The effectiveness of trace element supplementation following severe burn injury: a systematic review

Rochelle Kurmis

Master of Clinical Science

Joanna Briggs Institute

School of Translational Health Science

Faculty of Health Sciences

The University of Adelaide

Contents

Abstract

Trace elements have an important physiological role following severe burn injury with patients routinely receiving supplementation. Although trace element supplementation is commonly prescribed after burn injury, variations exist between supplement composition, frequency and the dosage administered. This objective of this research was to identify, assess and synthesise the available evidence on the effectiveness of trace element supplementation on clinically meaningful outcomes, including mortality, length of stay, rate of wound healing and complications in patients who have sustained a severe burn injury.

Following development of an *a priori* protocol, the effectiveness of selenium, copper and zinc supplementation, either alone or combined, compared to placebo or standard treatment, was investigated via systematic review and meta-analysis. A comprehensive search strategy was designed and employed to identify published and unpublished research. Methodological quality of eligible studies was critically appraised and relevant data extracted for synthesis.

Eight studies were included in the review: four randomised controlled trials and four non-randomised experimental trials, representing 398 participants with an age range of six to 67 years.

Results of this research indicate that the use of parentally-administered combined trace elements following burn injury confers positive effects in decreasing infectious complications. Combined parenteral trace element supplementation and combined oral and parenteral zinc supplementation have potentially clinically significant implications on reducing length of stay. Oral zinc supplementation shows possible beneficial effects on mortality. Further studies are required to accurately define optimal trace element supplementation regimens, dosages and routes, and to determine cost-effectiveness.

Declaration

I, Rochelle Kurmis, certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and, where applicable, any partner institution responsible for the joint-award of this degree.

I give consent to this copy of my thesis when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the *Copyright Act 1968*.

The author acknowledges that copyright of published works contained within this thesis resides with the copyright holder(s) of those works.

I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library Search and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

Rochelle Kurmis

May 2015

Acknowledgements

The undertaking of this masters thesis would not have been possible without the wonderful support and encouragement of many significant people whom I would like to acknowledge and thank.

Firstly, I would like to thank my supervisors Associate Professor Edoardo Aromataris and Associate Professor John Greenwood for their continued support and guidance. I would particularly like to thank Associate Professor Edoardo Aromataris for his time, often outside of formal “business hours”, which I appreciate, and for his support and patience, especially with my often poorly edited drafts and chaotic and unexpected external family challenges.

I would also like to thank Dr Zachary Munn for his persistent encouragement for me to undertake this masters degree in the first instance and for his presence, patience and assistance throughout. Thank you to Siang Tay for her assistance with copyediting of the thesis chapters to assist with grammatical consistency and punctuation. Also, a big thank you to my work colleagues for their support and understanding throughout this process.

To my wonderful family, thank you for supporting me throughout this undertaking and being there, especially when stress and turmoil seemed to rain. Happily, we all survived and achieved many milestones together, despite my often side-tracked and disorganised thoughts.

Finally, I would like to commend all of the study authors who have contributed to this field. It is with passion rather than financial gain that you achieve what you do, and each small step forward contributes towards improving patient care and outcomes globally.

Chapter 1: General introduction

1.1 Introduction

Nutrition support is recognised as an essential part of patient management following a severe burn injury.[1] Nutritional deficiencies exacerbate complications of severe burn injury such as infections, delayed wound healing and muscle catabolism, leading to deconditioning and increasing need for physical rehabilitation.[1, 2] Infective complications, such as wound sepsis and pneumonia, remain a major cause of mortality in the burn injury population.[1, 2]

Trace element deficiencies are recognised as part of the sequelae following severe burn injury.[2, 3] A survey of American Burn Association (ABA) Burn Centres indicated that 92% of responding centres routinely supplement patients with vitamins and/or minerals.[4] This common practice of vitamin and mineral supplementation following burn injury prompted the conduct of the research detailed in this thesis to assess the available evidence on the effectiveness of trace element supplementation on clinical outcomes, including mortality, length of stay (LOS) (hospital and intensive care unit (ICU)), and infective complications following severe burn injury. This chapter provides a background to burn injury and trace element supplementation, with particular focus on selenium (Se), copper (Cu) and zinc (Zn). The methodology for the systematic review is introduced along with how this topic relates to current and potential future clinical contexts within burn injury management. Chapter 2 presents the published systematic review protocol[5], whilst Chapter 3 presents the final systematic review as accepted for publication by the *Journal of Burn Care and Research*.[6] Finally, Chapter 4 discusses the findings of the review and presents the related implications for practice and future research. Due to the nature of this thesis by publication and abiding to the guidelines of the School of Translational Health Science, the need for repetition of some information, for example, introductory information in chapters 1, 2 and 3, is unavoidable. Chapters 2 and 3 are presented in their respective published and accepted for publication formats.[5, 6]

1.1.1 Disease burden related to burn injuries

In the United States (US) it has been estimated that each year, around 450,000 people seek medical treatment for burn injuries, and 3400 people die of burns as a direct result of fires.[7] In the US, Australia and New Zealand, fire/flame and scalds are the most common causes of burn injury.[8, 9]

Longer term societal economic costs of burn injury are also important factors to recognise.[10] Only 50-67% of people who are actively employed at the time of their burn injury return to paid

employment.[11] Globally, fire related burns have been estimated to account for 10 million Disability Adjusted Life Years (DALYs) each year.[12] In addition, physical rehabilitation from burn injury is often more prolonged than that of other types of injuries.[11] As a result, the financial burden of care for burn injury management is significant.[9, 12] To minimise this burden decreasing the amount of time for wound healing to occur is one of many strategies. Early wound healing generally facilitates earlier discharge from the acute hospital sector and decreases ongoing scar management requirements, resulting in significant cost and related resource savings.[13-15]

The average hospital LOS for patients who survive a burn injury is just over one day per percent of total body surface area burned.[9] For example, this equates to around 20 days expected hospital admission for a person who sustains a 20% total body surface area (TBSA) burn injury. Economic costs of burn injury vary by country and demographic group, with direct care for paediatric burn injuries alone exceeding US$211 million in the US in 2000, whilst in 2007 hospital burn management costs in Norway were over EUR€10.5 million.[10]

Mortality in the burn injury population can be either directly associated with the initial severity of the injury, or as a result of subsequent clinically related complications.[8, 9] Pneumonia is the most commonly reported clinical complication related to burn injury, with an incidence of 5.9% in fire/flame injury admissions[9] compared to a usual hospital acquired pneumonia rate of 0.08%.[16] Mechanical ventilation for four or more days increases the risk of acquiring pneumonia for burn injury patients.[9] The Baux score was developed in 1961 to assist the prediction of mortality following burn injury.[17] This simple equation was described as: Percent Mortality = Age + Percent Body Burned. Due to the improvement in survival rates as a result of advancements in the management of burn injury, the Baux Score was revised in 2010 to include inhalation injury due to its association with mortality.[17] Mortality rates for patients admitted from fire/flame injury in the US have been reported as 5.9%, with an average of three weeks hospital LOS for patients with less than 70% TBSA burns who do not survive.[9] For this patient group the average daily hospital expense is around US$14,000, which is more than that for burn injury survivors.[9] Total average hospital charges for a burns survivor in the US are $86,146 versus $285,225 for an in hospital death.[9]

1.1.2 Physiological function of selenium, copper and zinc

Se is an antioxidant and achieves this function as an essential component of the active site of the enzyme glutathione peroxidase (GSH-Px).[4] GSH-Px is active in the antioxidant defences of both the intra- and extra-cellular environments.[2] Depleted endogenous stores of antioxidants have been associated with an increase in free radical generation and heightened systemic inflammatory

responses.[18] In the ICU population, decreased antioxidant capacity is associated with increased morbidity and mortality.[18] As Se also plays an important role in the rate limiting step of the biosynthesis of GSH-Px, Se deficiency directly influences antioxidant responses.[4] Se also contributes to tissue oxygenation, protection against lipid per-oxidation, phagocytic activity of neutrophils[4], activation and regulation of thyroid hormones, DNA synthesis, and cell viability and proliferation.[18]

Similarly, the trace elements Cu and Zn also promote wound healing, as components of several metalloenzymes.[3, 4] Cu is a component of lysyl oxidase which is necessary for cross linking of collagen fibres[4]; this is important for wound healing rates and healed wound integrity. Cu is also a component of the antioxidant enzyme superoxide dismutase.[3, 4] Low levels of Cu decrease synthesis of superoxide dismutase, allowing for increased oxidative damage as a result of inflammation.[4] Zn is required for the function of over 200 metalloenzymes as well as for normal cell replication and growth.[4] Immune function is also influenced by Zn status, with deficiency leading to thymic atrophy, loss of T-helper cell function and alterations to the normal profiles of serum immunoglobulins.[4]

1.1.3 Trace element requirements

In Australia and New Zealand, Nutrient Reference Values (NRVs) have been determined for both macro- and micro-nutrients.[19] These represent the average daily requirements of healthy individuals.[19] The Recommended Dietary Intake (RDI) is defined as the average daily nutrient consumption required to meet the needs of 97-98% of healthy individuals for a particular gender and age group.[19] The Upper Level of Intake is defined as the highest average daily consumption level likely to cause no adverse reactions, such as toxicity, to nearly all individuals in the general population.[19] Where it is not possible to determine an RDI, an Adequate Intake (AI) level is applied.[19] Adequate Intake is defined as the average daily nutrient consumption based on observed or experimentally determined estimates or approximations of a group (or groups) of apparently healthy people where their nutrient intake is assumed to be sufficient.[19] The currently recognised NRVs for Se, Cu and Zn in Australia and New Zealand for children and adults are summarised in Table 1.

Table 1. Trace element nutrient reference values for specified age and gender groups[19]

Age group and gender		Selenium µg/day		Copper mg/day		Zinc mg/day	
		RDI	UL	AI	UL	RDI	UL
Children	1-3 years	25	90	0.7	1	3	7
	4-8 years	30	150	1	3	4	12
Boys	9-13 years	50	280	1.3	5	6	25
	14-18 years	70	400	1.5	8	13	35
Girls	9-13 years	50	280	1.1	5	6	25
	14-18 years	60	400	1.1	8	7	35
Men	≥19years	70	400	1.7	10	14	40
Women	≥19years	60	400	1.2	10	8	40

Note: Excludes values for infants (<1 year old), pregnancy, and lactation
Abbreviations: RDI=Recommended Dietary Intake, UL=Upper Level of Intake, AI=Adequate Intake

1.1.4 Trace element status from a population perspective

Se status in humans is directly affected by dietary intake and is sensitive to changes in the food chain.[20, 21] Wheat production and supply alone may contribute up to half of the available Se for adult Australians.[20-22] The majority of Se that people ingest from their food is dependent on the Se concentration of the soil in which crops are grown.[20, 23] The level of Se in the soil varies greatly with geography.[20, 21] Factors that improve Se content of soils include weathering of Se-containing rocks, volcanic activity and agricultural use of Se-containing fertilisers. In contrast, acid rain, burning of fossil fuels and fertilisers containing a high content of sulphur (sulphur acts as an antagonist with Se), heavy irrigation and soil acidification all contribute to the decreased availability of Se in the food chain.[20-22]

Pre-existing medical co-morbidities, such as gastrectomy procedures, have been reported to be associated with acute trace element deficiencies, including Cu deficiency.[4, 24] This is important to consider in the context of a recent increase in bariatric surgeries for weight loss in developed countries.[25] In Australia alone these procedures reportedly increased from 500 in 1998-1999 to 17,000 in 2007-2008.[25] In addition the elderly population are at higher risk of inadequate dietary intakes of both Cu and Zn, most notably where lower socio-economic factors are present.[26]

1.1.5 Trace element status following burn injury

As previously mentioned, trace elements, such as Se, Cu and Zn, play an important physiological role in immune function as well as wound healing; however they are acutely depleted following severe burn injury.[2, 3] The mechanism of trace element depletion following burn injury appears to be multi-

modal.[4, 27, 28] Trace elements are thought to be primarily lost through extensive exudative losses following injury.[27, 28] Concomitant increases in urinary excretion of these metals following burn injury contribute significantly, whilst additional causes of losses include thermal destruction of skin, repeated surgeries and removal of burn eschar.[2-4, 27, 28] The reported antagonistic relationship between endogenous Se and the silver used in burn dressings may also contribute to observable losses of Se.[3, 4] It has been reported that 5-10% of total body Zn stores and 20-40% of total body Cu stores may be lost within seven days of a severe burn injury.[4] This burn induced deficiency may also be further compounded by deficiencies as a result of pre-existing conditions or poor nutritional intake prior to injury.[4, 29]

Serum trace element concentrations following burn injury should be interpreted with caution.[29] Circulating levels may not be truly reflective of total body stores due to the pronounced inflammatory state following a severe burn injury[30] and the potential use of albumin as part of fluid resuscitation.[31] Se and Zn are recognised as negative acute phase reactants.[32] This means that in the face of acute inflammation, such as that elicited by trauma, circulating serum concentrations will decrease. Approximately 55-90% of circulating Zn in the body is bound to albumin.[30, 32] During acute phase reactions, circulating albumin drops significantly; however the decrease in Zn is often larger than that of the albumin, indicating transfer of Zn from its carrier protein to some other site.[30, 32, 33] Therefore, clinical interpretation of plasma Zn concentration should be performed in conjunction with the concentrations of circulating albumin and C-reactive protein (CRP), a marker of acute phase inflammation.[30, 33, 34] In burn injury, however, the use of albumin as part of fluid resuscitation may artificially alter the circulating levels of this plasma protein so that it is no longer reflective of total body stores.[31] Conversely, Cu is a known acute phase reactant (i.e. levels increase following trauma)due to increased synthesis of ceruloplasmin by the liver, which is thought to act as an anti-oxidant during illness.[30, 32] In addition to these complexities in interpretation, laboratory reference ranges for trace elements are influenced by the analytical methods used to process samples, and hence individual variances for each centre exist.[35] Reference ranges are reflective of the statistical normal distribution within a population and represent 95% of that population.[35] Laboratory reference ranges for Se, Cu and Zn reported in the burn injury literature are: Se 0.64-1.5 µmol/L; Cu 11.75-22 µmol/L; Zn 9.6-20 µmol/L.[2]

1.1.6 Trace element supplementation

Trace element supplementation may be provided by either the enteral (gastrointestinal tract) or the parenteral (intravenous (IV)) route. Enteral supplementation may be oral, such as tablet form, separate boluses flushed down a feeding tube, or as part of the enteral nutrition support provided

(i.e. components of tube feed or oral drink formulations). Parenteral supplementation may be provided as a component of IV formulations used for nutrition support or administered as separate boluses via the IV catheter.[18, 33] Supplements via either route may be given as single agents or as combined therapies.[36, 37]

Due to elevated requirements following injury, trace element supplementation in excess of standard nutritional requirements for healthy populations is sometimes provided.[36, 38] Recently published guidelines suggest that supplementation may be required for varying durations of time depending on the size of the burn injury: seven to eight days for 20-40% TBSA burned, two weeks for 40-60% TBSA burned, and one month (30 days) for >60% TBSA burns.[38]

Although published guidelines strongly support the supplementation of Se, Cu, and Zn, and provide recommendations for the duration of this supplementation, no guidance regarding dosage is provided.[38] Research investigating the effectiveness of IV supplementation of trace elements reports variations in dosage from 0.43-2.9 µmol Se, 15.04-42 µmol Cu and 194.44-406 µmol Zn.[39, 40] Due to postulated antagonism of Cu and Zn in the gastrointestinal lumen, trace element supplementation via the enteral route is controversial. Some proponents of supplementation hence prefer parenteral provision of trace elements, which directly negates this issue.[38] Other groups, however, have reported that in the burn injury population, high dose enteral Zn supplementation does not interfere with serum Cu concentrations or cause gastrointestinal disturbances.[34] Regardless of route, due to the supra-normal dosages of trace elements administered and the potential for toxicity to occur, monitoring of supplemented trace elements is warranted.[29, 34] Despite the limitations with interpretation of serum concentrations[30], they remain the most practical and readily accessible clinical tool for monitoring serum concentrations.

