Changes in the Concentrations of Plasma Selenium and Selenoproteins after Minor Elective Surgery: Further Evidence for a Negative Acute Phase Response? Colin Nichol,1 Jacqueline Herdman,2 Naveed Sattar,2 Patrick J. O’Drury,3 Denis St. J. O’Reilly,5 David Littlejohn,1 and Gordon Fell2∗

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The acute phase plasma protein response is part of the complex series of physiological, hematological, and biochemical events that constitute the inflammatory response after tissue injury or infection. The magnitude and duration of the response are related to the nature and severity of the injury and the presence of sepsis (1). We have previously reported alterations in plasma iron, transferrin, zinc, albumin, copper, and ceruloplasmin concentrations after major surgery to a marked rise in plasma C-reactive protein (CRP) concentration (2). In both acute (3, 4) and chronic (5) illnesses, the plasma concentration of selenium also decreases in proportion to the magnitude of the inflammatory response. There is concern about the decline in dietary intake of selenium in some areas of the world (6), because the antioxidant activities of several selenoproteins may be important in preventing free radical damage (7). If plasma selenium concentrations decrease during an inflammatory response, independently of dietary intake, then this would have important implications for the interpretation of the plasma selenium values reported in a wide range of illnesses. In this study, total plasma selenium concentration and changes in plasma selenoproteins after minor elective surgery (inguinal hernia repair) were determined and related to the accompanying alterations in plasma CRP.

Ten male patients (mean age, 51 years; range, 18–90 years) requiring inguinal hernia repair were recruited to the study. All patients were healthy before surgery, and none were taking any relevant medication. Samples of head hair and toe nails (80–100 mg) were obtained 24 h before surgery as a measure of long-term selenium nutritional status, along with venous blood collected into plain (10 mL) and lithium heparin tubes (20 mL). Blood samples were taken on the mornings of day 1 and day 6 after surgery. None of the patients received intravenous fluids or blood products post-surgery, and all had uncomplicated clinical courses. Routine ward diet was resumed the day after surgery. The study was approved by the local Ethics Committee, and all subjects gave their informed consent. Plasma and serum were separated as soon as possible and stored in plastic tubes at −20 °C.

For selenium determination, a Perkin–Elmer 1100B atomic absorption spectrometer, equipped with a PE HGA 700 programmer, a PE AS 70 autosampler, and an Epson FX800 printer, was used to obtain integrated absorption signals. A selenium electrodeless discharge lamp (5 W) was used, and atomic absorption measured at the 196.0 nm selenium line, with palladium as a matrix modifier (8). This method has a within-batch imprecision of 2.3%. The limit of detection is 0.05 μmol/L. Hair and nail samples were prepared by microwave digestion using concentrated nitric acid and hydrogen peroxide; the selenium content was then determined by electrothermal atomic absorption spectrometry (9). The selenoproteins in human plasma were separated by column affinity chromatography (10). Blue Sepharose CL-6B (Pharmacia) was used to bind both selenoprotein-P (Se-P) and albumin, thus releasing the glutathione peroxidase (GSHPx) fraction. Heparin Sepharose CL-6B (Pharmacia) was used to separate Se-P from albumin, and was then eluted from the column. The selenium content of the separated fractions was then determined by electrothermal atomic absorption spectrophotometry. The plasma GSHPx protein concentration was measured by a commercial enzyme-linked immunosorbent immunoassay kit, using polyclonal antibodies that were specific for human plasma GSHPx (pl-GPx-EIA kit from Bioxytech S.A.). The detection limit was 2.5 mg/L, and the within-batch imprecision was 5.1% at 5.4 mg/L. Plasma and red cell GSHPx activity was measured by a rate reaction method using t-butyl peroxide as substrate (11). The plasma GSHPx assay had a within-batch imprecision of 3.0%, and red cell GSHPx activity measurement had a between-batch imprecision of 7.3%. Serum albumin (colorimetric), transferrin and ceruloplasmin (immunoturbidimetric), iron (colorimetric), zinc and copper (inductively coupled plasma atomic emission spectrometry), and CRP (immunoturbidimetric) were measured as described previously (5).

