Low zinc and selenium concentrations in sepsis are associated with oxidative damage and inflammation

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Abstract

Background: Oxidative stress with dysregulated inflammation are hallmarks of sepsis. Zinc and selenium have important antioxidant functions, such that they could be important in patients with sepsis. We used an in vitro approach to assess the effect of zinc and selenium on oxidative stress, mitochondrial function, and inflammatory responses in conditions mimicking sepsis and related the findings to plasma concentrations and biomarkers in patients with and without sepsis.

Methods: Human endothelial cells were exposed to a range of zinc and selenium concentrations in conditions mimicking sepsis. Zinc, selenium, and a series of biomarkers of oxidative stress and inflammation were measured in plasma from critically ill patients with and without sepsis.

Results: Culturing cells with different concentrations of zinc caused altered zinc transporter protein expression and cellular zinc content, and selenium affected glutathione peroxidase 3 activity. Although zinc or selenium at physiological concentrations had no effect on interleukin-6 release in vitro, higher concentrations of the trace elements were associated with improved mitochondrial function. Plasma zinc and selenium concentrations were low in patients [zinc: median (range) 4.6 (2.1–6.5) µM in control patients without sepsis and 3.1 (1.5–5.4) µM in patients with sepsis, P=0.002; and selenium: 0.78 (0.19–1.32) µM in control patients and 0.42 (0.22–0.91) µM in sepsis patients, P=0.0009]. Plasma concentrations of interleukin-6, other biomarkers of inflammation, and markers of oxidative damage to proteins and lipids were elevated, particularly in patients with sepsis, and were inversely related to plasma zinc and selenium concentrations.

Conclusions: Zinc and selenium concentrations were reduced in critically ill patients, with increased oxidative stress and inflammatory biomarkers, particularly in patients with sepsis. Oxidative stress as a result of suboptimal selenium and zinc concentrations might contribute to damage of key proteins.

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Key words: critical illness; mitochondria, oxidative damage; selenium; sepsis; zinc

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About 37,000 patients in the UK die annually from severe sepsis, which is characterized by a systemic, dysregulated, and highly exaggerated inflammatory response, accompanied by oxidative stress and mitochondrial dysfunction. Zinc is an essential micronutrient that has numerous biological roles, and its deficiency increases susceptibility to infection. Zinc has also been suggested to have a role in antioxidant defence in an indirect manner, through the antioxidant metalloenzyme copper-zinc superoxide dismutase, by binding of zinc to redox active sites in place of more damaging metals, such as copper or iron, and also by its regulation of metallothioneins that have roles in free radical scavenging and inflammatory processes. Selenium is also an essential trace metal, which has direct antioxidant activity as a result of its incorporation into selenoproteins, such as glutathione peroxidases (GPx), thioredoxin reductase, and some isoforms of methionine sulphoxide reductase. Thus zinc or selenium status could influence oxidative stress and inflammatory responses in patients with sepsis. The aim of this study was to use an in vitro approach to determine the effects of a range of zinc and selenium concentrations on human endothelial cells exposed to conditions mimicking sepsis, in terms of oxidative stress, inflammatory cytokines, and mitochondrial function. We related these findings to a comprehensive assessment of zinc and selenium concentrations and biomarkers of oxidative and inflammation in intensive care unit (ICU) patients with and without sepsis to determine the potential for zinc or selenium status to influence oxidative stress and inflammation in sepsis.