1.1.7 Why this systematic review is needed

Currently many international, evidence-based nutrition support guidelines are available for clinicians, providing practice recommendations for the ICU setting.[41-43] These nutrition guidelines are commonly adopted for burn injury patients, as specific guidance for this sub-population may not be available.[41] More often, recommendations for burn injury patients are extrapolated from critical care research.[41] As previously mentioned, supplementation of vitamins and trace elements is common practice following burn injury[36, 38]; however the lack of uniformity in this practice reflects a lack of clear evidence-based guidance for this clinical practice.[33, 36]

A recent systematic review by Landucci and colleagues[44] investigated the efficacy of parenteral supplementation of Se as a monotherapy in ICU patients on antioxidant status, infection, organ failure, LOS and mortality. This systematic review focused on randomised controlled trials (RCTs) and quasi-randomised controlled trials where parenteral Se supplementation was administered in addition to routine care. Supplementation in conjunction with other anti-oxidant nutrients (including Cu and Zn) was excluded. Nine RCTs, including a total of 921 participants, were included in the meta-analysis of this review and reported a significant reduction in 28-day mortality with Se supplementation (RR=0.84, 95% CI 0.71, 0.99, p=0.04).[44] No association of Se supplementation with hospital LOS or increased risk of pulmonary infections could be determined.[44] The limitations identified in this review were that included studies had small sample sizes (six of the nine included studies involved less than 100 participants) and the large variety of methods and duration of Se administration.[44] This review included predominantly studies investigating mixed or medical ICU patients, with one included study including septic and trauma patients.[44] None of the included studies specifically investigated a burn injury population as a subgroup of their cohort.[44]

In their systematic review and meta-analysis of the effectiveness of antioxidant micronutrients on selected clinical outcomes in ICU patients, Manzanares and colleagues[45] included two studies investigating supplementation following burn injury, with the remaining 19 included trials investigating other subgroups of the ICU population, including those with medical, surgical and trauma diagnoses. This systematic review reported that combined anti-oxidant supplementation (including Se) was associated with significantly reduced mortality in the heterogeneous ICU population (RR=0.82, 95% CI 0.72, 0.93; p=0.002), with no significant effects on reducing infections or LOS (hospital and/or ICU).[45] Sub-group analysis of parenteral Se supplementation studies indicated that there were trends towards decreased mortality (RR=0.89, 95% CI 0.77, 1.03; p=0.11) and decreased infectious episodes (RR=0.87, 95% CI 0.74, 1.02; p=0.08).[45] Additional sub-group analyses demonstrated a trend towards reduced mortality when an initial loading dose of Se was provided prior to supplementation (RR=0.81, 95% CI 0.65, 1.02; p=0.07), although this administration strategy did not have an effect on infectious complications (RR=0.96, 95% CI 0.69, 1.33; p=0.80).[45]

Strengths of both of these reviews included their clearly documented search strategies, with Manzanares et al.[45] performing a comprehensive search, including grey literature, well developed inclusion criteria, and recognised methods of critical appraisal and data synthesis.[44, 45] Neither of these two reviews appeared to follow an *a priori* published protocol, although both described employing pre-specified sub-group analyses.[44, 45]

A search for systematic reviews on the effectiveness of trace element supplementation following severe burn injury in MEDLINE, the *Cochrane Library*, and the *Joanna Briggs Institute* (JBI) *Database of Systematic Reviews and Implementation Reports* failed to identify any similar previous publication. Synthesis of the current evidence regarding trace element supplementation following severe burn injury has the potential to influence and improve consistency in evidence-based care internationally. In comparison to many other interventions following burn injury, such as surgical procedures, modern wound dressings and antibiotics, nutritional intervention is relatively inexpensive.[46] As a result, the specific objective of this review was to assess the effectiveness of Se, Cu, and Zn supplementation on mortality, length of ICU/hospital stay, wound healing and infection rates (wound and nosocomial) in patients who had sustained a severe burn injury.

1.2 Methodological basis for the review

1.2.1 Methodology

Undertaking a quantitative systematic review of effectiveness, in keeping with JBI[47] and Cochrane[48] methodologies, was considered the most appropriate approach to address the objective of this research. Development of the search strategy is outlined in Chapter 2 (Section: Search Strategy). The search strategy employed across all pre-defined databases is outlined in Chapter 3 (Section: Search Strategy, Table 1) and additional supplementary information is provided in Appendix 1.

Inclusion of grey literature searches as a component of the search strategy aimed to minimize publication bias and selection bias in the review through the identification of unpublished studies.[48] Due to the probability that unpublished data is likely to show weaker effect estimates or unfavourable side effects of treatments, identification and inclusion of this data in this systematic reviews is important to ensure the validity of resultant aggregated data.[48]

1.3 Current clinical context

The mixed ICU population represents diverse surgical and medical diagnoses and illness severities.[49] Patients with burn injury, however, are a specific sub-group of this critical care population, characterised by severe hypermetabolic, inflammation, endocrine and immune responses.[33, 38] In combination these characteristic responses have a pronounced effect on nutritional requirements.[38] A recently published set of recommendations for nutritional therapy in major burns by the European Society for Parenteral and Enteral Nutrition (ESPEN) “strongly suggested” that micronutrient substitution, including Se, Cu and Zn, be included for both adults and children.[38] This was provided as Grade C evidence (based on the GRADE [Grade of Recommendation, Assessment, Development and Evaluation] methodology[50]), indicating that the contributing evidence supporting this

recommendation was of low quality[51]; however due to strong agreement between experts it was supported as a moderate strength recommendation.[38] The duration for Se, Cu and Zn supplementation was recommended as: seven to eight days for 20-40% TBSA burns, 14 days for 40-60% TBSA burns, and 30 days for >60% TBSA burns.[38] Other burns specific nutrition guidelines have been published[52]; however these failed to evaluate the quality of included evidence or based their recommendations solely on expert opinion.[53] Clinically this topic appears to be of interest, with a recent narrative review of the evidence for micronutrient supplementation, including trace element supplementation published in burns literature.[33]

1.4 Potential clinical impact

Translation of evidence into clinical practice may be achieved through the adoption of synthesised results in future clinical practice guidelines.[54] For centres that do not routinely supplement burn injury patients with trace elements, this systematic review may assist with provision of evidence to support changes to current practice through the aggregation of efficacy data which can be used to influence local policy, such as pharmacy formulary choices and agreed upon safe prescription dosages for this population. Should trace element supplementation following severe burn injury prove effective, significant cost savings could be achieved through its potential to decrease hospital LOS with reductions in wound healing time.

Chapter 2: The systematic review protocol

The following chapter contains the content of the protocol as published in the *JBI Database of Systematic Reviews and Implementation Reports*, 2013; 11(11) 44-53. *doi: 10.11124/jbisrir-2013-1134*

Kurmis, R., Aromataris, E. & Greenwood, J. (2013). The effectiveness of trace element supplementation following severe burn injury: A systematic review protocol *JBI Database of Systematic Reviews and Implementation Reports, v. 11 (11), pp. 44-53*

Chapter 3: The systematic review

The following chapter contains the content of the systematic review submitted to the *Journal of Burn Care and Research* on 9 October 2014 and accepted for publication, following peer review and revisions, as of 3 December 2014.

The authors wish to acknowledge The Journal of Burns Care and Research, In Press 2015 Vol xxx, Page 00-00, DOI: 10.1097/BCR.000000000000259. Wolters Kluwer Health Lippincott Williams & Wilkins©.

ORIGINAL ARTICLE

Trace element supplementation following severe burn injury: A systematic review and meta-analysis

Rochelle Kurmis BND APD, CF JBI[1, 2]
John Greenwood AM, BSc(Hons), MBChB, MD, DHlthSc, FRCS(Eng), FRCS(Plast), FRACS[2]
Edoardo Aromataris BSc(Hons), PhD[1]

1. The Joanna Briggs Institute, School of Translational Health Science, The University of Adelaide, 5005
2. Adult Burns Center, Royal Adelaide Hospital, Adelaide, 5000

Corresponding author
Rochelle Kurmis, Allied Health Project Manager, Burns Unit, Royal Adelaide Hospital, North Terrace, Adelaide 5000, South Australia, Australia
Fax: +61 8 8222 5676
Phone: +61 8 8222 2614
Email: **Rochelle.Kurmis@health.sa.gov.au**

October 2014

Acknowledgements

We would like to thank Anna Pearce for her assistance with critical appraisal of studies meeting eligibility criteria, Maureen Bell, Research Librarian, Barr Smith Library, The University of Adelaide, South Australia for her assistance with search strategy design and all of the staff at the Joanna Briggs Institute and Adult Burns Service, Royal Adelaide Hospital who supported this project.

Funding

No funding was received for the development, writing, or submission of this manuscript was received. The authors have no known conflicts of interest to declare.

Abstract

Objective

Trace elements have an important physiological role following severe burn injury with patients routinely receiving supplementation. Although commonly prescribed after burn injury, variation exists between supplement composition, frequency and the dosage administered. This review aims to assess the effectiveness of trace element supplementation on clinically meaningful outcomes in patients who have sustained a severe burn injury.

Methods

Supplementation of selenium, copper and zinc, either alone or combined, compared to placebo or standard treatment were eligible for inclusion. Pre-determined primary outcome measures were mortality, length of stay, rate of wound healing, and complications. A comprehensive search strategy was undertaken. Methodological quality of eligible studies was appraised and relevant data extracted for meta-analysis.

Results

Eight studies met eligibility criteria for the review; four RCTs and four non-randomized experimental trials, including a total of 398 participants with an age range of 6-67 years. Parenteral supplementation of combined trace elements was associated with a significant decrease in infectious episodes (Weighted Mean Difference -1.25 episodes, 95% Confidence Intervals -1.70, -0.80, $p<0.00001$).

Conclusions

The results of this review indicate that the use of parentally-administered combined trace elements following burn injury confer positive effects in decreasing infectious complications. Combined parenteral trace element supplementation and combined oral and parenteral zinc supplementation have potentially clinically significant findings on reducing length of stay. Oral zinc supplementation

shows possible beneficial effects on mortality. Definitive studies are required to accurately define optimal trace element supplementation regimens, dosages and routes following burn injury.

Key Words
Burn injury, trace elements, nutrition support

Introduction

Pronounced inflammatory responses along with severe metabolic disturbances are observed following severe burn injury.[1, 2] Nutritional deficiencies exacerbate the complications of severe burn injury including infection, delayed wound healing and muscle catabolism. This is important since infective complications, such as wound sepsis and pneumonia, remain a major cause of mortality following hospitalisation due to burn injury.[1, 2] Trace elements, such as copper (Cu), selenium (Se), and zinc (Zn), play an important physiological role in immune function as well as wound healing, and all are acutely depleted following severe burn injury.[2-4] The cause of these deficiencies appears to be multi-modal. Trace element deficiencies appear to arise primarily due to extensive exudative losses following injury, repeated surgeries[2, 3, 5, 6], and burn baths commonly administered as part of burn injury management.[6] Reports suggest that 5-10% of total body Zn stores and 20-40% of total body Cu stores are lost within seven days of severe burn injury, with increases in urinary excretion of these metals contributing significantly to their depletion.[7] Additional loss of trace elements occurs through thermal destruction of skin and with removal of burn eschar. The reported antagonistic relationship between endogenous Se and silver used in antimicrobial burn dressings may contribute to observable losses of Se.[3, 7]

In a survey of American Burn Centers, 92% routinely supplemented patients with vitamins and/or minerals.[8] Although common practice, variation exists between the supplements administered. International evidence-based nutrition support guidelines are available for clinicians and provide global recommendations for the Intensive Care Unit (ICU) setting.[9-11] These guidelines are commonly adopted for burn injury patients as burn- specific guidance for this sub-population may or may not be available. More often, recommendations for burn injury patients are extrapolated directly from critical care data. The critical care population is recognised as a heterogenous group. Burn injury however, is a specific sub-group, characterised by the severe hypermetabolic, inflammatory, endocrine and immune responses. These combine to have a pronounced effect on nutritional requirements, and therefore evidence-based recommendations for nutritional supplementation in burn injury should be separate from the "general" critical care population. Recently published recommendations for nutritional therapy for patients with major burns suggested that micronutrient substitution, including Zn, Cu and Se, should be included for both adults and children.[12] This was provided as Grade C evidence (based on the GRADE methodology [13]) with strong agreement between experts.[12] A search for systematic reviews on this topic in MEDLINE, the Cochrane Database of Systematic Reviews and The Joanna Briggs Institute (JBI) Database of Systematic Reviews and Implementation Reports, failed to identify any existing publication on this specific topic. This apparent gap between primary research and translation into evidence based practice prompted this

study, the objective of which was to review currently available evidence assessing the effectiveness of trace element supplementation on clinically meaningful outcomes following severe burn injury in children and adults. More specifically, to assess the effectiveness of Se, Cu and Zn supplementation on mortality, length of intensive care unit (ICU)/ hospital stay, wound healing and infection rates (wound and nosocomial) in patients who have sustained severe burn injury.

Methods

Protocol & Registration

In keeping with the PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) guidelines[14], an *a priori* protocol was published[15] (PROSPERO registration number CRD420140007049).

Eligibility Criteria

Population

This review considered studies that included children (2-18 years of age) and adults (≥ 18 years of age) who sustained severe burn injury (defined as burn injury ≥ 10% Total Body Surface Area (TBSA) in children and ≥ 15% TBSA in adults) and had been admitted to an ICU, Burns ICU (BICU), or burns unit for surgical management of their injury. Studies that included patients with significant multi-trauma in addition to burn injury were excluded.

Intervention & Comparison

Studies that evaluated enteral or parenteral supplementation of Se, Cu and Zn, either alone or combined and compared to placebo or regular treatment were eligible for inclusion, where treatment and control groups received standard nutrition intervention including enteral or parenteral nutrition and multi-vitamin supplements. Studies that included trace element supplementation in combination with other predefined nutrient supplementations were also considered for inclusion.

Outcome Measures

Pre-determined primary outcome measures for this review were mortality; length of stay (LOS)(ICU/ hospital); rate of wound healing (time to first donor site healing or time to wound closure); complications (e.g. wound infection, hospital acquired pneumonia). Secondary outcome measures were defined as tissue (measured from skin biopsies) and serum (measured via blood sampling) Se, Cu and Zn concentrations.

Studies

This review primarily considered experimental study designs including randomised controlled trials (RCTs) however both experimental and epidemiological study designs including non-randomised controlled trials, quasi-experimental, before and after studies, prospective and retrospective cohort studies and case control studies were also considered for inclusion. The decision to include observational studies rather than RCTs alone was made due to the low number of eligible studies anticipated and the lack of evidence for significant differences in effect estimates between these two study designs.[16]

Information Sources

A three-step search strategy in keeping with the JBI methodology was developed to find published and unpublished studies investigating the effectiveness of trace element supplementation following burn injury. An initial search of PubMed and CINAHL was conducted, followed by analysis of text words contained in the title and abstracts of relevant articles, along with index terms and key words. A second comprehensive search using all the identified keywords and terms was then performed across all pre-defined databases and sources.[15]. Table 1 lists databases accessed to identify published data, while the following clinical trial registries and grey literature repositories were searched to identify un-published data: clinicaltrials.gov (US Clinical Trials Register), www.australianclinicaltrials.gov.au (Australian clinical trials register), www.anzctr.org.au (Australian and New Zealand Clinical Trials Register), www.controlled-trials.com (European Clinical Trials Register), Mednar, www.opengrey.eu, DART-Europe E-thesis Portal and www.openthesis.org.

Search Strategy

The detailed search strategy employed, including key words and limits, is shown in Table 1. At the time searches were conducted, auto-alerts were set-up based on the search parameters and any additional publications were considered up to July 2014.

Holistic burn injury management prior to 1980 appears significantly different to current practice, and hence nutritional interventions from this period may not translate in regards to effectiveness measures. All citations retrieved from database and searching sources of grey literature were exported into the bibliographic citation management software EndNote® X6.0.1 (Thomson Reuters). Following removal of duplicates and screening of titles and abstracts against the eligibility criteria for the review, potentially relevant full text articles were retrieved and assessed as to their suitability for inclusion in the review.[15] Where required, corresponding authors were contacted via email to request further information to assist with this process.

Reference lists of all retrieved studies were searched manually to attempt to locate any additional, relevant citations that were not identified as part of the first and secondary search strategies.

Table 1. Detailed database search strategy including key words and limits

Database (search platform indicated where relevant)	Search terms	Filters/ Limits
PubMed	(((Burns[mh:noexp] OR burn*[tiab] OR thermal injur*[tw])) AND (Trace elements[mh] OR trace element*[tiab] OR selenium[mh]OR selenium[tiab] OR copper[mh] OR copper[tiab] OR zinc[mh] OR zinc[tiab] OR antioxidants[mh] OR antioxidant*[tiab] OR nutrition*[tiab] OR nutritional support[mh])) NOT sunburn[tiab]	From 1980/01/01 to 2014/12/31
Embase (OVID)	#1 Trace element.de. or trace element*.ti. or trace element*ab.mp. or selenium.de. or selenium.ti. or selenium.ab. or copper.de. or copper.ti. or copper.ab. or zinc.de. or zinc.ti. or zinc.ab. or antioxidant.de. or antioxidant*.ti. or antioxidant*.ab. or nutrition*.ti. or nutrition*.ab. or nutritional support.de. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword] #2 Burn.de. or burn*.ti. or burn*.ab. or thermal injur*.de.	yr="1980 - Current"
CINAHL (EBSCO)	(TI+(%26quot%3btrace+element*%26quot%3b)+OR+AB+(%26quot%3btrace+element*%26quot%3b)+OR+MH+%26quot%3btrace+elements%26quot%3b+%2b+OR+TI+(selenium)+OR+AB+(selenium)+OR+TI+(copper)+OR+AB+(copper)+OR+TI+(zinc)+OR+AB+(zinc)+OR+TI+(antioxidant*)+OR+AB+(antioxidant*)+OR+TI+(nutrition*)+OR+AB+(nutrition*)+OR+MH+%26quot%3bnutritional+support%26quot%3b+%2b+OR+TI+(mineral*)+OR+AB+(mineral*))+AND+(MH+burns%2b+OR+TI+(burn*)+OR+AB+(burn*)+OR+TI+(%26quot%3bthermal+injur*%26quot%3b)+OR+AB+(%26quot%3bthermal+injur*%26quot%3b))+NOT+(TI+(sunburn)+OR+AB+(sunburn))&cli0=DT1&clv0=198001-201401&type=1&site=ehost-live">(TI "trace element*" OR AB "trace element*" OR MH "trace elements"+ OR TI selenium OR AB selenium OR TI copper OR AB copper OR TI zinc OR AB zinc OR TI antioxidant* OR AB antioxidant* OR TI nutrition</A>	
Web of Science	#1 TS=Trace element* OR TI=trace element* OR TS=selenium OR TI=selenium OR TS=copper OR TI=copper OR TS=zinc OR TI=zinc OR TS=antioxidant* OR TI=antioxidant* OR TS=nutrition* support OR TI=nutrition* support #2 TS=Burn* OR TI=burn* OR TS= burn injur* OR TI=burn injur* OR TS=thermal injur* OR TI=thermal injur* #3 TS=Sunburn #4 #2 AND #1 #5 #4 NOT #3	DocType=All document types; Language=All languages; 1980- 2013

Study Selection & Methodological Assessment

Papers that met the pre-determined eligibility criteria for the review[15] were assessed by two reviewers (RK and AP) independently for methodological validity prior to inclusion in the review using standardised and piloted critical appraisal instruments from the JBI Meta Analysis of Statistics Assessment and Review Instrument (JBI-MAStARI, Joanna Briggs Institute, University of Adelaide, South Australia).[17] Studies achieving an appraisal score of ≤4 were excluded due to their high risk of bias. Any discrepancies that arose between the reviewers were resolved through discussion, or with a third reviewer where required. We attempted to contact corresponding authors where additional information not published was required, and critical appraisal scores were adjusted subsequently where appropriate.

