathophysiologic significance than MDA plasma adducts. Evidence of increased plasma HNE-protein adducts was obtained in human alcoholics still showing normal liver function.[27] Moreover, a consistent association of increased HNE steady-state levels with chronic liver diseases characterized by steatosis and/or fibrosis has been proved.[16,17] The link between early detection of increased plasma HNE-protein adducts and actual aldehyde involvement in the pathogenesis of fibrosis is strongly supported by the demonstrated proinflammatory and profibrogenic effect of this lipid peroxidation end-product.[7,15]

Now, considering the here reported cohort of long-term HPN patients, laboratory and echographic characterization allowed to define a still relatively low incidence of the typical complications of this nutritional regimen. In our casistic, mean AST and ALT levels are still inside the reference ranges and indexes of cholestasis are borderline, so that an appropriate correlation with higher MDA and HNE values is not possible yet. But, despite the present lack of conclusive demonstrations, the hypothetical involvement of HNE and related molecules in the pathogenesis of HPN complications remains quite likely.

Indeed, the evidence of higher HNE-plasma protein adduct levels in HPN patients before the occurrence of clinical complications may be an useful marker for prevention, e.g. by prompt and suitable correction of the micronutrient supplementation.

Of note, HPN patients showed lower hematic levels of selenium, vitamins E, C and total GSH as to control, despite their special dietary regimen appeared at least able to maintain the blood level of all these nutritional elements within a range of normality (see Tables II and III). In other words, a normal but relatively low level of antioxidants may become inadequate to prevent oxidative stress in the case of HPN of long duration. While at the present stage it is not possible to draw any conclusion on the effect of oxidative stress on the nutritional status, a further antioxidant supplementation of HPN patients is indicated.

As regards the antioxidants to be supplemented in higher amounts, the here reported findings and statistical analyses point to vitamin E and selenium as the main candidates (Table IV). By the way, the significant inverse correlation obtained for erythrocyte GSSG and plasma HNE (Table IV) should be considered an additional index of unbalanced oxidation reactions rather than a further need of GSH precursors.

Another dietary amendment that could be suggested is a slight, possibly periodic, reduction in the amount of PUFA given by standard HPN. In this connection, the fast and highly reproducible assay of HNE- and MDA-protein adducts in peripheral blood could be of great help in identifying the most appropriate PUFA supplementation level.

In conclusion, plasma level of HNE-protein adducts appears to be a particularly reliable marker of oxidative status in HPN. In fact, of the two lipid peroxidation indexes found to be markedly higher in HPN patients, i.e. plasma protein adducts with MDA and 4-hydroxynonenal, respectively, only the second was statistically correlated with all the antioxidant-related changes consistently detected in the patients, namely decreased plasma α-tocopherol and selenium intake and increased erythrocyte oxidized glutathione (Table IV). MDA-protein adducts resulted to be statistically correlated only with selenium uptake ($p < 0.05$). Analysis of the further potential changes of HNE- and MDA-protein adducts in the single HPN patients with the prolongation of parenteral nutrition is in progress. Suitable supplementation with α-tocopherol and selenium is also under consideration.

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