Methods

Cell culture and treatment

Human umbilical vein endothelial cells (HUVEC-C cell line; ATCC/LGC Standards Ltd, Teddington, Middlesex, UK) were maintained in Dulbecco’s modified Eagle’s growth medium (GE Healthcare UK Ltd, Little Chalfont, Buckinghamshire, UK) containing the following: glucose, 1 g litre\(^{-1}\) and glutamine, 4 mmol litre\(^{-1}\), and supplemented with fetal bovine serum (FBS), 10%; gentamicin, 50 µg ml\(^{-1}\) (Lonza Group Ltd, Basel, Switzerland), and amphotericin B, 2.5 µg ml\(^{-1}\). Cells were cultured in a humidified atmosphere of 5% CO\(_2\)–95% air at 37°C. Most of the zinc in culture medium is found in FBS, and so to prepare zinc-depleted growth medium the FBS was treated with Chelex 100 resin (Bio-Rad, Hemel Hempstead, Hertfordshire, UK) at 50 mg litre\(^{-1}\) for 2 h. The zinc concentration was measured using flame atomic absorption spectroscopy (F-AAS; iCE 3000 series AA spectrometer; Thermo Scientific, Cambridge, UK). Zinc chloride was then added to give a range of physiologically relevant zinc concentrations of 1–20 µM in the complete medium (confirmed by F-AAS).

The effect of zinc on cells was confirmed by analysis of mRNA expression of the zinc transporter protein ZIP2. Cells were cultured in medium containing Chelex-treated FBS to which zinc had been added for 3 days, then harvested in TRIzol\(^{\text{®}}\) reagent (Invitrogen, Paisley, UK), and RNA was isolated using chloroform/isoopropanol extraction followed by semi-quantitative RT-PCR for mRNA expression using standard techniques (primer sequences are given in the Supplementary Material).

Dulbecco’s modified Eagle’s growth medium is deficient in selenium, and although FBS contains selenoproteins, selenium in this form cannot be used by cells, and so there was no necessity to remove selenium before culture. Glutathione peroxidase (GPx3) is one of the family of antioxidant peroxidase enzymes that contains a selenocysteine centre and is very sensitive to selenium availability. Glutathione peroxidase activity was determined in cell lysates using two-dimensional electrophoresis.

Cells were maintained in medium containing Chelex-treated FBS (containing 0.4 µM zinc) for 2 weeks before seeding onto 96- or six-well plates in medium containing 1–20 µM zinc, or were maintained in medium to which 0–100 nM sodium selenite was added, for 2 weeks. Cells were then treated with lipopolysaccharide, 2 µg ml\(^{-1}\) (LPS, Sigma Aldrich Ltd, Poole, Dorset, UK) from Escherichia coli 0111:B7 plus peptidoglycan, 20 µg ml\(^{-1}\) (PepG; prepared as described previously) or vehicle control for up to 7 days. Cell viability was assessed using the acid phosphatase assay. To investigate the effect of severe acute zinc depletion or zinc excess, in some experiments cells were treated with N,N,N′,N′-tetrais(2-pyridylmethyl)ethylenediamine, 5 µM (TPEN; Sigma) or zinc pyrithione, 0.5 µM (Sigma) for 24 h before LPS/PepG treatment. The TPEN strips intracellular zinc from cells, whilst zinc pyrithione floods cells with free zinc.

Interleukin (IL)-6 and IL-8 are key cytokines produced early in the sepsis process and have been shown to be associated with sepsis severity. Interleukin-6 and IL-8 were measured in medium after 24 h treatment of cells with or without LPS/PepG over a range of zinc or selenium concentrations, using commercially available enzyme immunoassays (R&D Systems, Abingdon, Oxfordshire, UK).

Nuclear factor κB (NFκB) is a redox-sensitive transcription factor that has a key role in regulation of cytokines and other inflammatory mediators in sepsis. Activated NFκB was measured as the amount in nuclear extracts, because only activated NFκB is present in nuclei. Nuclear extracts were prepared from cells treated as above for 4 h (peak of NFκB activation; data not shown). Details of the methods for nuclear fractionation are provided in the Supplementary Material. A commercially available enzyme immunoassay (PhosphoTracer NF-κB p65 ELISA kit; Abcam, Cambridge, UK) was used to quantify nuclear phosphorylated NFκB.