Data Extraction

Data extracted from included papers used the standardised data extraction tool from JBI-MAStARI.[17] Data extracted included specific details about the interventions (trace element supplemented and dosage, mode (enteral/ parenteral) and duration of administration, single agent/combined therapies) populations (age, gender, burn severity, location of treatment), study methods (study design, recruitment, sample size, randomization, methods and timing of measurements) and outcomes of significance to the review question and specific objectives where available. Serum trace element concentrations were pooled using the units µmol/L. Where study data was presented as µg/L, the following conversion factor was applied: µmol/L x (molar mass) g/mol = µg/L where the molar mass of Cu =63.546 g/mol, molar mass of Zn =65.39 g/mol, and the molar mass of Se= 78.96 g/mol. Where relevant data was missing (e.g. standard deviation), this data was calculated where possible using information from the relevant publications. Where participant numbers in treatment versus experimental groups were lacking (ie. only total participant numbers provided), allocated groups were assumed to be equal.[18, 19]

For the purpose of meta-analysis only intervention and burned control groups were compared. Where included citations presented results of additional groups including alternative treatments not of interest to this review or “healthy” (non-burned) controls, this data was excluded from extraction, along with outcome measures reported in all studies that were not pre-defined as of interest to this review.

Authors were contacted for complete data where relevant data was omitted or not reported in the published manuscript (such as hospital LOS, standard deviations, number of participants allocated to each treatment group)[2, 18, 19] or where data was presented as conference abstracts or for completed

registered trials without related publications being identified through the search strategy (un-published data).[20-22]

Data Synthesis

Quantitative data, where possible, was pooled for statistical meta-analysis using Review Manager (RevMan) 5.3 software.[23] Effect sizes are expressed as risk ratios (RR) with 95% confidence intervals (CI) for mortality data. Weighted mean differences (WMD) and their 95% CI for serum trace element concentrations, infectious complications, and LOS were calculated for analysis. Due to inadequate data available, wound healing could not be included in the meta-analyses. Risk ratios were calculated using the Mantel-Haenszel method whilst an Inverse Variance approach was employed for WMD estimates. A random-effects model, as described by DerSimonian and Laird[24] was applied to estimate variances for the Mantel-Haenszel and Inverse Variance estimations.[25] Heterogeneity was assessed statistically using the standard chi-square (Chi^2) test and inconsistency quantified by the I^2 statistic, between-study variance was estimated using tau-squared (Tau^2).[25] Due to the small number of studies in each analysis (<10), potential for publication bias was not tested using funnel plot asymmetry due to the insufficient power to determine chance from real asymmetry.[25] For the purpose of this review we considered $p < 0.05$ for reporting statistical significance for overall effect, however due to the small number of included studies and small sample sizes, to avoid misinterpretation of heterogeneity $p < 0.10$ was considered statistically significant for the results of the Chi^2 test.[25]

Results

Study Selection

Of 13,029 potential citations identified via electronic and hand searches, as well as citations identified via auto-alerts, 50 full text articles were assessed for eligibility. Critical appraisal scores for all studies that met the inclusion criteria are provided in Table 2. Of the 15 relevant studies, seven were excluded on the basis of low methodological quality. Of the eight remaining studies, seven studies were able to be included in meta-analyses[2, 18, 19, 26-29] whilst one study could only be presented as a narrative synthesis.[20] The full process of study selection is detailed in Figure 1.

Study Characteristics

The included studies were four prospective, randomized, blinded, control trials [2, 18, 19, 28] and four non-randomized experimental trials.[20, 26, 27, 29] Characteristics of included studies and extracted outcomes are provided in Table 3. Overall, studies in this review included 398 participants with an age range of 6-67 years.

Participants

One study from the oral Zn supplementation group included pediatric patients as part of their cohort[26, 30], whilst the remaining studies only investigated effectiveness of trace element supplementation in adult burn injury patients.

Both the included studies investigating oral Zn supplementation included a "healthy control" comparison group[26, 29], whilst one also included additional comparison groups investigating alternate anti-oxidant compounds out of the scope of this review.[29] One of the combined trace element supplementation studies also included a "healthy control" group for comparison of tissue trace element concentrations.[2] Only one study defined the burn aetiology of their cohort[26] (flame and scald), whilst three studies defined thermal burn injury as part of their inclusion criteria.[19, 27, 28]

Interventions

Five studies, conducted in Switzerland, investigated varying doses of combined trace elements (Cu, Se and Zn) provided by the parenteral route (83 participants).[2, 18, 19, 27, 28, 30] Two studies, both conducted in Iraq, investigated the administration of a single daily oral dose of Zn (sulphate) (250 participants).[26, 29] The remaining study, conducted in the United States of America, compared enteral administration of Zn with combined enteral and intra-venous (IV) Zn supplementation (65 participants).[20] All included studies were published between 1994 and 2014 and reported on at least one of the pre-defined primary outcome measures[2, 18-20, 26-30], whilst six reported on at least one secondary outcome measure.[2, 18, 20, 26, 27, 30]

Details of nutritional management, excluding intervention/control, were specified for all of the parenteral combined trace element supplementations studies[2, 18, 19, 27, 28, 30] and omitted from both of the oral supplementation studies and oral versus oral and IV study.[20, 26, 29] Four of the included studies described their wound management/surgical management practices.[2, 26, 27, 29] Four[2, 19, 20, 28] of the seven studies presenting an intervention versus a comparison group reported the distribution of inhalation injuries between groups, all of which were not statistically significant, whilst one study identified five participants (equating to 50% of the included cohort) as having an inhalation injury but did not specify as to which group they were allocated.[27] This last study introduces the potential for a high risk of selection bias due to alternate allocation type method for group allocation employed, as the cohort is such a small sample size (n=10, five in each group), especially if the distribution of inhalation injury were significantly different between the intervention and control group. These factors need to be considered as limitations when interpreting LOS, mortality and infective complications (primarily pneumonia). Of the four included RCTs[2, 18, 19, 28], none detailed their randomisation methods.

Table 2. Critical Appraisal Scores for studies that met eligibility criteria for the review.

	Citation	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Total
Included Studies	Al-Kaisy et al. 2006[26]	No	Yes	No	No	Yes	Yes	Yes	Yes	Yes	-	6
	Berger et al 1994[27]	No	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	8
	Berger et al, 1996[19]	Unclear	Unclear	Unclear	Yes	Unclear	Yes	Yes	Yes	Yes	Yes	6
	Berger et al 1997[18]	Unclear	Yes	Unclear	Unclear	Yes	Yes	Yes	Yes	Yes	Yes	7
	Berger et al, 1998[28]	Unclear	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	9
	Berger et al, 2007 [2]	Unclear	Yes	Unclear	Yes	Unclear	Yes	Yes	Yes	Yes	Yes	7
	Nordlund et al, 2014*[20]	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	-	8
	Sahib et al, 2010[29]	No	Unclear	Unclear	Yes	Unclear	Unclear	Yes	Yes	Yes	Yes	5
Excluded studies	Al-Jawad et al, 2008[31]	No	Unclear	No	No	Unclear	Yes	Yes	Unclear	No	-	2
	Barbosa et al, 2009[32]	Unclear	Unclear	Unclear	Unclear	Yes	Yes	Unclear	Yes	Yes	Unclear	4
	Caldis-Coutris et al, 2012[33]	No	No	No	No	No	Yes	No	No	Yes	-	2
	Cunningham et al, 1991[34]	No	No	No	Yes	n/a	Yes	Yes	No	No	-	3
	Cunningham et al, 1993[35]	No	No	No	Yes	n/a	No	No	No	No	-	1
	McClain et al, 1993*[22]	No	No	No	No	No	Unclear	No	Unclear	No	-	0
	Pochon, 1981[36]	No	Yes	No	Yes	No	Yes	No	Yes	No	-	4

Studies that scored four out of nine/ten or less were rated as being at high risk of bias and were excluded from data extraction and synthesis. * Abstracts only, authors contacted for additional information, scores adjusted following additional information from authors where provided. n/a = not applicable. Please refer to priori published protocol for full critical appraisal question descriptions.[15]

Figure 1: Flow diagram outlining systematic review process, following PRISMA criteria with modifications [14]

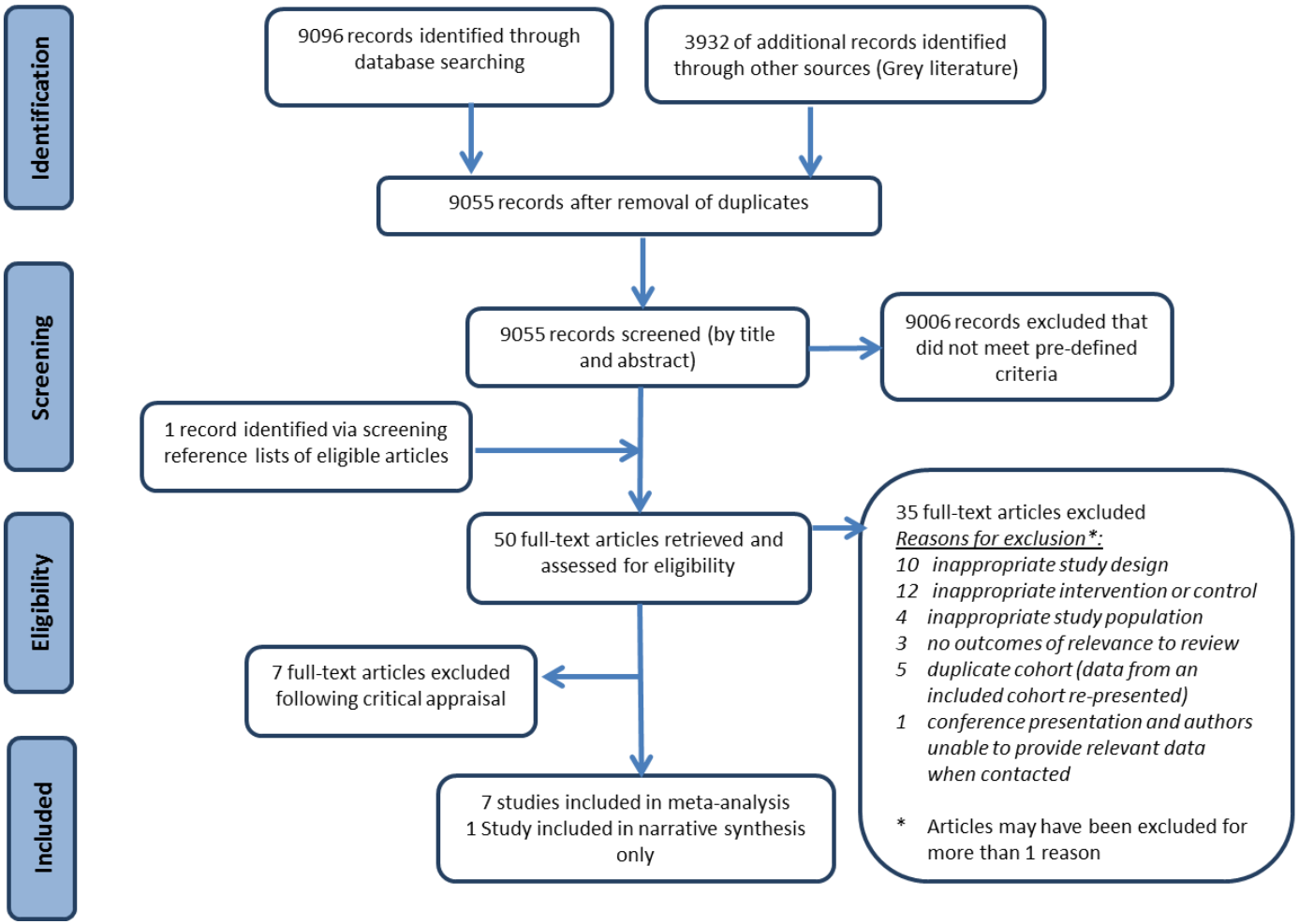


Table 3. Characteristics of included studies

Study	Methods	Participants, setting	Intervention	Outcome measures
Al-Kaisy et al. 2006[26]	**Study design:** Non-randomized experimental trial **Duration of follow-up:** Until Discharge	**Participants** Total *n* =70 Intervention *n* =15 Control *n* = 43 (Healthy control comparison *n*=12 – excluded from analysis) 27 males and 31 females Age (6-67 yrs.) (mean, 35.6 ± 19.4, ± SD) Burn % : 15 to 70% estimated according to the rule of nine Burn degree of first to third. Cause of burns was direct flame in 45 patients (77.5%) and hot water in 13 patients (22.5%). **Setting:** Burn unit, Department of Surgery in Baquba General Hospital, Diyala, Iraq **Inclusion criteria:** Nil stated	**Intervention group:** Standard hospital therapeutic policy + single daily oral dose of a capsule containing 66 mg Zn sulphate, (equivalent to 15 mg elemental Zn) from day of admit until discharge **Control group:** Standard hospital therapeutic policy	Serum Zn (µg/dl) Serum Cu (µg/dl) Wound infection (%) Healing time (days) Mortality rate (%)
Berger et al. 1994[27]	**Study design:** Non-randomized experimental trial Sequential allocation, first 5 standard treatment, second 5 intervention Patient & surgeons blinded, PI not blinded due to risk of Cu toxicity **Duration of follow-up:** Laboratory measures until day 25, others until discharge	**Participants** Total *n* =10 Intervention *n* =5 Control *n* = 5 9 males, 1 female Age; TE group 29±6, Control group 38±2 Inhalation injury in 5 patients **Setting:** Burns Centre of the Centre Hospitalier Universitaire Vaudois in Lausanne, Switzerland **Inclusion criteria:** Thermal burns 30-55% TBSA, >18 years, <65 years of age	Parenteral TE supplementation infused daily over 12h vi peripheral catheter or CVC in addition to standard EN/PN for 7 days followed by all patients receiving 1 oral multi-Vitamin daily until end of week 4 post injury. **Intervention group:** 2.4mg Cu, (15.04µmol Cu) 82 µg Se, (0.434 µmol Se) 26.5 mg Zn (194.44µmol Zn) **Control group:** 0.3mg Cu, (1.88 µmol Cu) 0µg Se, 1.4mg Zn (10.273µmol Zn)	Serum Cu, Zn, Se concentration Length of stay Wound healing Infectious complications mortality

Study	Methods	Participants, setting	Intervention	Outcome measures
Berger et al. 1996[19]	**Study design:** RCT, DB, Placebo controlled Randomization: Methods not reported; randomly assigned to two groups **Duration of follow-up:** 30 days post injury	**Participants** Total *n* = 12 Intervention *n* = unspecified Control *n* = unspecified Age 18-65 years 30-85% TBSA burns 3 cases inhalation injury in each group **Setting:** Burns Centre, Lausanne, Switzerland **Inclusion criteria:** admitted to the Burns Centre	Standard TE + intervention/placebo Day 1-8. After D8, all patients continued to receive standard parenteral TE supplementation **Intervention group:** Standard TEs + additional Cu, Se and Zn, providing mean daily prescription for Cu (40.4µmol), Se (2.9µmol) Zn (406µmol) **Control group:** Standard TEs 20µmol Cu, 0.4 µmol Se, 100µmol Zn	Serum Cu, Se, and Zn Length of stay burn unit and in the hospital Infectious complications mortality
Berger et al. 1997[18]	**Study design:** RCT, DB, placebo controlled Randomization: Methods not reported **Duration of follow-up:** 30 days	**Participants** Total *n* = 20 Intervention *n* = unspecified Control *n* = unspecified Age 41±15 years Burns 49±17 % TBSA (30-87) **Setting:** burns unit of the adult Intensive Care Medicine Department of CHUV in Lausanne, Switzerland, a tertiary university hospital **Inclusion criteria:** Nil stated	Standard recommended parenteral TE +/- additional supplementation, from Day 1-8. In addition standard EN via NJ tube commenced within 12 hrs. of injury. All pts received IV recommended vitamin intakes (Cernevit) + 500mg Vitamin C/day **Intervention group:** Standard TEs + additional IV 1.3 mg Cu,(16.29µmol Cu total) 200µg Se, (1.228µmol Se) 20 mg Zn (194.44 µmol Zn) **Control group:** 1.3 mg Cu, (8.14µmol Cu) 32 µg Se, (0.169µmol Se) 6.5 mg Zn (47.69 µmol Zn)	Serum Se, Zn, Cu concentrations Infections