Results are presented as the mean and observed range. Comparisons between different days after surgery were made by a paired t-test (Minitab statistical software).

Selenium concentrations found in hair and nail samples taken from the patients were 0.64 ± 0.05 mg/kg and 0.35 ± 0.11 mg/kg, respectively. These results were similar to those found in the local population (0.52 ± 0.11 mg/kg and 0.44 ± 0.10 mg/kg, n = 25) (9). The minor surgery of inguinal hernia repair caused a modest rise in plasma CRP concentration (mean concentration increased to 20.7 mg/L; P <0.01; Table 1). This is less than the increase in CRP concentration found in more major surgical procedures (12). Nevertheless, this increase in plasma CRP was associated with significant alterations in the plasma concentrations of trace metals and carrier proteins in a manner similar to that seen after major surgical procedures (2). The plasma concentrations of iron, transferrin, zinc, and albumin decreased as CRP
increased; all significantly fell (by 64%, 8%, 10%, and 7%, respectively) one day after surgery (P < 0.05). By day 6, concentrations of all these analytes had returned towards the starting values (P < 0.05). Copper and ceruloplasmin concentrations showed no significant change at day 1; however, by day 6, significant increases in both were observed (P < 0.05).

Given the above expected changes in established acute phase reactants, it was interesting to note that plasma selenium concentrations followed a similar pattern, decreasing by 10% (P < 0.01) at day 1, followed by a subsequent increase (P < 0.05) toward the starting concentrations by day 6. Table 1 also shows the selenium concentrations found in the plasma selenoproteins during the study period. The amount of selenium found in the combined selenoprotein fractions was 92–95% of the starting total plasma selenium concentration. The concentration of selenium in the Se-P fraction declined by 12% (P < 0.05) at day 1, subsequently increasing to baseline concentration (P < 0.05). Although a trend toward a reduction in the amount of selenium associated with plasma GSHPx was also observed, this was not statistically significant, nor could we detect significant alterations in the protein concentration or enzymatic activity of plasma GSHPx. There was, however, a significant (P < 0.05) increase in the amount of selenium associated with plasma GSHPx when comparing day 1 and day 6. For selenium associated with albumin, we could not detect any significant alteration during the study. However the low concentrations of selenium in this fraction were close to the detection limit of the method. Cytosolic red cell GSHPx enzyme activity showed no change over the time course of the study (Table 1).

What are the potential mechanisms for the observed perturbations in plasma selenium concentration? One possibility is a dilutional effect. However, this was unlikely because none of the patients received intravenous fluids postoperatively or experienced hemodynamic complications. A second factor could have been increased urinary excretion of selenium; however, other studies (3, 13) do not suggest that urinary selenium losses are the cause of low plasma selenium concentrations commonly seen in ill patients. Another possibility is lack of dietary intake; however, the rapidity of the fall in plasma selenium concentration would make nutritional depletion unlikely. Furthermore, little evidence of preexisting selenium depletion was found, as judged by measurement of hair/nail selenium content or the activity of red cell GSHPx. Could the changes, therefore, be caused by redistribution of plasma selenoproteins in a fashion analogous to the behavior of albumin in the acute phase response? (14). The demonstration of specific endothelial receptors for plasma Se-P (15) does suggest an extravascular role for this protein, which may have important antioxidant functions.

Whatever the mechanism, the observation that plasma selenium decreases as part of the inflammatory response is important. Low plasma selenium concentrations have been described in a variety of acute clinical conditions such as myocardial infarction, severe burns (13), acute pancreatitis, and in intensive care patients (3). Because these conditions are accompanied by variable degrees of inflammatory response, the low plasma selenium concentration may be caused, in part, by an appropriate acute phase response. Low plasma selenium concentrations have also been described in chronic conditions. In a group of terminally ill cancer patients, we found a strong negative correlation between CRP and plasma selenium concentration, patients with CRP values >30 mg/L having the lowest plasma selenium results (5). It has been suggested that marginal selenium deficiency may increase susceptibility to disorders such as cancer and cardiovascular disease (16), but it is noteworthy that the early stages of such degenerative disease may also have a subclinical inflammatory component (17).
We conclude that when interpreting plasma selenium concentrations, a marker of the inflammatory response, such as CRP, should be included to distinguish true nutritional depletion from the inherent effects of disease.