Mitochondrial function was assessed in cells cultured in 96-well plates treated for 7 days as above. The cationic fluorescent dye JC-1 (Invitrogen) was used to quantify mitochondrial membrane potential as described. Reduction of the dye AlamarBlue\(^{\text{®}}\) was used as an indicator of mitochondrial metabolic activity as described. The mitochondrial complex I inhibitor rotenone (50 µM for 5 h) was used as positive control treatment for all mitochondrial assays to ensure that the assays were detecting mitochondrial damage. Adenosine triphosphate was measured as described\(^{\text{°}}\) in cells grown on 96-well plates for 7 days and treated as described above.

Clinical study

Following ethical approval by the Scotland A Research Ethics Committee (REC reference 11/AL/0137) and written informed
consent or assent from a near relative, 39 consecutive patients aged >16 yr were recruited. The sepsis cohort comprised those who were within 24 h of fulfilling the consensus criteria for sepsis according to clinical suspicion of infection plus two of the following: tachycardia (>100 beats min⁻¹), tachypnoea (>20 bpm or ventilated) or leucocyte count <4 or >12×10⁹ litre⁻¹. Those patients who had no evidence of infection were recruited as control subjects. Patients were excluded if they were <16 yr, pregnant or lactating, were human immunodeficiency virus positive, receiving steroids (>20 mg day⁻¹) prednisolone or equivalent orally, used regularly for more than 2 weeks before ICU admission), or had cancer or any autoimmune disorder. Patient characteristics, diagnosis/site of infection, admission acute physiological and chronic health evaluation (APACHE) II and daily sequential organ function assessment (SOFA) scores, and plasma albumin and C-reactive protein (CRP) were recorded.

Venous blood samples were drawn into lithium heparin tubes and processed within 2 h. Total leucocytes were isolated using sedimentation through Gelofusin as described, and nuclear extracts were prepared for NFκB analysis as described above. C-Reactive protein is a classic acute phase protein from the same family as pentraxin 3. Although non-specific, high concentrations of both markers are indicative of inflammation and are particularly elevated in infection. Plasma IL-6, IL-8, and pentraxin 3 were measured using enzyme immunoassays (R&D Systems) as described. Lipid hydroperoxide (LPO) is a marker of oxidative stress previously shown to be associated with sepsis severity and outcome and is involved in numerous physiological and pathological pathways. Elevated concentrations are related to disease severity regardless of infection type. This was measured using the suPARnostic™ kit (ViroGates, Copenhagen, Denmark).

Myeloperoxidase (MPO) protein was quantified by enzyme immunoassay (Immundiagnostik AG, Bensheim, Germany). Thiocyanate concentrations were determined by ion-exchange chromatography ( Dionex Corp., Sunnyvale, CA, USA); details of the methods are described in the Supplementary material. Protein oxidation was assessed by quantification of protein-bound amino acids and their oxidation products after hydrolysis of proteins (using methane sulphonic acid) as described. Concentrations of protein methionine and methionine sulfoxide are expressed relative to the non-oxidizable amino acid isoleucine (to correct for losses during processing) and are corrected for protein concentrations in the samples. Thiols were quantified by spectrophotometry using 5,5'-dithiobis-(2-nitrobenzoic acid) as described, using glutathione as a standard. All samples and standards for all assays were analysed in duplicate or greater using independently prepared samples.

Plasma zinc was determined using F-AAS, and plasma selenium was determined using inductively coupled plasma mass spectrometry. Plasma GPx3 activity was measured using a coupled enzyme system assay where the change in absorbance at 340 nm was proportional to GPx3 activity. All measures were undertaken by researchers who were unaware of whether samples were from a control or sepsis patient.

**Statistical analysis**

For in vitro assays, three to six independent experiments were performed. No assumptions were made about data distribution, and statistical analysis was performed using Kruskal–Wallis analysis with post hoc Mann–Whitney U-test as appropriate. For clinical data that were not normally distributed, differences between sepsis and control patients were assessed using the Wilcoxon–Mann–Whitney test. Spearman testing was used to determine whether variables were independent. A P-value of <0.05 was considered to be significant. Data are presented as box- and-whisker plots showing median, interquartile, and full range or individual data points. Some in vitro data are expressed as the percentage of results in cells exposed to LPS/PepG in normal medium to enable comparison between both selenium and zinc and between different measures.