Study	Methods	Participants, setting	Intervention	Outcome measures
Berger et al. 1998[28]	**Study design:** RCT, DB, Placebo controlled Randomization**:** Methods not reported; On admission randomly allocated to 1 of 2 groups. Ethics & informed consent **Duration of follow-up:** 30 days post injury	**Participants** Total *n* = 20 Intervention *n* =10 Control *n* = 10 Age 40±16 TBSA burned % 48±17 7 cases of inhalation injury, 5 in control group and 2 in TE group **Setting:** Burns Centre, Lausanne, Switzerland **Inclusion criteria:** thermal burns covering > 30% of their body surface areas	Standard amounts of TE commenced as soon as possible after admission Additional intervention/control from day 1 -8 **Intervention group:** Standard TE + additional IV 40.4µmol Cu, 2.9µmol Se, 406 µmol Zn **Control group:** Standard TE 20µmol Cu, , 0.4µmol Se, 100 µmol Zn	Serum Cu, Se, and Zn Length of stay burn unit and in the hospital Infectious complications
Berger et al. 2007[2]	**Study design:** Prospective RCT, placebo controlled patients were stratified according to 3 criteria: burned surface (< or >/=50% BSA), inhalation injury confirmed by bronchoscopy (yes or no), and age (<or ≥50 y) Randomization: Method not stated **Duration of follow-up:** Laboratory markers measured for 20 days, other parameters until discharge	**Participants** Total *n* =21 Intervention *n* =11 Control *n* = 10 15 males and 6 females Age: Intervention group 46±15, Control group 38±16 % TBSA burn: 16-92% Inhalation injuries:Intervention group = 5, Control group = 4 **Setting:** burns unit of the adult Intensive Care Medicine Department of CHUV in Lausanne, Switzerland, a tertiary university hospital **Inclusion criteria:** admission within 6 h of injury; age of 16–65 y; burns on>20% BSA, including >/=10% of the BSA burns assessed as 2nd intermediate to deep or 3rd degree on admission; and informed consent.	**Intervention group:** daily 250 mL of a 0.9% saline solution over 12 h containing 59 µmol Cu, 4.8 µmol Se, and 574 µmol Zn per day, IV route **Control group:** daily 250 mL of a 0.9% saline solution over 12 h containing glucagon-like peptide 1, IV route	length of ICU and of hospital stay, Serum Zn Serum Cu Serum Se Tissue (skin) Zn, Cu & Se concentrations Infections Wound healing Mortality
Nordlund et al. 2014[20]	**Study design:** Non-randomized experimental trial (before and after study design)l **Duration of follow-up:** Until ICU discharge	**Participants** Total *n* = 65 Intervention *n* = 27, 46±15 years of age Control *n* = 38, 45±19 years of age Either sex, **Setting:** Admitted to burns ICU, Harborview Medical Center, Seattle, USA **Inclusion criteria:** Admitted to burns ICU between March 2010 and July 2011	**Intervention group:** 20mg IV elemental Zn for 14 days +220mg/d Zn sulphate until Zn concentrations normalized **Control group:** 220mg/d Zn sulphate (50mg elemental Zn) until Zn concentrations normalized	Length of stay burns ICU (survivors) Mortality Infectious complications Serum Zn concentration

Study	Methods	Participants, setting	Intervention	Outcome measures
Sahib et al 2010[29]	**Study design:** Non-randomized experimental trial Allocated to one of six groups, no detail on methods Consent obtained on admission Ethics approval **Duration of follow-up:** Until discharge	**Participants** Total *n* = 180 Intervention *n* = 30 Control *n* = 30 (4 other intervention groups not of interest to protocol ∴ excluded from analysis, *n*=30 each) Either sex, age range 20-45 **Setting:** Admitted to burns unit, Dept. Surgery, Al-Kindy Medical College, Bagdad, Iraq **Inclusion criteria:** Burn size 15-40% TBSA calculated using rule of nine	**Intervention group:** standard management as per hospital policy + 75mg/d Zn sulphate capsule **Control group:** standard management as per hospital policy	Wound infection Healing time Mortality

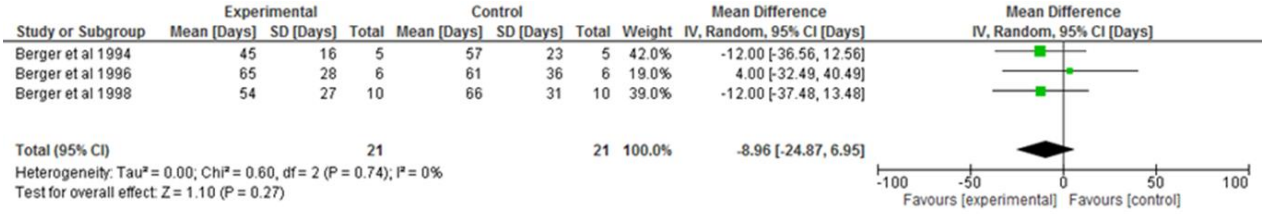
Zn=zinc, Cu=copper, Se=selenium, RCT=randomized control trial, DB= double blinded, TBSA=total body surface area, BSA=body surface area, IV=intravenous, ICU=intensive care unit, TE=trace element

Analysis

Effect of combined parenteral trace element supplementation on length of stay, wound healing, infectious complications, and mortality

Results of the three studies[19, 27, 28] reporting on LOS following parenteral administration of combined trace elements were pooled, demonstrating that trace element supplementation was not associated with a significant decrease in LOS (Figure 2)(WMD -8.96, 95% CI -24.87, 6.95, p=0.27, heterogeneity I^2=0%). These results are clinically significant however, with a mean decrease in LOS of approximately nine days which would represent overall cost savings. When sensitivity analysis was performed, omitting the trial providing lower trace element dosages[27], due to the assumption that there may be a dose related response, results continue to indicate that there is no effect of supplementation on LOS (WMD -6.76, 95%CI -27.65, 14.13, p=0.48, heterogeneity I^2=0%).

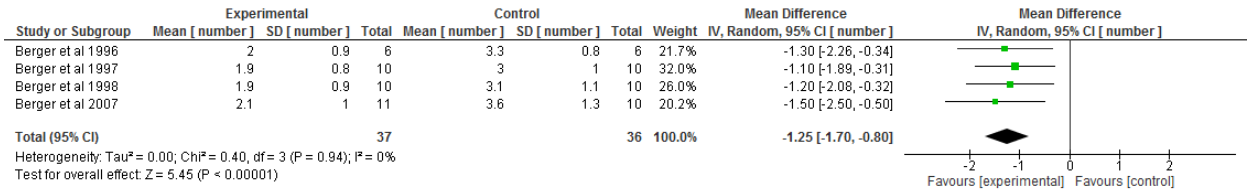
Figure 2. Parenteral combined trace element supplementation and Length of Stay (days)



Wound healing measures were unable to be aggregated due to the significant differences in how this outcome was measured by the two studies where it was reported (surface requiring re-grafting[27] and grafting index (%BSA operated per % BSA requiring surgery)[2]). Individually significant improvements were reported (50% mean decrease with supplementation (p=0.02) and 35% mean decrease in the supplemented group (p=0.01) respectively), however samples sizes in both studies were small.

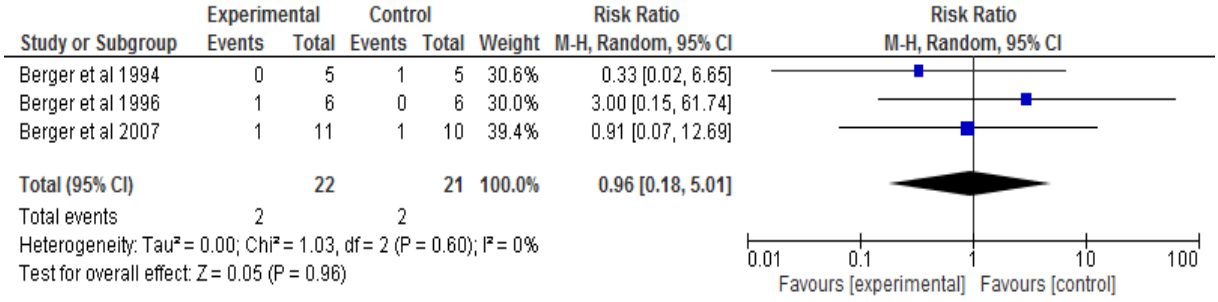
With regards to total infectious complications, when the mean and SD of the four studies[2, 18, 19, 28] are pooled, evidence supports a significant decrease in infectious episodes with trace element supplementation (Figure 3) (WMD -1.25, 95%CI -1.70, -0.80, p<0.00001, heterogeneity I^2=0%). Two studies reported incidence of individual sites of infection.[2, 28] As this data was provided as total counts and ranges only, without mean or SD provided, it was not possible to aggregate subgroups of sites of infections. Both studies reported significantly lower pulmonary infections in the intervention groups (50% reduction (p=0.03) and 80% reduction (p=0.016) respectively), however no statistical differences in incidence of cutaneous, urinary or blood (bacteremia) infections were seen in either trial.

Figure 3. Parenteral combined trace element supplementation and Infectious episodes



Three studies[2, 19, 27] were identified reporting mortality as an outcome following parenteral supplementation of combined trace elements. When aggregated there is no evidence to support an effect of supplementation on overall mortality (Figure 4) (RR 0.96, 95% CI 0.18, 5.01, p=0.96, heterogeneity I^2=0%). When sensitivity analysis was performed, omitting the trial providing lower trace element dosages[27], results continue to support the lack of effect of supplementation on mortality (RR 1.52, 95%CI 0.21, 11.10, p=0.68, heterogeneity I^2=0%).

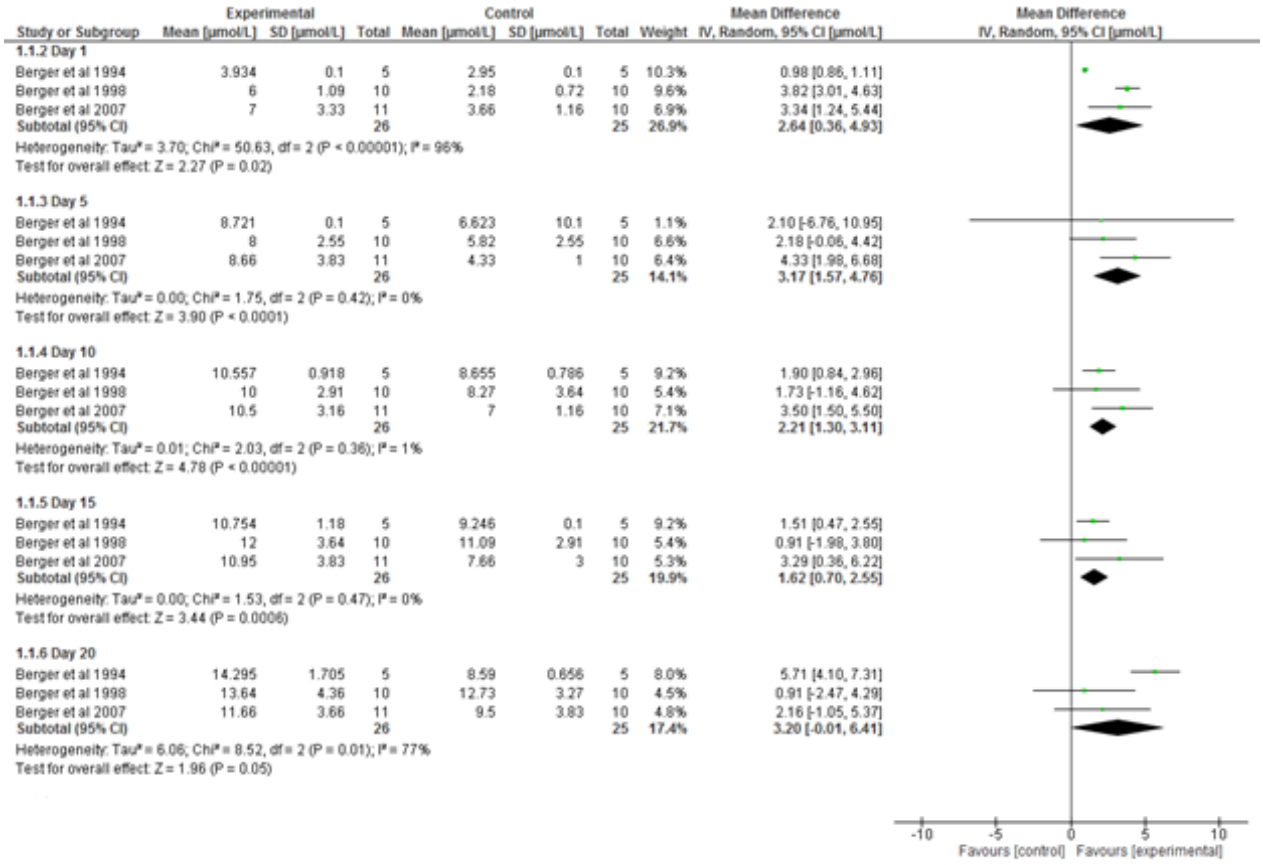
Figure 4. Parenteral combined trace element supplementation and Mortality



Effect of combined parenteral trace element supplementation on serum zinc, copper, and selenium concentrations and tissue trace element concentrations

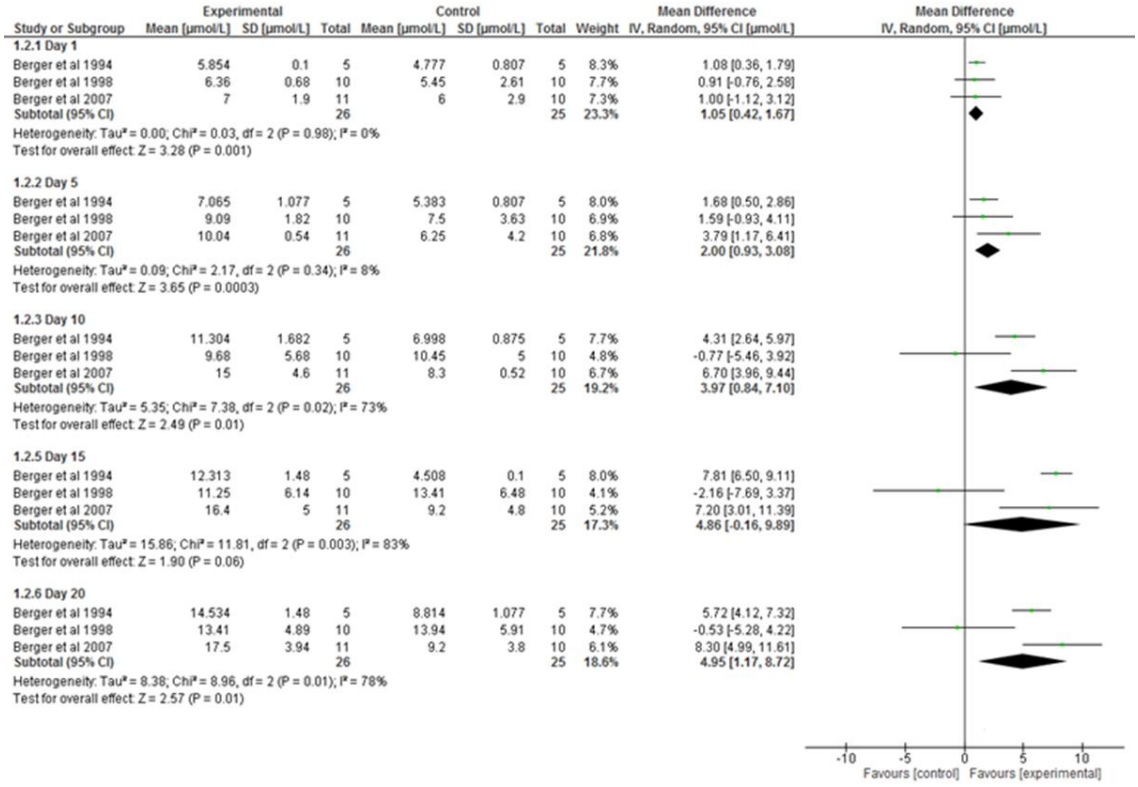
Three studies identified report on serum Zn and Cu concentrations following parenteral supplementation of combined trace element solutions.[2, 27, 28] When data was pooled at common time points across these studies (Figure 5), there was evidence for an effect of supplementation on increasing Zn concentrations at each time point. At time points where statistical heterogeneity was not present, the magnitude of effect provides greater support for the efficacy of supplementation on serum concentrations [Day 5: WMD 3.17, 95% CI 1.57, 4.76, p<0.0001, heterogeneity I^2=0%; Day 10: WMD 2.21, 95% CI 1.30, 3.11, p<0.00001, heterogeneity I^2=1%; Day 15: WMD 1.62, 95% CI 0.70, 2.55, p=0.0006, heterogeneity I^2=0%].

Figure 5. Parenteral combined trace element supplementation and serum zinc concentration



Analysis of serum Cu concentrations (Figure 6) reveals evidence to support an effect of supplementation on serum concentrations at days 1,5,10 and 20. At time points where statistical heterogeneity is not present the magnitude of effect provided greater support for the efficacy of supplementation on serum concentrations [Day 1: WMD 1.05, 95% CI 0.42, 1.67, p=0.001, heterogeneity I^2 0%; Day 5: WMD 2.0, 95% CI 0.93, 3.08, p=0.0003, heterogeneity I^2 8%]. Interpretation of these results must also consider that the control administered in the study performed by Berger et al in 1998[28] provided more Cu than the intervention administered by Berger et al in 1994.[27] When sensitivity analysis was performed omitting the latter trial [27], evidence continues to support a positive effect of supplementation on serum Cu concentrations (WMD 2.81, 95% CI 0.84, 4.78, p=0.005, heterogeneity I^2=77%).

Figure 6. Parenteral combined trace element supplementation and serum copper concentration



Analysis of data for reported serum Se concentrations (Figure 7) supports an effect of supplementation on concentrations at days 1, 5, 10, and 15. At time points where statistical heterogeneity is not present the magnitude of effect provides greater support for the efficacy of supplementation on serum concentrations [Day 1: WMD 0.16, 95% CI 0.07, 0.25, p=0.0004, heterogeneity I^2 0%; Day 10: WMD 0.2, 95% CI 0.11, 0.30, p=0.0001, heterogeneity I^2 0%].

Positive effects of supplementation on all serum trace element concentrations were demonstrated over time when compared with baseline measurements, as can be seen in Figure 8. One study reported tissue trace element concentrations as an outcome measure[2], preventing any further analysis. Results suggested that burned skin tissue levels of Se and Zn are significantly higher by day 20 of admission when compared with baseline (day 3) measures (mean increase of 7.88 nmol/g dry weight (p=0.05) and mean increase of 773.7 nmol/g dry weight (p=0.004) respectively). Again the small sample size included in these results needs to be considered during interpretation.