References

Mutation Screening by Denaturing Gradient Gel Electrophoresis in North American Patients with Acute Intermittent Porphyria, Niels Erik Petersen,¹ Henrik Nissen,¹ Mogens Hørder,¹ Janine Senz,² Azim Jamani,³ William E. Schreiber²,³ (¹ Department of Clinical Biochemistry and Clinical Genetics, Odense University Hospital, DK-5000 Odense C, Denmark and The Danish Centre of Porphyria; ² Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, British Columbia, V6T 1W5 Canada; ³ Division of Clinical Chemistry, Vancouver Hospital and Health Sciences Centre, Vancouver, British Columbia, V5Z 1M9 Canada; * author for correspondence: fax 45-65-41-19-11, e-mail nep@dou.dk)

Acute intermittent porphyria (AIP) is a dominantly inherited disorder of heme metabolism caused by a 50% decrease in the activity of porphobiligen deaminase (EC 4.3.1.8.). The estimated prevalence of the disease is 1:1000 to 1:5:100 000, making it the most common of the neurologic porphyrias (1). Although its penetrance is only 10–20% (2), acute attacks of AIP are disabling and can be life-threatening. Prevention of acute attacks is the most important principle in managing the disease; however, it requires prior identification of individuals at risk. Because AIP has a genetic basis, molecular analysis for this disease is ultimately the most reliable way to identify gene carriers.

More than 130 mutations in the porphobiligen deaminase gene have been characterized to date (3). The majority have been restricted to a single family, or at most a few families, and no mutation accounts for more than a small percentage of all cases. With the exception of selected geographic areas where single mutations predominate (4, 5), testing for known mutations in a patient who has not previously been investigated is likely to be negative. Molecular analysis therefore requires an efficient strategy for finding the causative mutation. Denaturing gradient gel electrophoresis (DGGE) has been used by several groups to screen the exons and flanking intronic segments of the porphobiligen deaminase gene for variations in the wild-type sequence (6–13). This work has been carried out in European laboratories, and the mutations that have been discovered reflect the molecular defects within that population. By contrast, relatively few mutations have been described in the North American population (14–19). The goal of this study was to further characterize the spectrum of mutations in North American patients with AIP, using a well-established DGGE assay with high sensitivity to identify exons/introns that required sequencing (8). DNA samples were also analyzed by heteroduplex analysis (16) to compare its sensitivity in detecting mutations to that of DGGE.

Fifteen patients with AIP from 12 unrelated families were studied. Geographically, these patients were referred from the provinces of Ontario, Manitoba, Saskatchewan, Alberta, and British Columbia in Canada and from the states of Kansas and Washington in the United States. The diagnosis was based on a combination of clinical and family history, increased excretion of δ-aminolevulinic acid and porphobiligen in urine, reference value fecal porphyrins, and decreased activity of porphobiligen deaminase in red cell lysates. Cross-reacting immunologic material status was determined in six patients from five kindreds; all were negative for cross-reacting immunological material. Procedures involving human subjects were performed in accordance with the Helsinki Declaration of 1975, as revised in 1983, and informed consent was obtained from all subjects before their inclusion in the study.

All 15 exons of the porphobiligen deaminase gene and their surrounding intronic sequences were analyzed by DGGE as previously described (8). DGGE patterns corresponding to known polymorphisms in introns 2 and 3 (20), intron 6 (8), intron 10 (21), and exon 10 (22) were present in many of these samples. Unique patterns not associated with known polymorphisms were found in 14 of the 15 patients, and sequencing of these regions re-