**Results**

**In vitro study**

Fetal bovine serum treated with Chelex resin had a zinc concentration of 0.4 µM, compared with 4 µM zinc before treatment. Magnesium and calcium concentrations were also reduced slightly (~13 and 11%, respectively, P=0.002) by Chelex treatment, but this was not considered to be a biologically important change. Iron concentrations of Chelex-treated FBS were unchanged, but the effect on copper was marked, with 60% reduction [from median (range) 2.2 (2.2–2.3) to 0.9 (0.9–1.0) µM, P=0.005]; therefore, treated FBS was replenished with copper sulphate, with final copper concentrations confirmed by F-AAS [2.2 (2.1–2.3) µM]. Cells cultured in Chelex-treated medium for up to 4 weeks were morphologically indistinguishable from those cells cultured in usual growth medium, and cells reached confluency at the same point. Cells treated with TPEN also had significantly lower total cellular zinc than control cells in untreated medium, whilst those treated with zinc pyrithione had significantly higher cellular zinc content (Fig. 1A). Cells cultured in medium with 1 µM zinc had significantly lower total cellular zinc content than cells that had been cultured in medium containing 20 µM zinc (Fig. 1A), and ZIP2 mRNA expression in cell lysates decreased with increasing zinc concentrations (Fig. 1B). Glutathione peroxidase activity in cell lysates increased significantly as selenium concentrations increased (Fig. 1C). Exposure of cells to LPS/PepG for 4 h resulted in a significant increase in NFκB activation [0.81 (0.78–0.90) compared with 2.13 (1.96–2.26) fluorescence units in cells without LPS/PepG, P=0.002], but was not affected by trace metal concentrations. Interleukin-6 and IL-8 concentrations were significantly higher in cells treated with LPS/PepG for 24 h in normal medium compared with untreated cells in all experiments (P<0.0001), as expected. Treatment of cells with TPEN had no effect on IL-6 concentrations, but exposure of cells to zinc pyrithione resulted in significantly lower IL-6 concentrations (Fig. 1D, P=0.002). However, zinc concentrations across a more physiological range did not affect IL-6. Likewise, IL-6 concentrations were similar at all zinc concentrations and also increased as selenium concentrations (Fig. 1D), and IL-8 was also unaffected by trace metal concentrations (data not shown).

Mitochondrial membrane potential and metabolic activity were all significantly lower in cells treated with LPS/PepG in normal medium for 7 days compared with untreated cells, as expected (P=0.002 and P=0.004, respectively). In cells exposed to LPS/PepG at a range of zinc concentrations, membrane potential increased as zinc concentration increased, but selenium had no effect (Fig. 2A). However, metabolic activity was elevated at the highest zinc concentrations and also increased as selenium increased (Fig. 2A). Cells treated with rotenone as a positive control had significantly lower membrane potential (P=0.008) and higher metabolic activity (P=0.02), as expected (data shown in online Supplementary material). Adenosine triphosphate concentrations were also significantly higher at the highest zinc concentration (Fig. 3).
Clinical study

Thirty-nine patients were recruited, of whom one was withdrawn because they were subsequently found to fulfill the exclusion criteria, leaving 20 patients with sepsis and 18 critically ill non-infected control patients. Demographic characteristics (summarized in Supplementary Table 1) and APACHE II and SOFA scores were similar in both groups. Patients in the sepsis group had a significantly longer length of stay on ICU compared with the control group ($P=0.001$), and higher ICU and 28 day mortality. An overview of the various diagnoses/sites of infection is given in Supplementary Table 2.