Figure 7. Parenteral combined trace element supplementation and serum selenium concentration

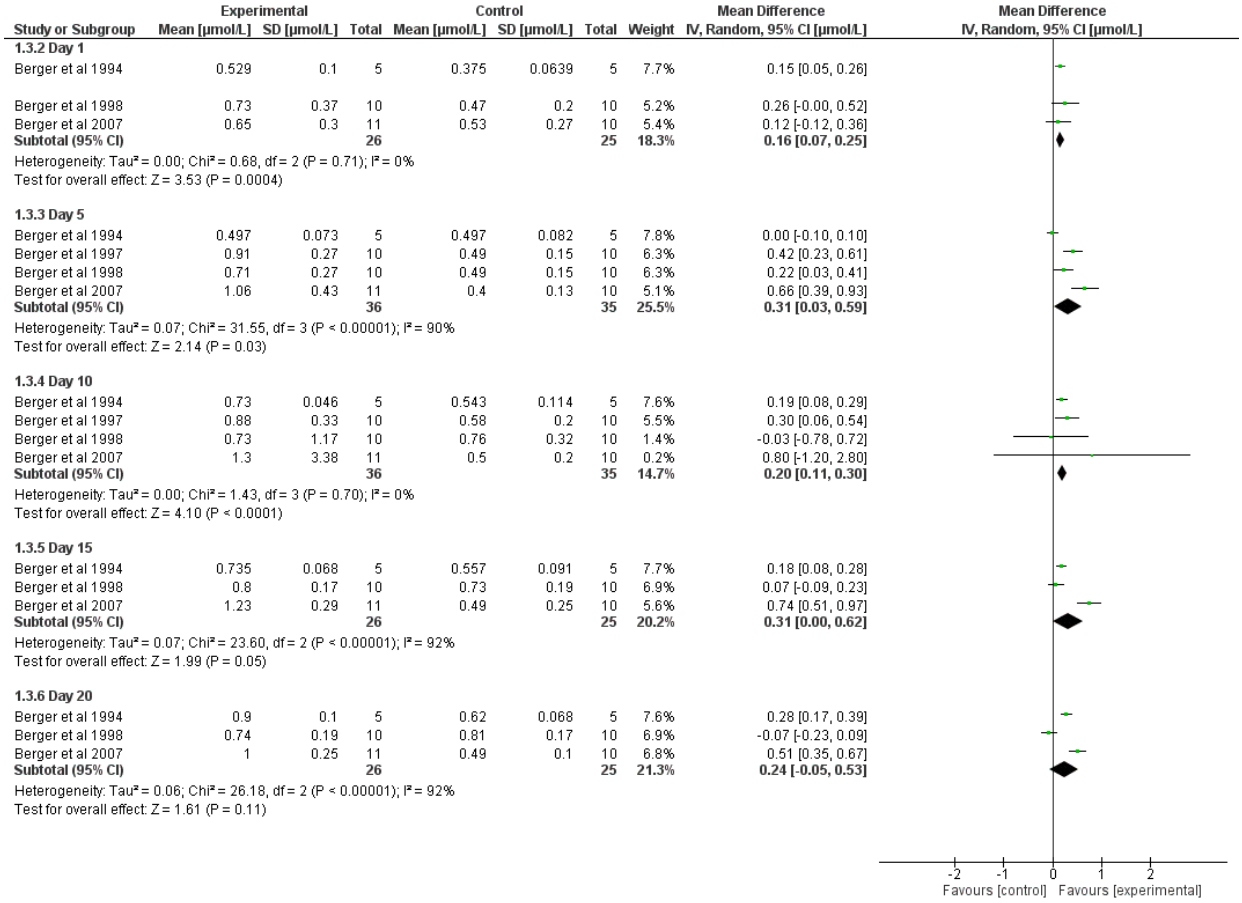
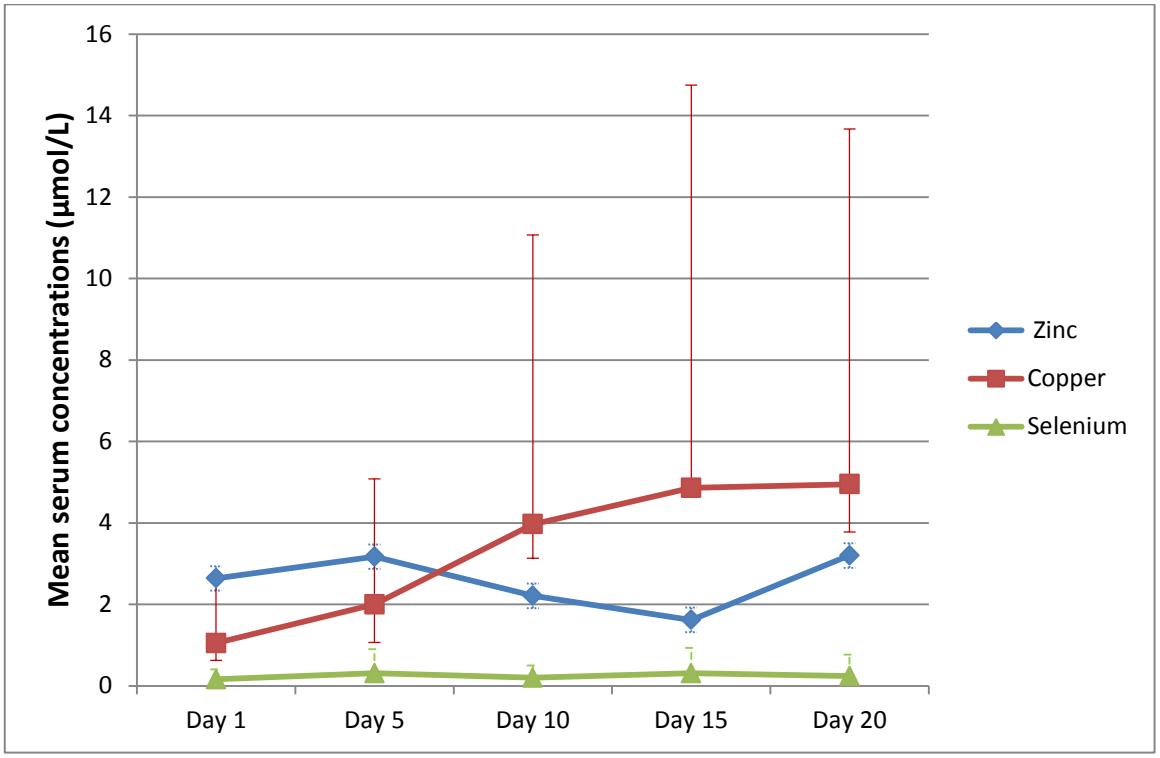


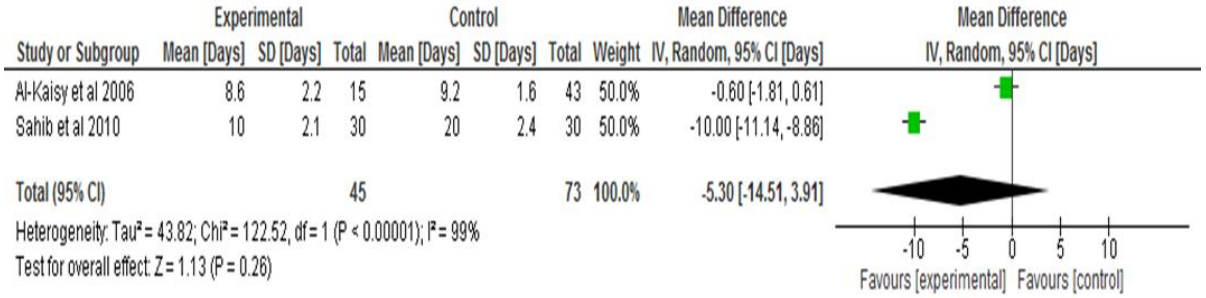
Figure 8. Overall effect estimates of serum zinc, copper, and selenium concentrations vs time



Effect of oral zinc supplementation on wound healing, infectious complications, and mortality

Two included studies[26, 29] investigated the effects of oral Zn supplementation following burn injury. When data for wound healing were aggregated (Figure 9), oral Zn supplementation was not associated with a significant decrease in time to wound healing (days) (WMD -5.30, 95% CI -14.51, 3.91, P=0.26, heterogeneity I^2=99%), however this result could be considered clinically significant when patients are healing five days earlier, which is likely to facilitate earlier discharge from hospital and result in significant overall cost savings.

Figure 9. Oral zinc supplementation and wound healing

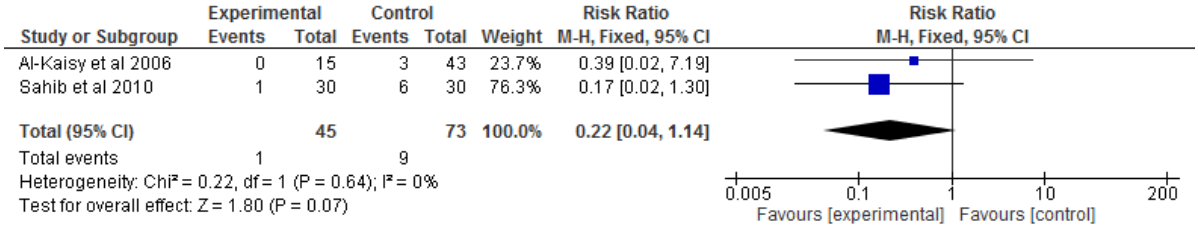


As only counts and percentages were reported for wound swabs (marker of wound infectious complications) in both studies, it was not possible to aggregate these results due to the continuous nature of this data. The study conducted by Al-Kaisy and colleagues[26] reportedly observed no

significant difference in wound infection rates, whilst the study conducted by Sahib and colleagues[29] reported a significant decrease in positive wound swabs at day 3 (13.33% vs 50%) and day of discharge (10% vs 16.66%) in the supplemented group (p<0.05).

Oral Zn supplementation was not associated with a decrease in mortality (Figure 10; RR 0.22, 95% CI 0.04, 1.14, p=0.07, heterogeneity I^2=0%). Length of hospital stay was not reported by either study, with patients' results only being expressed as an undefined "discharge day" in both studies.[26, 29] Al-Kaisy et al.[26] reported significantly higher serum Zn concentration in the supplemented group, mean difference of 38µg/dL by day of discharge, when compared with the burned control group (p<0.05). No significant differences were observed between serum copper concentrations in the supplemented (mean 191 ±33 µg/dl) group versus the burned control group (mean 198 ±18 µg/dl), indicating that oral zinc supplementation does not have an antagonistic effect on copper metabolism.

Figure 10. Oral zinc supplementation and mortality



Effect of combined oral and parenteral zinc supplementation

One included study investigated the effect of combined enteral and parenteral Zn supplementation versus enteral Zn supplementation alone.[20] Results of this study suggest no significant effect on mortality between interventions (15% versus 7%, p=0.38). No significant decreases in ICU LOS were reported when only survivors were included in the analysis (p=0.42), however the mean difference was seven days less in the combined supplementations group (mean ± SD: 30±15) when compared to the oral Zn supplementation group (mean ± SD: 37±24), which represents a clinically significant decrease. Significantly lower total infectious complications are reported in the enteral and IV supplemented group (52 versus 87, p=0.002), characterized by significantly lower wound (26 versus 57, p=0.02) and urinary tract infections (22 versus 57, p=0.006) however no difference in rates of pulmonary infections (pneumonia) were observed (37 versus 27, p=0.39). Although serum concentrations of Zn over time were not presented, time to normalisation of Zn concentrations are reported as not significantly difference between groups (p=0.55).

Discussion

In this systematic review with meta-analyses, three distinct forms of trace element supplementation in burn injury patients were identified; combined trace element supplementation (Cu, Se and Zn) administered via the parenteral route, Zn alone supplemented via the oral route, and combined oral and parenteral supplementation of Zn. This is the first systematic review conducted on this specific topic and resulting evidence supports parenteral supplementation of combined trace elements and combined oral and parenteral Zn administration in decreasing overall infectious complications. Although pooled analysis of specific sites of infections was not possible, individual study data suggests that combined Cu, Zn and Se supplementation confers a protective effect against pulmonary infections, whist combined oral and IV Zn supplementation appears to have a protective effect against wound and urinary tract infections. Challenges exist in attributing direct individual nutrient benefits when interpreting results for the combined trace element supplementation (Cu, Zn, and Se together), as direct comparisons of effect for each nutrient are not possible. No single modality supplementation trials investigating Se or Cu fitting inclusion criteria for this review were identified, making direct nutrient effects impossible to determine. A recent systematic review by Landucci and colleagues investigating Se supplementation in critical care patients identified 9 studies representing a total of 921 participants, 28 day mortality was shown to be reduced when parenteral Se supplementation was administered (RR=0.84, 95% CI 0.71-0.99, p=0.04, heterogeneity I^2=0%) however no positive effect on LOS (WMD 2.12, 95% CI -0.33, 4.57, p=0.09, heterogeneity I^2=0%) or pulmonary infection risk (RR=1.11, 95% CI 0.69, 1.77, p=0.671, heterogeneity I^2=6.8%) could be elucidated.[37] Another recent systematic review, conducted by Manzanares and colleagues, investigated antioxidant nutrients, including Se alone or in combination with other antioxidant nutrients (including Cu and Zn) and also demonstrated a decrease in mortality (RR=0.82, 95% CI 0.72, 0.93, p=0.002, heterogeneity I^2=3%) with again no significant effect on LOS (ICU LOS: WMD=0.07, 95% CI -0.08, 0.22, p=0.38; hospital LOS: WMD= -0.13, 95% CI -0.35, 0.09, p=0.25, heterogeneity I^2=0%) or overall infective complications (RR=0.88, 95% CI 0.76, 1.02, p=0.08, heterogeneity I^2=0%).[38] Extrapolation of these systematic review results from the mixed ICU population to the burns population should be made with caution. While these results suggest that there are likely to be beneficial effects from these supplementation strategies that may translate to the burns population, inclusion of burns patients in the systematic review by Landucci and colleagues is not apparent.[37] In regards to the systematic review by Manzanares and colleagues, two of the studies[2, 28] included also met inclusion criteria for this present review. As a result extrapolation to the burn injury population should not be made directly. No effect of trace element supplementation on mortality could be determined as part of the current review, however due to the small number of included studies and small samples sizes within the included studies any inferences of effect on mortality need to be

interpreted with caution. It is possible that the larger ICU cohort reflect results for the burn injury population as well, however the hypermetabolic response, trace element losses (through skin loss and wound exudate), along with the nutritional requirements for such massive wound healing are unique to burns, and as such should be taken into consideration. None of the included studies in this present systematic review reported on statistical power calculations, so were all likely underpowered to detect an effect on improving mortality, especially within the burn injury population where multiple confounders on mortality (such as inhalation injury, burn size and age) exist. Importantly no increases in mortality were demonstrated, indicating the apparent safety of the trace element regimens administered.

Although no statistically significant evidence was found to support an effect of any form of trace element supplementation on length of hospital or ICU stay, both of the regimens including parenteral administration of trace elements demonstrated a clinically significant decrease in LOS. Again due to the small sample size, even after pooling data where possible, it is likely that adequately powered studies may demonstrate a statistical significance in the future. The cost savings for this clinically significant decrease in LOS would more than outweigh the cost of the related intervention, and inclusion of these cost versus benefit analyses should be considered as part of future studies as this will assist clinicians in justifying implementation of study results, especially given the current restrictions on health budgets. Effects of oral Zn supplementation on infectious complications and LOS could not be examined. When secondary outcome measures of this review were assessed, evidence supported the effectiveness of combined parenteral trace element supplementation on increasing serum Cu, Se and Zn concentrations however no effect was seen for the combined oral and IV Zn supplementation regimen.

Although this review included a comprehensive literature search for both published and unpublished data, it is limited by the inclusion of mostly published data due to the inability to access unpublished trial data identified, despite correspondence with relevant authors. Another limiting factor when interpreting the results of this review, are the small number of studies included and their small sample sizes. This introduces the possibility for type II errors in subgroup analyses. As all studies reporting on parenteral supplementation originated from the same research centre in Switzerland and both studies investigating oral Zn supplementation originated from the same research centre in Iraq, there is potential for duplicate publication bias despite the best intentions of the reviewers and primary authors through omitting duplicate publications[2, 39-41] as part of the screening process. Location and language bias are also limitations of this review. Both articles investigating oral Zn supplementation originated from a country experiencing a period of international military conflict during the period of publication, which may have had an effect on the adequacy of baseline

nutritional status at time of presentation. As ethnicity of participants was not conveyed for either cohort, there is also the potential that these results may pertain to a specific genotype that is not represented widely in the context of an international burn population.

Another consideration when interpreting the results of this review is the inclusion of only single centre studies, due to the lack of identified multi-centre trials in this area. In the broader field of Intensive Care Medicine, it has been identified that the inclusion single centre trials in practice guidelines be viewed with caution due to their frequent lack of scientific rigor or external validity, and their context should be compared with the local setting before adopting changes in clinical practice.[23] Ordinarily the process of a meta-analysis would account for this factor, however in this review all of the combined parenteral trace element supplementation studies came from the same centre in Switzerland whilst both of the oral Zn supplementation studies came from the same centre in Iraq. In the area of burns care this has potential for significant implications due to the great difference in surgical and medical management techniques between centres, regions and countries.
Due to changes in surgical practices over time and the increased use of skin substitutes in some centres along with the shift away from hydrotherapy, exudative losses may have been reduced. Early balance studies quantifying losses of trace elements following burn injury identified wound exudate losses and hydrotherapy following burn injury as significant contributors. [2, 3, 5, 6] As a result in these wound management practices, dosages of trace elements based on this earlier data may need to be reviewed. This is difficult to quantify however, due to the lack of trace element dosage vs effect studies in this population. Interestingly, despite the concern surrounding the benefits of oral zinc supplementation due to its antagonistic relationship with copper in the lumen of the gastro-intestinal tract, no differences were seen in serum copper concentrations in the study conducted by Al-Kaisy and colleagues, suggesting that this may not be an issue in the burn injury population with supplementation doses of 66mg zinc sulphate daily.[26] This dose was however significantly less than the doses provided by Nordlund and colleagues[20], however as serum copper concentrations were not an outcome measure of this study, whether this paradox persists with higher supplementation levels remains unknown.

Current guidelines for the nutritional management of burn injury patients support the use of micronutrient supplementation[12, 42], despite the current lack of large, multicentre definitive trials or systematic reviews in this area. Recommendations in these guidelines are mostly based on expert consensus, through observed small scale clinical studies and their clinical practice over time. Although current evidence is weak in supporting trace element supplementation following burn injury, this review does indicate that it appears safe (no mortality effect or other reported adverse

side effects, although this has not been consistently addressed in all studies) and may confer protective benefits against infectious complications. Although the effect on LOS was not statistically significant, the clinical significance seen within the individual studies should be considered in future studies as part of a cost vs benefit analysis (pooled WMD -8.96 days), due to the large discrepancy between the cost of one day in burns intensive care versus a full course of trace element supplementation. One potential confounder to the current aggregation presented, is the variation in trace element dosages provided to the intervention and control groups. In two studies[19, 28] the control group received higher prescriptions of Cu than the treatment group of two other included studies.[18, 27] This may potentially decrease any overall effect seen, however when this study was excluded in sensitivity testing based on study design, no significant effects were elucidated. A large, multicentre study, stratified for burn size, severity, age, and trace element dosage is required at present to provide definitive evidence regarding this topic, however omission of trace element supplementation in the management of this population, based on the current evidence at hand, is not supported.

Conclusions

The results of this review indicate that the use of parentally administered combined trace elements (Cu, Se, and Zn) following burn injury confer positive effects in decreasing overall infectious complications. Pulmonary infections are most likely to be reduced with combined trace element supplementation along with improvements supplemented serum trace element concentrations. Combined oral and IV Zn supplementation may be more beneficial in reducing wound and urinary infections. Combined parenteral trace element supplementation and combined oral and IV Zn supplementation have potentially clinically significant findings on reducing LOS. Oral Zn supplementation shows potential beneficial effects on mortality. Although these results are very weak, when weighed against the low cost and apparent patient tolerance of this therapy and clinically significant decrease in time to wound healing, oral zinc supplementation should be considered in burn injury patients. No adverse outcomes of trace element supplementation, such as increased mortality or poor patient tolerance that outweighs the potential benefits of supplementation, have been reported to date. Current evidence for all forms of trace element supplementation following burn injury is limited and underpowered, hence further large-scale, multi-centre, definitive studies are required to accurately define optimal trace element supplementation regimens, dosages and routes following burn injury.

References

1. Deitch EA. Nutritional support of the burn patient. *Critical care clinics*. 1995; 11(3):735-50.
2. Berger MM, Baines M, Raffoul W, et al. Trace element supplementation after major burns modulates antioxidant status and clinical course by way of increased tissue trace element concentrations. *American Journal of Clinical Nutrition*. 2007;85(5):1293-300.
3. Klein G.L., Przkora R. and D.N. H. Vitamin and trace element homeostasis following severe burn injury.In: *Total Burn Care*. 3rd ed.: Elsevier Inc, Philadelphia, USA, 2007, p. 387-90.
4. Berger MM, Baines M, Raffoul W, et al. Trace element supplementation after major burns modulates antioxidant status and clinical course by way of increased tissue trace element concentrations. *The American journal of clinical nutrition*. 2007; 85(5):1293-300.
5. Berger MM, Cavadini C, Bart A, et al. Selenium losses in 10 burned patients. *Clinical nutrition*. 1992; 11(2):75-82.
6. Berger MM, Cavadini C, Bart A, et al. Cutaneous copper and zinc losses in burns. *Burns*. 1992; 18(5):373-80.
7. Gamliel Z, DeBiasse MA and Demling RH. Essential microminerals and their response to burn injury. *Journal of Burn Care and Rehabilitation*. 1996; 17(3):264-72.
8. Graves C, Saffle J and Cochran A. Actual Burn Nutrition Care Practices: An Update. *Journal of Burn Care & Research*. 2009; 30(1):77-82.
9. Heyland D.K., Dhaliwal R., Drover J.W., et al. Clinical practice guideline for nutrition support in the mechanically ventilated, critically iIll adult patient. June 2013; available from: www.criticalcarenutrition.com. html; accessed July 27, 2013.
10. McClave SA, Martindale RG, Vanek VW, et al. Guidelines for the provision and assessment of nutrition support therapy in the adult critically ill patient: Society of critical care medicine (SCCM) and American society for parenteral and enteral nutrition (A.S.P.E.N.). *Journal of Parenteral and Enteral Nutrition*. 2009; 33(3):277-316.
11. Kreymann KG, Berger MM, Deutz NEP, et al. ESPEN guidelines on enteral nutrition: intensive care. *Clinical Nutrition*. 2006; 25(2):210-23.
12. Rousseau A-F, Losser M-R, Berger MM and Ichai C. ESPEN endorsed recommendations: Nutritional therapy in major burns. *Clinical Nutrition*. 2013; 32(4):497-502.
13. Kavanagh BP. The GRADE system for rating clinical guidelines. *PLoS medicine*. 2009; 6(9): e1000094.
14. Moher D, Liberati A, Tetzlaff J. et al. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *BMJ*. 2009; 339:b2535.
15. Kurmis R, Aromataris E and Greenwood JE. The effectiveness of trace element supplementation following severe burn injury: A systematic review protocol. *JBI Database Systematic Reviews and Implementation Reports*. 2013; 11(11):44-53.
16. Anglemyer A, Horvath HT and Bero L. Healthcare outcomes assessed with observational study designs compared with those assessed in randomized trials. *The Cochrane Database of Systematic Reviews*. 2014;4:MR000034.
17. The Joanna Briggs Institute. Joanna Briggs Institute Reviewers' Manual: 2014 edition. Adelaide, Australia: The Joanna Briggs Institute; 2014..
18. Berger MM, Shenkin A, Spertini F, et al. Large trace element supplements reduce the number of infectious episodes after burns. *Trace Elements in Man and Animals - 9*.1997:433-5..
19. Berger MM, Spertini F, Shenkin A, et al. Clinical, immune and metabolic effects of trace element supplements in burns: a double-blind placebo-controlled trial. *Clinical nutrition*. 1996; 15(2): 94-6.
20. Nordlund M, Pham TN, Honari SE, et al. Dual enteral and intravenous zinc supplementation in severe burns. *Journal of Burn Care and Research*. 2014;35(3) Suppl:S143.
21. Rollins C, Moore A, Neumeister M, et al. Burn Micronutrient Pilot Repletion Study: Characterizing relationships between vitamin and mineral supplementation and health

outcomes of adult burn patients. *Journal of the American Dietetic Association*. 2010; 110(9):A34.
22. McClain CJ, McClain ML, Boosalis MG, et al. Zinc and the stress response. *Scandinavian Journal of Work, Environment & Health*. 1993;19 Suppl 1:132-3.
23. The Nordic Cochrane Centre. Review Manager (RevMan) v. 5.3. Copenhagen, Denmark: The Cochrane Collaboration; 2014.
24. DerSimonian R and Laird N. Meta-analysis in clinical trials. *Controlled Clinical Trials*. 1986;7:177-88.
25. Higgins JPT., (editors) GS. Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 [updated March 2011]: The Cochrane Collaboration; 2011. Available from: www.cochrane-handbook.org.
26. Al-Kaisy AA, Salih Sahib A and Al-Biati HA. Effect of zinc supplement in the prognosis of burn patients in iraq. *Annals of Burns and Fire Disasters*. 2006;19(3):115-22.
27. Berger MM, Cavadini C, Chiolero R, et al. Influence of large intakes of trace elements on recovery after major burns. *Nutrition*. 1994;10(4):327-34+352.
28. Berger MM, Spertini F, Shenkin A, et al. Trace element supplementation modulates pulmonary infection rates after major burns: a double-blind, placebo-controlled trial. *The American Journal of Clinical Nutrition*. 1998;68(2): 365-71.
29. Sahib AS, Al-Jawad FH and Alkaisy AA. Effect of antioxidants on the incidence of wound infection in burn patients. *Annals of Burns and Fire Disasters*. 2010;23(4):199-205.
30. Stucki P, Perez M-H, Cotting J, et al. Substitution of exudative trace element losses in burned children. *Critical Care*. 2010; 14(1):439.
31. Al-Jawad FH, Sahib AS and Al-Kaisy AA. Role of antioxidants in the treatment of burn lesions. *Annals of Burns and Fire Disasters*. 2008;21(4):186-91.
32. Barbosa E, Faintuch J, MacHado Moreira EA, et al. Supplementation of vitamin E, vitamin C, and zinc attenuates oxidative stress in burned children: A randomized, double-blind, placebo-controlled pilot study. *Journal of Burn Care and Research*. 2009;30(5):859-66.
33. Caldis-Coutris N, Gawaziuk JP and Logsetty S. Zinc supplementation in burn patients. *Journal of Burn Care and Research*. 2012;33(5):678-82.
34. Cunningham JJ, Lydon MK, Briggs SE, et al. Zinc and copper status of severely burned children during TPN. *Journal of the American College of Nutrition*. 1991;10(1):57-62.
35. Cunningham JJ, Leffell M and Harmatz P. Burn severity, copper dose, and plasma ceruloplasmin in burned children during total parenteral-nutrition. *Nutrition*. 1993; 9(4):329-32.
36. Pochon JP. Zinc- and copper-replacement therapy--a must in burns and scalds in children? *Progress in pediatric surgery*. 1981;14:151-72.
37. Landucci F, Mancinelli P, De Gaudio AR, et al. Selenium supplementation in critically ill patients: a systematic review and meta-analysis. *Journal of Critical Care*. 2014;29(1):150-6.
38. Manzanares W, Dhaliwal R, Jiang X, et al. Antioxidant micronutrients in the critically ill: a systematic review and meta-analysis. *Critical Care*. 2012;16(2):R66.
39. Berger MM, Binnert C, Chiolero RL, et al. Trace element supplementation after major burns increases burned skin trace element concentrations and modulates local protein metabolism but not whole-body substrate metabolism. *The American Journal of Clinical Nutrition*. 2007;85(5):1301-6.
40. Berger M, Binnert C, Baines M, et al. Trace element supplements influence protein metabolism and tissue levels after major burns. *Intensive Care Medicine*. 2004;30(Suppl):S61.
41. Berger MM, Eggimann P, Heyland DK, et al. Reduction of nosocomial pneumonia after major burns by trace element supplementation: aggregation of two randomised trials. *Critical Care*. 2006; 10(6):R153.
42. Prelack K, Dylewski M and Sheridan RL. Practical guidelines for nutritional management of burn injury and recovery. *Burns*. 2007;33(1):14-24.

Chapter 4: Discussion and conclusions

4.1 Discussion

To date there appears to be surprisingly little evidence available to support the almost universal supplementation of trace elements on burn injury patients currently practised.[15, 36, 37] Results of the research presented in this thesis suggest potentially clinically significant implications for practice.[6] For example the mean decrease is almost nine days in hospital LOS with parenterally administered combined trace element supplementation, five days in time of healing with oral Zn supplementation, and seven days in ICU LOS with combined oral and parenteral Zn supplementation.[6] Statistically significant results support supplementation of trace elements and a decrease in infectious episodes (pulmonary infections for combined parenterally administered combined trace element supplementation [Se, Cu and Zn], and urinary tract infections for combined oral and parenteral Zn supplementation).[6] However as will be discussed in this chapter, the strength of the studies supporting these results is lacking.

As previously mentioned (see sections 1.1 and 1.3), strong agreement exists among experts in this area that supplementation is advantageous and safe[38], and as such it has become "standard practice" in many burn centres.[37] This practice may lead to difficulties in conducting future robust experimental research due to the ethical dilemma of withholding "standard" care to control for supplementation versus non-supplementation in order to truly elucidate effectiveness. In addition, enteral (and total parenteral) nutritional formulae administered as part of burn injury care often contain concentrations of trace elements above that of a "usual" diet.[36] In almost all of the studies included in the systematic review (Chapter 3), the total amount of trace elements administered via nutritional formulae between groups was not specified.[6] Although the provision of "standard nutrition care" was assumed or stated as part of the study protocols, differences in actual amounts received may have differed significantly between groups, which potentially influenced reported outcomes, especially given the small sample sizes of included studies and often poorly described randomisation methods.[6] This is particularly relevant to the burns population, as it has been documented previously that patients often do not receive the prescribed amount of nutrition.[55-57]

Although the internal validity of these included studies was assessed through the critical appraisal process[5], external validity is difficult to assess for the three supplementation interventions presented within this review. This is due to all of the parenteral combined supplementation trials being conducted in the one Swiss centre[39, 40, 58-60], both of the oral Zn supplementation trials being conducted in the same unit in Iraq[61, 62], and the only combined parenteral and enteral Zn supplementation study being conducted in the US.[63] These three populations are geographically very

distinct, preventing extrapolations of the results within each supplementation group type to other populations. Characteristics, such as potential significant variations in baseline nutritional status inherent from their food supply, and genetic, cultural or ethnic, and environmental influences, cannot also be generalised. In addition to this, potential for performance bias also exists as there are significant variations in medical and surgical management of the burn injury in current practice.[12, 15] If one centre utilises skin substitutes to temporise open wounds, preventing wound exudation, whilst another centre utilises dressings requiring daily/twice daily burn baths, there may be significant differences in trace element losses between these groups.[27, 64] This may alter the dosage of supplementation required to have a similar effect on patient outcomes or the resulting impact of the supplementation effectiveness itself.

In order to ensure transparency in the assessment of the quality of the body of evidence for each supplementation intervention grouping identified in Chapter 3, Summary of Findings tables were developed for the main comparisons within each group.[48, 65] These provide concise presentation of key findings from the systematic review.[48, 66] Employing the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) system[67], they were produced using the GRADEprofiler© (GRADEpro©) software version 3.6.1 (The Nordic Cochrane Centre, The Cochrane Collaboration, Copenhagen, Denmark, 2014).[68] Summary of Findings tables are a formalised way of weighing the available evidence to allow for factors, such as within-study risk of bias (methodological quality), publication bias, inconsistency, indirectness, imprecision of evidence, precision of effect estimates, dose-response relationships, and confounders of findings, to be accounted for in the ranking of the evidence.[48, 68] This process allows for improved accessibility and understanding of the systematic review results in a standardised and transparent way.[48, 65] The Summary of Findings tables presented in this chapter refer to the results of the systematic review (Chapter 3).[6] A Summary of Findings table is presented for each mode of trace element supplementation grouping presented in the systematic review (Chapter 3).[6] The remainder of this discussion will focus on presenting these Summary of Findings tables; however, references to the findings of the systematic review (Chapter 3) will be made.[6]

4.1.1 Effectiveness of parenteral combined trace element supplementation

Five included studies[39, 40, 58-60] investigated the effect of parenterally administered combined trace element supplementation, making it the most researched modality of trace element supplementation in the current burn injury literature. As can be seen in the Summary of Findings table for this method of supplementation (Table 2), evidence supporting the specified outcome measures varied from low to very low. Both the LOS and infectious episodes outcome measures scored “low” according to the GRADE system. This indicates that further robust research is likely to

improve the confidence in these effect estimates. Increasing the sample size studied will increase the precision of the resulting confidence intervals, as well as decreasing the risks of bias in the currently available literature, as highlighted in Table 2.

Table 2. GRADE profile table: Combined parenteral trace element supplementation following severe burn injury

Outcomes	No of Participants (studies) Follow up	Quality of the evidence (GRADE)	Relative effect (95% CI)	Anticipated absolute effects *Time frame is supplementation commenced within 48 hours of injury*	
				Risk with Control	Risk difference with IV Combined Trace Element Supplementation (95% CI)
Length of Stay Days. Scale from: 0 to 365.	32 (3 studies) 0-149 days[2]	⊕⊕⊖⊖ **LOW**[3,4,5,6,7,8] due to risk of bias, imprecision		The mean length of stay in the control groups was **61 Days**[1]	The mean length of stay in the intervention groups was **8.96 days lower** (24.87 lower to 6.95 higher)
Mortality Number of deaths	43 (3 studies) 0-149 days	⊕⊖⊖⊖ **VERY LOW**[3,4,5,6,7,8,9] due to risk of bias, imprecision	**RR 0.96** (0.18-5.01)	**95 per 1000**	**4 fewer per 1000** (from 78 fewer to 382 more)
Infectious episodes number [10]. Scale from: 0 to 5.	73 (4 studies) 0-30 days	⊕⊕⊖⊖ **LOW**[4,5,6,7,12] due to risk of bias, imprecision		The mean infectious episodes in the control groups was **3.25 infective episodes**[11]	The mean infectious episodes in the intervention groups was **1.25 lower** (1.7-0.8 lower)
Serum Zinc Levels[13] µmol/L	51 (3 studies) 0-20 days	⊕⊕⊖⊖ **LOW**[4,5,6,7,8] due to risk of bias, imprecision		The mean serum Zn levels in the control groups was **7.975 µmol/L**	The mean serum Zn concentration in the intervention groups was **2.21 µmol/L higher** (1.3-3.11 higher)
Serum Copper Levels µmol/L	51 (3 studies) 0-20 days	⊕⊖⊖⊖ **VERY LOW**[4,5,6,7,12] due to risk of bias, indirectness, imprecision		The mean serum Cu levels in the control groups was **8.58 µmol/L**[13,14]	The mean serum Cu concentration in the intervention groups was **3.97 µmol/L higher** (0.84-7.1 higher)
Serum Selenium Levels[13] µmol/L	71 (4 studies) 0-20 days	⊕⊖⊖⊖ **VERY LOW**[4,5,6,7,8,9,12] due to risk of bias, inconsistency, imprecision		The mean serum Se levels in the control groups was **0.596 µmol**[13]	The mean serum Se concentration in the intervention groups was **0.20 µmol/L higher** (0.11-0.3 higher)

*The basis for the **assumed risk** (e.g. the median control group risk across studies) is provided in footnotes. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).
CI: Confidence interval; **RR:** Risk ratio;

GRADE Working Group grades of evidence
High quality: Further research is very unlikely to change our confidence in the estimate of effect.
Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
Very low quality: We are very uncertain about the estimate.