Trace metal status was compromised in all patients, with extremely low plasma zinc concentrations, at around only 25% of the normal physiological range ($\sim10–17$ $\mu$M) and low
The normal range for selenium depends on geographical location because of the local soil selenium content. However, GPx3 requires circulating selenium concentrations of \( \sim 1 \mu M \) for optimal activity, and concentrations were below this in most patients, with many sepsis patients having selenium concentrations below 0.5 \( \mu M \), which would be considered severely depleted (Fig. 4). Glutathione peroxidase activity was also low in all patients (activity is usually expected to be \( >0.4 \text{ U ml}^{-1} \)). Zinc, selenium, and GPx3 activity were significantly lower in the patients with sepsis than in the control patients (Fig. 4). Plasma zinc and selenium concentrations, and selenium and GPx3, were positively correlated (\( P=0.0008 \) and \( P=0.0007 \), respectively).

Marked inflammation was seen, with increased cytokine concentrations, but there was no significant difference in IL-8 between sepsis patients and control patients (Fig. 5A). Interleukin-6 was more variable, particularly in the sepsis patients, with again no different between the two groups (Fig. 5A). Pentraxin 3, CRP, and suPAR were all elevated, whilst CRP and suPAR concentrations, but not pentraxin 3, were higher in patients with sepsis than in control patients (Fig. 5C). Plasma selenium was correlated significantly with both CRP (\( P=0.0008 \)) and IL-6 (\( P=0.0007 \), respectively).

ATP production in endothelial cells exposed to LPS/PepG in normal medium (control) or LPS/PepG and a range of zinc concentrations for 24 h. Results are expressed as the percentage of LPS/PepG-treated control cells in normal medium. \( P \)-values shown are from Kruskal-Wallis test. *Significantly different from control (\( P<0.05 \), Wilcoxon-Mann-Whitney test). Box-and-whisker plots show median, interquartile and full range (\( n=6 \)).

Mitochondrial membrane potential (MMP; A) and mitochondrial metabolic activity (B) in endothelial cells exposed to LPS/PepG in normal medium (control) or LPS/PepG and a range of zinc concentrations for 24 h. Results are expressed as the percentage of LPS/PepG-treated control cells in normal medium. \( P \)-values shown are from Kruskal-Wallis test. *Significantly different from control (\( P<0.05 \), Wilcoxon-Mann-Whitney test). Box-and-whisker plots show median, interquartile and full range (\( n=6 \)).

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There was also evidence of oxidative stress; LPO concentrations were elevated in patients with sepsis and were significantly higher than in the control patients (Fig. 6A). Likewise, concentrations of MPO and thiocyanate (a major substrate for the MPO enzyme) were higher in the sepsis group (Fig. 6A and C). There was also a loss of oxidation-sensitive thiols and a suggestion of loss of methionine residues on plasma proteins, with a corresponding increase in the concentrations of the oxidation product methionine sulfoxide, in the sepsis patients compared with control patients (Fig. 6D and F). Concentrations of all other protein amino acids were not altered significantly. Glutathione peroxidase activity was correlated with MPO (\( P=0.04 \)) and LPO (\( P=0.02 \)) concentrations. Nuclear phosphorylated NFkB was detected in all
patients, indicating active translocated NFκB, but concentrations were not different between groups and there was no relationship with cytokine concentrations or markers of oxidative stress (data not shown).

Discussion

Markedly compromised plasma zinc and selenium concentrations occur in critically ill patients, and are associated with oxidative damage and inflammation. Most of these parameters were more affected in ICU patients with sepsis compared with control ICU patients who had no evidence of infection. In addition, using an endothelial cell model of sepsis, zinc or selenium concentrations within the physiologically achievable range had no effect on cytokine profiles but did affect aspects of mitochondrial dysfunction in conditions mimicking sepsis.

We used Chelex treatment of FBS to create zinc-depleted cell culture medium. Culturing cells in a low-zinc environment resulted in decreased total cellular zinc content without affecting cell viability and resulted in altered zinc transporter protein expression. Despite previous suggestions that low zinc concentrations would promote oxidative stress and result in more pronounced inflammatory responses, we found no difference in levels of activated NFκB or in IL-6 and IL-8 concentrations when zinc supply was varied within a physiologically achievable range. In contrast, using the same method of cellular zinc depletion as used here, it was reported previously that phorbol myristate acetate or LPS-induced cytokine production was higher in HL-60 cells and endothelial cells cultured in 1 µM compared with 15 µM zinc. Likewise, zinc depletion of human lung fibroblasts, achieved using Chelex, was reported to result in oxidative stress. Using a variety of human lung cell lines exposed to tumour necrosis factor α plus LPS, zinc was recently reported to promote NFκB activation by interacting directly with inhibitor of nuclear factor κB (IκB) kinase. As expected, in our study, NFκB activation, IL-6, IL-8, and oxidative damage markers were higher in endothelial cells exposed to LPS/PepG, as we have previously reported, yet there was no difference over a wide range of zinc concentrations, except after exposure to unphysiologically high concentrations using zinc pyrithione. These results suggest that the endothelial cells were very efficient at maintaining inflammatory mediator production, despite limited zinc availability, in conditions mimicking sepsis.

The effect of low zinc on inflammation is likely to depend on cell-specific zinc transporters, through which zinc homeostasis and cellular zinc concentrations are regulated. We observed down-regulation of the zinc importer ZIP2 as the zinc concentration in the culture medium was increased, along with increased cellular zinc content, but cells were very tolerant of low zinc availability. Exposure of cells to differing selenium concentrations in conditions mimicking sepsis also did not affect IL-6 concentrations, despite sensitivity of the cells to selenium as shown by marked effects on GPx3 activity. However, we did find that the zinc or selenium environments influenced aspects of LPS/PepG-induced mitochondrial dysfunction. Mitochondrial damage plays a pivotal role in the pathophysiology of sepsis, characterized by loss of membrane potential and metabolic activity, finally resulting in impaired ATP production. Our data suggest that the zinc or selenium environment can affect mitochondrial function in conditions of sepsis, but generally the cells were able to tolerate low zinc availability.

We found differences in the key oxidant-generating enzyme MPO (and its major substrate, thiocyanate), biomarkers of inflammation, and multiple markers of oxidative damage (particularly cysteine and methionine residues on plasma proteins, which are major targets for damage generated by MPO and its oxidants because of their abundance and susceptibility to oxidation) between patients with sepsis and control patients, despite similar APACHE II and SOFA scores. Oxidative stress has been consistently described in patients with sepsis, but the extent of the oxidative damage and the relationship to trace metal status and inflammatory biomarkers have not been reported. Peroxidases, including MPO, play an important role in the innate immune system because they catalyse formation of antimicrobial agents such as hypochlorous or hypothiocyanous acid from hydrogen peroxide and halide ions, which can cause host cell and mitochondrial damage. Myeloperoxidase-derived oxidants target selenoenzymes, including GPx and thioredoxin reductase, and can also damage zinc–cysteine or zinc–histidine clusters in proteins, resulting in irreversible damage and derangement of zinc homeostasis. Myeloperoxidase-derived oxidants have been implicated in several inflammatory conditions, and high MPO concentrations have been linked to decreased survival after myocardial infarction. We found the highest MPO concentrations in
those patients with sepsis, which was negatively associated with GPx activity, and we found loss of oxidation-sensitive thiols and increased methionine sulfoxide, confirming oxidative protein damage. Likewise, LPO was elevated in patients with sepsis, indicating oxidative lipid damage. Such comprehensive and broad-ranging oxidative damage has not been reported previously, and it is interesting to speculate that not only will suboptimal zinc and selenium contribute to oxidative stress and inflammation, but oxidative damage may also contribute to low selenium and zinc and further oxidative stress.