[1] Mean of control group mean hospital length of stay results
[2] Based on length of stay combined range for intervention and control groups, Berger et al. 1996
[3] Case-control and randomised control study designs together
[4] Randomisation methods unclear or alternate allocation method used (risk of selection bias)
[5] Single centre studies from a single research group (risk of location bias)
[6] Blinding of allocator unclear or not addressed (allocation bias)
[7] Unclear blinding of outcome assessor
[8] Small sample size (n=42)
[9] Point estimates and confidence intervals include 'no effect' AND appreciable harm or benefit
[10] Infectious events/ complications as defined by primary authors
[11] Mean of control group mean infectious episodes
[12] No explanation was provided
[13] Based on Day 10 serum levels following 8 days of supplementation
[14] Mean of control group mean scores

Visual inspection of a forest plot is a simple way to determine the possible effects of potential outliers in a meta-analysis.[48] Considering Chapter 3, Figure 2, there appears to be no studies causing the results to sway greatly in favour of either the experimental or control group; in essence, the results are homogenous between studies.[6] This visual inspection is supported by statistical analysis of heterogeneity also (Chapter 3, Figure 2).[6] Due to the small sample size of only 32 participants and 95% confidence intervals around the pooled effect estimate including both no effect and potential benefit of the intervention or control[6], the quality rating for LOS was downgraded in keeping with GRADE principles.[68] There is greater uncertainty regarding the effect estimates for the outcome measures of mortality and serum Cu and serum Se levels, as the quality of the evidence supporting these outcome measures was graded as very low. These results are not surprising given the risk of bias, small sample sizes of the included studies and the number of external confounding factors for these outcome measures following burn injury, such as age, severity of burn and baseline nutritional status, which may also be dependent on the micronutrient adequacy of the food supply within a geographic location.

Surprisingly there were no articles identified investigating the parenteral supplementation of Se as a single agent within the burn injury population. This is in stark contrast to the critical care literature.[18, 44, 45, 69] A systematic review conducted by Huang and colleagues[69] included studies investigating the effects of parenteral Se supplementation on mortality. Twelve studies meeting their inclusion criteria were identified, with nine studies representing 965 participants included in their meta-analysis.[69] This analysis demonstrated a statistically beneficial effect of Se supplementation on mortality for patients with systemic inflammatory response syndrome or sepsis.[69] Further subgroup analysis demonstrated that supplementation for at least seven days was required for beneficial effects to be evident.[69] Given the beneficial results demonstrated with Se administered in the ICU population when inflammation and systemic infection (sepsis) was present, it is surprising that this intervention has not been replicated in the burn injury population. Aggregating this critical care research has allowed duration of supplementation and effective dosages to be explored further by subgroup analysis.[69]

4.1.2 Effectiveness of oral zinc supplementation

Two studies[61, 62] investigating the oral supplementation of Zn were identified as part of this systematic review.[6] As can be seen from the GRADE profile table for this intervention group (Table 3), the quality of the evidence supporting this method of supplementation was rated as very low. Again this is due to the small sample sizes of the included studies and multiple external confounding factors such as location bias and the issue of these studies being conducted in Iraq during a period of significant international conflict. Visual inspection of the forest plot for wound healing outcomes

(Chapter 3, Figure 9) indicates that whilst the individual confidence intervals for each included study[61, 62] are small, when aggregated, there is great heterogeneity between the effects seen.[6] This is represented visually with the study conducted by Sahib and colleagues[62] supporting a pronounced effect with supplementation whilst the results reported by Al-Kaisy and colleagues[61] indicate that the intervention is no more effective than the control.[6] This disparity is supported by the statistical calculation for heterogeneity (Chapter 3, Figure 9).[6] The results for the outcome measure of mortality (Chapter 3, Figure 10) are more homogenous; however both studies have wide confidence intervals for their estimates of effect.[6] Another methodological weakness is possible lack of ethical approval in one study.[61] The ethics approval process often allows for internal peer review of a study to ensure rigour as well as patient rights and confidentiality are observed. Without this process it is not possible to ensure that patients were consented for their participation in the study or offered the right of refusal and/or withdrawal, which may influence compliance and eventual study outcomes, as these factors were not reported on by the authors.[61] There was also a lack of baseline data for comparison between groups for both studies[61, 62], preventing further evaluation for possible confounders of external validity from being identified.[48] In keeping with GRADE principles, the quality ratings for both mortality and wound healing were downgraded due to this combination of factors.[68]

Table 3. GRADE profile table: Oral zinc supplementation following severe burn injury

Outcomes	No of Participants (studies) Follow up	Quality of the evidence (GRADE)	Relative effect (95% CI)	Anticipated absolute effects *Time frame is Supplementation commenced within 48 hours of admission*	
				Risk with Control	**Risk difference with Oral Zn Supplementation** (95% CI)
Mortality number of events	118 (2 studies) 15 days	⊕⊖⊖⊖ **VERY LOW**[1,2,3,4,5,6] due to risk of bias, imprecision	**RR 0.22** (0.04- 1.14)	**123 per 1000**	**96 fewer per 1000** (from 118 fewer to 17 more)
Wound healing days to complete healing	118 (2 studies) 15 days	⊕⊖⊖⊖ **VERY LOW**[1,2,3,4,7,8] due to risk of bias, inconsistency, imprecision		The mean wound healing in the control groups was **14.6 days**	The mean wound healing in the intervention groups was **5.3 days lower** (14.51 lower to 3.91 higher)

*The basis for the **assumed risk** (e.g. the median control group risk across studies) is provided in footnotes. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).
CI: Confidence interval; **RR:** Risk ratio;

GRADE Working Group grades of evidence
High quality: Further research is very unlikely to change our confidence in the estimate of effect.
Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
Very low quality: We are very uncertain about the estimate.

[1] No randomisation to groups (risk of allocation bias)
[2] Unclear blinding of participants, allocator, or outcome assessor
[3] Baseline group characteristics not presented
[4] Single centre studies, both from same centre (location bias)
[5] Indirect comparison due to dosage differences in supplementation.
[6] Small sample size and wide confidence intervals.
[7] Large amount of heterogeneity despite similar population and protocols.
[8] Small sample size.

In contrast to parenteral combined trace element supplementation (Section 4.1.1), the oral supplementation of Zn has not been investigated as thoroughly in the ICU population. A systematic review of antioxidant nutrients in the ICU population[18], conducted in 2005, only identified one pilot study investigating Zn alone.[70] This study was conducted in head injury patients and employed a protocol where supplementation was initially provided via the parenteral route for 15 days before being continued orally until three months post injury.[70] Another review conducted in 2008 identified four randomised controlled trials investigating the effectiveness of Zn supplementation in the critical care population[71]; however three of these included Zn as part of combined parenteral supplementation and the remaining study was the aforementioned study by Young and colleagues[70], investigating parenteral prior to oral Zn supplementation.[70]

4.1.3 Effectiveness of combined oral and parenteral zinc supplementation

The remaining study identified as part of the systematic review[6] presented in Chapter 3 was the only one to combine the modalities of oral and parenteral administration.[63] Despite rating as very low quality evidence, according to the GRADE system (Table 4)[68], the study presents a novel method for supplementation that may confer both local gastrointestinal tract and systemic benefits.[33] The single retrospective cohort study design and small sample size were key factors contributing to the downgrading of this study in keeping with GRADE principles.[33, 68] Again, extrapolation to the global burns community is limited by the single centre nature of this study even though baseline group comparisons were presented.[33]

In one study by Young and colleagues[70] investigating Zn supplementation in closed head injury patients, participants were initially commenced on parenteral Zn supplementation and then transitioned to oral Zn supplementation. Although this research was not a study of combined modality supplementation, as identified by Nordlund and colleagues[33] as part of this systematic review[6], it is more reflective of supplementation in clinical practice within the ICU setting. Interestingly, this study reported improved retinol-binding protein and pre-albumin levels with supplementation (markers of overall protein status).[70] The authors did acknowledge that there was a larger surgical requirement in the control group, which may have biased these results.[70] This study suggests that Zn supplementation has a role in protein metabolism[70], which may be the reason why improved wound healing was observed in the studies investigating oral Zn supplementation alone.[61, 62] Although direct extrapolations between the head injury and burn injury populations cannot be made, similarities such as inflammation and possible hyper-metabolism do exist.[72] Further research into Zn supplementation in burn injury may benefit from the use of pre-albumin as a marker of effectiveness, in addition to serum levels and clinical endpoints such as mortality and LOS.

Table 4. GRADE profile table: Combined oral and parenteral zinc supplementation

Outcomes	No of Participants (studies) Follow up	Quality of the evidence (GRADE)	Relative effect (95% CI)	Anticipated absolute effects *Time frame is supplementation commenced within 48 hours of injury*	
				Risk with Oral Zn supplementation	Risk difference with Combined oral and parenteral Zn supplementation (95% CI)
ICU Length of Stay Days	65 (1 study) 37 days[1]	⊕⊖⊖⊖ **VERY LOW**[2,3] due to imprecision		The mean ICU length of stay in the control groups was **37 days**	The mean ICU length of stay in the intervention groups was **7 days lower** (16.50 lower to 2.50 higher)
Infectious Episodes Number	65 (1 study) 37 days	⊕⊖⊖⊖ **VERY LOW**[4] due to imprecision	**RR 0.60** (0.41-0.88)	**Study population**	
				87 per 100	**35 fewer per 100** (from 10 fewer to 51 fewer)
Mortality Number	69 (1 study) 37 days	⊕⊖⊖⊖ **VERY LOW**[4] due to imprecision	**RR 2.07** (0.5- 8.55)	**Study population**	
				7 per 100	**8 more per 100** (from 4 fewer to 54 more)

*The basis for the **assumed risk** (e.g. the median control group risk across studies) is provided in footnotes. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).
CI: Confidence interval; **RR:** Risk ratio;

GRADE Working Group grades of evidence
High quality: Further research is very unlikely to change our confidence in the estimate of effect.
Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
Very low quality: We are very uncertain about the estimate.

[1] Mean of control group length of ICU stay results
[2] No serious limitations
[3] Small sample size (<400)
[4] No explanation was provided

4.2 Implications for practice

4.2.1 Translation of review results into practice

Results of the systematic review presented in Chapter 3 of this thesis are not strong enough to support practice change. The JBI model of Evidence-Based Healthcare outlines the framework required for change implementation.[73] This conceptual, cyclic model encapsulates four main domains of the evidence-based healthcare process in order to influence global heath.[73] These domain categories are: healthcare evidence generation, evidence synthesis, evidence (knowledge) transfer, and evidence utilisation.[73] For the purpose of this current systematic review[6], the primary studies identified as part of the search process fulfill the domain of healthcare evidence generation, whilst the review process and meta-analyses provide evidence synthesis regarding this topic.[54, 73] Evidence (knowledge) transfer will be achieved through the publication of the review findings in an international, highly regarded journal within the burn care community, as demonstrated by its affiliation with the ABA.[6, 73] In regards to evidence utilisation, currently there is no evidence as part of the current review to recommend the cessation of trace element supplementation in centres where it is currently practised.[6] This is supported by the absence of documented adverse effects identified

within the current evidence synthesis[6], literature supporting the benefits of trace element supplementation in the ICU population and its comparatively low cost of provision.[18, 45] Possible clinically significant benefits, such as the trends towards decreases in LOS, time to wound healing and decreases in infectious episodes with supplementation, also support its continued use.[6]

The findings of the research presented in this thesis[6] provide further support to the results of previously published surveys of clinical practice in this area which suggested wide variation in clinical practice among burn centres.[36, 37] This is possibly due to guidelines based on expert consensus that are commonly referred to by clinicians failing to provide a level of evidence that is strong enough to influence change in practice.[38, 52, 54] Although published research at present is unable to strongly support trace element supplementation following severe burn injury, or provide clear recommendations regarding dosage and duration of supplementation, the small amount of literature at present does not refute it either.[6] In contrast, the potential cost savings from the clinically significant results in decreased LOS with supplementation presented in Chapter 3 would offset the comparably low cost of the supplementation itself.[6] This is important in the context of overall cost savings. Australian high acuity beds, such as those in the ICU setting where severe burn injury patients are often accommodated for extended periods, have been reported to cost AU$2760 (~US$2135) per day.[74] Although local cost data for trace element supplementation is currently unavailable, the cost of supplementing parenteral nutrients in Flemish ICUs has been reported to cost a mean of EUR€28 (~AU$38, ~US$29) per patient.[75] For low to middle income countries such as India (per capita income < US$1000-4000 [~AU$1292-5169]), the mean cost of burn care per patient has been reported as US$134.96 (~AU$174) per day or US$1060.52 (~AU$1370) per patient.[76] In addition, LOS can be viewed as a surrogate marker of time to healing as patients are usually discharged from hospital upon healing.[15] This indicates that trace element supplementation may increase the healing rate of wounds following burn injury.[6] This has potential to decrease the longer-term burden of care for patients. This burden includes ongoing scar management using pressure garments and managing psychological wellbeing due to body image changes, as burn wounds that heal in less than 21 days are at much lower risk of developing hypertrophic scarring.[13]

4.2.2 Dosages of trace element supplementation

The prescription of vitamin and trace element supplementation in the burn injury literature has been compared to the requirements of the "healthy" general population (RDIs).[36] In this context, the use of RDIs as a comparator can only be used as a starting point and the need for increased trace element requirements should be considered due to the increased metabolic demand as well as to replace previously described losses as a result of injury (see Chapter 1, Section 1.1.6).[27, 28, 31]

Early studies by Berger and colleagues[27] investigating the losses of trace elements following burn injury indicated that the primary mode of Cu depletion is via the cutaneous route (skin and exudative losses) accounting for 20-40% of total body stores.[27] Total cumulative Cu loss from cutaneous, urine and faecal sources was observed to be 37mg in the first week post injury. [27] Similar to Cu, the primary mode of Zn depletion is also cutaneous, with total losses of almost 210mg from all sources in the first week post injury, representing 5-10% of total body stores.[27] In contrast, the main source of Se depletion was observed to be via urinary losses. [28] This excretion was reported as 41±13µg/24 hours during the first week post injury, compared to a normal population reference range of 35±7µg/24 hours.[28] Based on the assumption of the need to correct these losses, a subsequent study by Berger and colleagues[39] investigated the difference between their standard supplementation regimen and supra-normal parenteral supplementation of Se, Cu and Zn, and its effect on cumulative balances and clinical outcomes (discussed in Chapter 3[6]).[39] Table 5 below shows the comparison of total enteral and parenteral trace element intake compared with the balances for both groups.[39]

Table 5. Mean total trace element intakes versus mean total balances for standard versus supra-normal parenteral supplementation as reported by Berger et al., 1994[39] for days 1-7 post burn

	Standard supplementation group		Supra-normal supplementation group	
	Mean total intake	**Mean balance (range)**	**Mean total intake**	**Mean balance (range)**
Cu (mg/day)	2.8*	-14.5 (-73.5-8.6)^	4.5*	14.1 (5.0-20.7)^
Se (µg/day)	92*	254 (-52-717)	187*	587 (217-990)
Zn (mg/day)	14.2*	-21.8 (-114.2-23.8)^	39.4*	166.3 (51.4-280.6)^

* Indicates significant difference between groups p<0.001

^ Indicates significant difference between groups p<0.03

Although dose response studies for trace element supplementation have not been identified in published burns literature[6], Se supplementation has been more widely investigated in critical care literature.[18, 44, 45, 69] Dose response of parenteral Se supplementation was investigated by sub-group analysis in the systematic review conducted by Manzanares and colleagues[45], with results of these analyses shown in Table 6.

Table 6. Statistical results of Se dosing sub-group analysis on specified outcomes as reported by Manzanares and colleagues[45]

	Se dose administered		
Reported outcome of interest	**<500µg/day**	**500µg/day**	**>500µg/day**
Mortality	RR=0.94, 95% CI 0.67, 1.33, p=0.75	RR=0.87, 95% CI 0.57, 1.32, p=0.51	RR=0.80, 95% CI 0.63, 1.02, p=0.07
Infectious episodes	RR=0.87, 95% CI 0.64, 1.19, p=0.39	RR=0.86, 95% CI 0.71, 1.05, p = 0.13	RR=0.76, 95% CI 0.35, 1.69, p=0.51

RR=Risk Ratio, 95% CI=95% Confidence Intervals, p=Probability

No participants representative of the burns population were included in any sub-group analysis conducted in this review[45]; as such, allowances for substitution of losses as a result of injury have not been factored into the included study designs. These findings indicate that there may be a greater effect of reducing mortality with Se doses above 500µg per day, whilst overall optimal benefits may be seen with dosages at 500µg per day.[45] Both Landucci and colleagues[44] and Manzanares and colleagues[45] determined that there were no significant effects detected in the general ICU population when dosages of Se <500µg per day were provided.[44, 45] These findings are in contrast with an earlier systematic review conducted by Heyland and colleagues[18], which included primary studies investigating ICU patients representing trauma, surgical, medical and head injury populations, along with two citations investigating supplementation in burn injury patients.[18] This review determined that evidence available at the time suggested there was a trend towards lower mortality rates when Se was supplemented, either alone or in combination with other antioxidant nutrients (RR 0.59, 95% CI 0.32, 1.08; p=0.09).[18] Upon further analysis, they reported that there was a trend towards lower mortality when Se doses provided were between 500-1000µg/day (RR 0.52, 95% CI 0.24, 1.14; p=0.10) compared to no effect on mortality when Se doses were below 500µg/day (RR 1.47, 95% CI 0.20, 10.78; p=0.7).[18] Both studies representing the burn injury population included in the review provided <500µg/day Se and were included in the latter subgroup analysis.[18] Both of these burn injury cohorts were represented in the current systematic review presented in Chapter 3.[6, 40, 60] When results for optimal dosage of Se supplementation in the ICU population[18, 44, 45, 77] are compared with the supplemented doses in the current review[6], as presented in Table 7, it can be seen that all included studies provided a potentially sub-therapeutic dose.[39, 40, 58-60] This is potentially confounded further in the burn injury population by additional trace element losses not present in the general ICU population. This may account for the lack of statistical effect seen in the meta-analysis of this outcome (Chapter 3, Figure 4) along with the large confidence intervals for this effect estimate.[6] The small sample size included in this analysis is a likely contributor to the large confidence intervals seen; however a dose-escalation study conducted prior to a more definitive trial in the ICU population has determined that supplementation of Se providing 800µg daily (500µg parenterally and 300µg enterally) is safe.[77] This strongly infers that dosages investigated by burn injury research on this issue to date may be sub-optimal to confer statistically significant effects on outcome measures. Whilst this study was limited methodologically by its non-randomised design and small sample size[77], it provided a basis for a subsequent clinical trial.[49] Results from the subsequent RCT cannot be compared to the current review due to the combination of other nutrients in the intervention group, including glutamine which was reportedly associated with a trend towards increased 28 day mortality (adjusted odds ratio 1.28; 95% CI 1.00, 1.64; p=0.05).