Plasma zinc concentrations were low in all patients and lowest in those with sepsis. Low plasma zinc has been reported previously in ICU populations, although in most studies the concentrations were not as low as reported here. Indeed, in some patients the concentrations found here were within the range of symptomatic zinc depletion in an experimental human model. We also found that the low zinc concentrations were associated with high LPO and high IL-6 concentrations, in agreement with others. Redistribution of trace metals from the circulation to tissues, such as the liver, as part of the acute phase response, resulting in low plasma concentrations of both zinc and selenium, has been known for some time and is thought to be mediated in part by cytokines. However, the exact mechanism and the benefit or harm of this is still unclear. It is known that, in contrast to iron, the main determinant of zinc absorption is current dietary intake rather than previous intake or existing whole-body zinc status. Zinc homeostasis is well controlled in the gastrointestinal tract so that when dietary intake is low, absorption is almost complete and urinary excretion of zinc is curtailed. During the acute phase response, however, urinary zinc excretion is increased regardless of zinc intake, implying that the homeostatic mechanisms controlling zinc may be impaired. The extremely low zinc concentrations seen here may therefore result from poor zinc availability attributable to both movement of zinc into tissues and increased zinc excretion in the urine, despite the low intakes common in ICU patients, and may be exacerbated by oxidative damage to homeostatic mechanisms.

Selenium has a direct role in antioxidant protection because it is present within the active centre of selenoproteins, which include GPx, thioredoxin reductase and some isoforms of methionine sulfoxide reductase, and MPO-derived oxidants target these selenium-containing enzymes. Furthermore, elevated concentrations of methionine sulfoxide were detected on plasma proteins, which may arise from a decreased concentration or

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**Fig 5** Plasma IL-6 (A), interleukin-8 (IL-8; B), and pentraxin 3 (PTX3; C) C-reactive protein (CRP; D), and soluble urokinase-type plasminogen activator receptor (suPAR) concentrations (E) in critically ill patients without infection (control, n=18) and patients with sepsis (n=20). Box-and-whisker plots show median, interquartile and full range (+=outliers). P-value is Wilcoxon–Mann–Whitney test.
activity of the selenium-dependent methionine sulfoxide reductase proteins or form decreased concentrations of its cofactor thioredoxin, which is maintained in its reduced form by selenium-dependent thioredoxin reductase. Concentrations of selenium were very low in plasma from all patients, lowest in patients with sepsis, and strongly associated with plasma zinc concentrations and GPx3 activity. Others have described low plasma selenium in ICU patients, associated with peak CRP and IL-6 concentrations, but few have reported the relationship between both selenium and zinc. Like zinc, selenium concentrations have also been reported to decrease as part of the acute phase response. The extent to which low plasma selenium concentrations are reflective of a selenium deficit, temporary re-distribution or uptake, or loss via oxidative damage to selenium-containing proteins, or all of these, is not clear.

Zinc or selenium supplementation trials of patients with sepsis have been largely inconclusive, with the best evidence for selenium. A systematic review in 2013 concluded that administration of selenium decreased mortality rates, but the largest study to date, of more than 1000 surgical ICU patients, found that adjuvant selenium treatment was not associated with a favourable outcome in patients with sepsis. Clinical zinc supplementation trials, mostly in conjunction with administration of other trace elements and antioxidants, have provided no strong evidence to support the benefit of zinc supplementation. Despite this, a survey of ICUs revealed that low plasma zinc was often used as a trigger to administer zinc regardless of the lack of evidence to support supplementation. Given that zinc absorption is regulated by intake not by whole-body status, it is likely that administration of high doses of zinc to patients on ICU may result in increased zinc excretion.

In summary, we report combined suboptimal zinc and selenium concentrations in critically ill patients that are associated with marked oxidative damage to proteins and lipids, which themselves may compound trace element status further.

**Supplementary material**

Supplementary material is available at British Journal of Anaesthesia online.
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Authors’ contributions

K.M. undertook all the zinc analyses, contributed to the ethics committee submission, collated and analysed the clinical data, and drafted the paper. J.T. and L.H. undertook oxidative stress analyses under the supervision of M.J.D., and L.H., J.T., and M.J. D. all contributed to writing the paper. D.A.L. supervised some of the laboratory work and critically revised the paper. N.R.W. contributed to study design and ethics committee submission, oversaw patient recruitment, and contributed to writing the paper. J.H.B. supervised zinc measurements and contributed to study design and conduct. H.F.G. conceived of the study, supervised the entire project, contributed to data analysis, and critically revised the paper. All authors approved and edited the final manuscript.

Declaration of interest

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