Table 7. Trace element supplementation doses provided by included studies in the combined parenteral trace element supplementation sub-group

Citation	Trace element intervention group dose/day	Trace element control group dose/day
Berger et al., 1994[39]	Se 82µg Cu 2.4mg Zn 26.5mg	Se 0µg Cu 0.3mg Zn 1.4mg
Berger et al., 1996[58]	Se 226µg Cu 2.6mg Zn 26mg	Se 30µg Cu 1.3mg Zn 6.4mg
Berger et al., 1997[59]	Se 232µg Cu 2.6mg Zn26.5mg	Se 32µg Cu 1.3mg Zn 6.5mg
Berger et al., 1998[40]	Se 226µg Cu 2.6mg Zn 26mg	Se 30µg Cu 1.3mg Zn 6.4mg
Berger et al., 2007[60]	Se 375µg Cu 3.75mg Zn 37.5mg	True placebo, trace element free comparator

4.2.3 Monitoring of serum trace element levels

Whilst baseline trace element requirements are not known within the burn injury population, effects of supplementation on the pre-specified outcomes reported in this review have demonstrated that administration of trace elements at the reported dosages is safe and of possible benefit.[6] Continued monitoring of serum trace element concentrations at baseline as well as throughout supplementation is warranted, irrespective of the modality of treatment, with titration of individual supplementation levels clinically required to achieve or maintain serum concentrations within the locally specified reference range.[29] This approach accounts for geographical baseline variations, as well as for nuances in individual requirements as a result of medical/surgical techniques employed.[29, 31] Variations in trace element provision, as a result of local feeding practices, nutritional formulae available and patient tolerance, can also be accounted for with continual monitoring.[29, 31, 36, 37]

Alongside monitoring of serum concentrations of trace elements and related biochemical parameters, the clinical picture and progress of the individual patient should always be considered.[31, 46] Markers of progress, such as wound healing and local and systemic infections, should also be included with overall nutritional adequacy when providing trace element supplementation at supra-normal levels.[31] A multi-disciplinary team approach is required for burn centres to deliver optimal patient care[78], including trace element supplementation.[15, 46]

4.3 Implications for research

4.3.1 Research design considerations

As previously discussed (see Section 4.1), extrapolation of results from each supplementation method grouping in this review is limited by potential location and performance bias. This prevented the process of data pooling from improving the external validity of the findings of this research. For this reason future research needs to take into account not only a multi-centre approach, but ideally, a multi-national approach as well to truly generate meaningful results that can be translated globally.

As highlighted by the Summary of Findings tables (Tables 2-4) and discussed in the systematic review (Chapter 3)[6], sample sizes for all modalities of supplementation examined were small, even where pooling of data was possible.[6] This is a likely cause for the weak effect estimates seen within the systematic review[6], especially when considered with the large number of confounders for research in the burn injury population, as sample sizes were too underpowered to account for this. Traditionally, recruitment of adequate numbers of patients into burn injury studies is problematic, but a multi-centre approach should aid this. Adequate patient numbers will further increase the likelihood of the ability of any research to stratify according to the Baux score, which may assist in identifying which patients benefit most from trace element supplementation.

In addition to adequately powered study designs, adequate dosage of trace element supplementation to elucidate an effect also warrants consideration. As discussed in section 4.2.2, current combined trace element supplementation levels may have been insufficient to demonstrate optimal effectiveness. As the safety of higher supplementation levels has been determined in the ICU population[77], these could be extrapolated to future burn injury studies.

Cost-benefit analysis is also required in any future trace element supplementation studies in the burn injury population, whether they be conducted in a high- or low-middle income country. This factor has been neglected by current research and has the potential to provide significant results, even if applied as a post-hoc analysis to the currently available literature. Cost effectiveness and cost-benefit analyses may assist in the justification for clinicians to adopt or continue current nutritional practice in this area, especially in today's environment of high cost healthcare and budgetary justification.[46, 79]

Issues surrounding the adequate funding to conduct future well designed, large scale research exist. The cost of undertaking the large multi-centre trials needed to determine the true efficacy of supplementation would be relatively significant, and due to the comparatively low cost of

supplementation, these trials would not be readily seen as advantageous for commercial financial support. Although the burden of disease attributed to burn injury worldwide is high, the number of burn injuries in developed countries is low compared with other high burden conditions such as cancer, obesity and diabetes.[10, 12] This makes funding through developed countries government funded research programs less attractive. In contrast the incidence (over 95% of fire-related burns) and burden of burn injury in developing countries is very high, so funding for burn injury prevention is preferable[12] to burn injury care research. This is partly due to the lack of resources to appropriately care for the majority of burn injuries as well as the high cost of morbidity from disability and disfigurement.[12] Non-government philanthropic organisations and foundations established to further burn injury and care may prove to be the best avenues for financial support of such research in the future.

4.3.2 Considerations for strengthening internal validity

Due to inherent baseline nutritional differences in people, future studies need to include a baseline nutritional comparison between groups, as well as at the end of the study. In addition, where serum trace element concentrations are reported, acute phase (CRP) and negative acute phase protein markers (pre-albumin) need to be included to assist interpretation of results in the context of severe inflammation. Methods for wound management and/or closure and nutritional management also need to be reported for transparent interpretation of study results; however intravenous fluid management as part of burn resuscitation may also be of importance. For burn injury studies, initial fluid provision (in both volume and type) is pertinent.[31] Both under and over fluid resuscitation may have profound effects on patient outcomes and potential effects of nutritional strategies at a gut mediated level as well as on the interpretation of laboratory results.[31, 37] Inadequate fluid resuscitation can cause hypovolaemia leading to haemorrhagic shock, whilst excessive fluid resuscitation may lead to abdominal compartment syndrome.[80, 81] This is important in the context of nutritional management for multiple reasons. Current research is investigating the link between hemorrhagic shock and gut barrier failure in the pathogenic pathways of acute lung injury.[82] It is thought that gut-derived factors such as toxins and inflammatory factors, released or produced by gut ischemia, are carried via the mesenteric lymphatic system to the lungs regardless of whether the intestines have been reperfused.[82] Although associated with organ failure, these gut-derived factors may also contribute to infection through their action of suppressing cellular defense functions.[83] This ischemia-reperfusion injury to the intestinal system is believed to act as a primary site for the formation of reactive oxygen species (ROS ["free radicals" containing the oxygen molecule])[83] whilst generating cytokines.[82] In addition, the mesenteric microcirculation can act as a priming site for circulating neutrophils.[82] This supports the hypothesis of the need for providing anti-oxidant and immune enhancing nutrient therapies via the enteral route, as there is some evidence to support

that antioxidant therapy delivered to the splanchnic region of the gut decreases the incidence of multi-organ failure.[82]

Descriptions of some or all of these potential confounding variables were frequently absent in the studies included in this systematic review[6], limiting the ability for direct comparison of future research results against the present data.

4.3.3 Considerations for modality of supplementation

Proponents for parenteral trace element supplementation support the rationale that this method of administration avoids the effects of gastric antagonism of bioavailability of some substrates.[38] This antagonism was not supported by the small number of studies investigating oral Zn supplementation in the presented systematic review[6, 61, 62]; however the limitations and confounders of these studies have already been discussed. The study by Nordlund and colleagues[63] was the only included study that investigated the provision of combined enteral and parenteral trace element supplementation.[33] This study did demonstrate beneficial effects in the combined modality supplementation group.[33] No studies investigating the enteral provision of Se and/or Cu were identified through the comprehensive search strategy.[6] This may provide a novel area of combined trace element supplementation in the future in order to elucidate whether there is a stronger effect of local (enteral) supplementation to the gut or systemic (parenteral) supplementation, or if there is an additive benefit with the combination of supplementation via both the enteral and parenteral modalities.

4.4 Conclusions

This systematic review aimed to synthesise the current evidence for the effectiveness of trace element supplementation following burn injury in order to better guide clinical recommendations based on the target population's specific needs. Current guidelines draw significantly on historical practices, expert opinion, anecdotal and heterogeneous critical care evidence. This has led to diverse local and global clinical practice within a relatively small patient population.[36, 37]

Results of this systematic review, although weak, should be considered in context of the significant cost savings to healthcare with these potentially clinically significant results compared to the low cost of the intervention, apparent patient tolerance and lack of adverse side effects.[6] Clinically significant decreases in LOS and possible decreased infectious episodes have been demonstrated with parenteral administration of combined trace elements and potentially with combined oral and IV Zn supplementation.[6] Oral Zn supplementation may decrease mortality and time to wound healing.[6] In this context, continued practice of trace element supplementation can be supported. Further, well designed, adequately powered, multi-centre studies are required in this field. Cost-benefit analysis of

trace element supplementation in burn injury should also be considered as part of future studies. These analyses should consider not only inpatient hospital savings, but rehabilitation and outpatient care costs as well. These factors are likely to be a major driver for implementation of any study findings in the future due to the growing global economic burden of healthcare provision.

Appendices

Appendix 1. Search strategy supplementary information

Keyword searches as appropriate were used for grey literature databases:

Database – Unpublished studies	
US Clinical trials Register	Search terms:Selenium OR copper OR zinc OR "trce element' Conditions: Burn OR "thermal injury"
Australian Clinical Trials Register	Search terms: zinc; burn injury; nutrition support; selenium; copper; thermal injury; trace element
Australian and New Zealand Clinical Trials Register	Search terms: burn* and nutrition; burn* and trace element; burn* and zinc; burn* and selenium; burn* and copper
European Clinical Trials Register	Search terms:burn*; burn and selenium; burn* and selenium; burn* and zinc; burn* and copper; burn* and "trace element*"; burn* and nutrition*; burn* and antioxidant*
MedNar	Keyword search: burn and nutrition; burn and trace element; burn and zinc; burn and selenium; burn and copper
Open Grey	Search terms: burn* AND zinc; burn* AND selenium; burn* AND copper; burn* AND trace element; burn* AND nutrition*; burn injury; thermal injury;
DART-Europe E-thesis Portal	Search terms: burn injury
Open Thesis	Search term: burn

Databases searched, dates of search conducted and number of citations identified

Database – *Published studies*	**Date search performed**	**Number of citations identified**
PubMed	15/11/2013	2401
CINAHL	15/11/2013	482
EMBASE	15/11/2013	3224
Web Of Science	15/11/2013	2988
Database – Unpublished studies	**Date search performed**	**Number of citations identified**
US Clinical trials Register	15/11/2013	17
Australian Clinical Trials Register	21/11/2013	4
Australian and New Zealand Clinical Trials Register	21/11/2013	0
European Clinical Trials Register	21/11/2013	20
MedNar	21/11/2013	6858
Open Grey	21/11/2013	28
DART-Europe E-thesis Portal	21/11/2013	30
Open Thesis	21/11/2013	68

Appendix II. Critical appraisal instruments

JBI critical appraisal tool – randomised and quasi-randomised trials

1. Was the assignment to treatment groups truly random?

Yes	Method by which randomization to intervention or control group described by author(s). (e.g. random allocation using number generator)
No	Methods other than randomization used to allocate patients to intervention or control groups (e.g. quasi randomisation/ stratification as appropriate for study design)
Unclear	General terms like "random" and "randomisation" used but method by which this was achieved not clearly described.

Reviewer's response/comment:

2. Were participants blinded to treatment allocation?

Yes	Participants unaware that they have been allocated to either the intervention or control group.
No	Participants aware of which group they have been allocated to even although blinding may have been possible
Unclear	Description of above unclear or unsatisfactory.

Reviewer's response/comment:

3. Was allocation to treatment groups concealed from the allocator?

Yes	Allocator unaware of whether they were allocating participants to intervention or control group.
No	Allocator aware of which group they were allocating participants (patients) to.
Unclear	Description of above unclear or unsatisfactory.

Reviewer's response/comment:

4. Were the outcomes of people who withdrew described and included in the results and analysis?

Yes	Withdrawn participants reported and reasons for the withdrawal described. All participants included in final calculations including withdrawn participants, regardless of whether their final outcomes were measured.
No	No explanation of withdrawn patients or the significance of these withdrawals. Withdrawn patients not analysed in the groups to which they were originally allocated.
Unclear	Withdrawn patients incompletely described. Numbers of included/withdrawn patients do not match result figures. Description of above unclear or unsatisfactory.

Reviewer's response/comment:

5. Were those assessing outcomes blind to the treatment allocation?

Yes	Data collectors were blinded for outcomes assessment? (If outcome is measured using an objective test, such as a lab value, this criterion is assumed to be met). In diagnostic study, were test results blinded to patient history and other test results?
No	Data collectors were aware of which group the patient belonged to.
Unclear	Description of above unclear or unsatisfactory.

Reviewer's response/comment

6. Were the control and treatment groups comparable at entry?

Yes	At a minimum, the following baseline data for the patients was reported: • Age • Sex • %TBSA • Baseline measurements for individual intended measurable outcomes
No	Baseline data between groups is clearly not comparable (ie. statisistical differences between groups at baseline that may affect the interpretation of trace element effectiveness – i.e. large discrepancies between average TBSA% burn/age.
Unclear	Description of above unclear or unsatisfactory. No or minimal reporting of baseline data i.e. only age and sex, no clear %TBSA reported with no indication of individual baseline measurements for intended outcome measures or no mention of statistical differences between groups where differences in baseline measures are apparent).

Reviewer's response/comment:

7. Were groups treated identically other than for the named interventions?

Yes	Participants in both the intervention and control groups were treated identically for all other aspects of care other than trace element supplementation.
No	Participants in each group were treated differently in respect to other aspects of care.
Unclear	Description of above unclear or unsatisfactory.

Reviewer's response/comment:

8. Were outcomes measured in the same way for all groups?

Yes	Description of how data was measured and collected provided and consistent between participant groups.
No	Description of how outcome data was measured and collected different for each group.
Unclear	Description of above unclear or unsatisfactory.

Reviewer's response/comment:

9. Were outcomes measured in a reliable way?

Yes	All outcomes measured using standardised methods or instruments: • Length of stay reported in days • Rate of wound healing clearly described in how measured (donor site/complete healing) and whether measure is objective or subjective (i.e. surgeon/nurse visually assessed subjectively or whether tools/scales were used) • Presence of infection clearly were clearly determined (positive blood/wound cultures/ need for antibiotic intervention/radiologic confirmation of pneumonia, etc.) • Clear objective description of how tissue/plasma levels are sampled and analysed and appropriate for reported results Authors mention the reliability and/or validity of the measurements they use (including trained data collectors) or piloted within the trial.
No	Estimates or self reported outcomes reported. Incorrect or non standard methods or instruments used, absence of clear definitions for measurements of outcome measures. No reporting on the reliability and/or validity of the methods used for measuring outcome or training provided for data collectors.
Unclear	Description of above unclear or unsatisfactory.

Reviewer's response/comment:

10. Was appropriate statistical analysis used and reported?

Yes	Appropriate statistical methods used, described and reported. Withdrawn participants analysed in the groups to which they were originally allocated (Intention to treat analysis/ITT).
No	Statistical methods not described or inappropriate methods used. Missing patient data not reported or accounted for.
Unclear	Description of above unclear or unsatisfactory.

Reviewer's response/comment:

JBI critical appraisal tool – cohort (with control)/case-controlled studies

1. Is the sample representative of patients in the population as a whole?

Yes	Authors describe the target population that they want to look at. Authors mention or describe how population was selected/recruited, is this representative of the whole population? Inclusion and exclusion criteria are defined. Baseline demographics of the participants are described (age, sex, morbidities, baseline measurements of whatever outcomes will be investigated (e.g. serum trace element levels, wound size %TBSA, etc). Additional information can include: • Geographical location (e.g. developing world/developed world), nutritional regimens in addition to intervention
No	No mention of how target population selected/recruited, or whether representative of the whole population. Inclusion/exclusion criteria not defined. Age or sex described only.
Unclear	Description of above unclear or unsatisfactory.

Note: descriptions should include both study and control groups, and an explanation of how comparable they are.

Reviewer's response/comment:

2. Are participants at a similar point in the course of their burn injury?

Yes	Participants have all sustained an acute thermal (scald/flame) burn injury. Participants commence trace element supplementation at the beginning of their medical/surgical burn injury management (i.e. within 48 hours of injury/admission). Participants all receive trace element intervention according to the same criteria (pre-determined) for supplementation duration.
No	Participants have not all sustained an acute thermal burn injury (may include reconstruction patients, sunburn). Participants commence trace element supplementation at different times of their burn injury management. Participants receive trace element supplementation for different lengths of time an not according to a pre-determined algorithm.
Unclear	Description of above unclear or unsatisfactory.

Reviewer's response/comment:

3. Has bias been minimised in relation to selection of cases and controls?

Yes	Description of how the study and control groups were selected (i.e. sequential thermal burn admissions with age/gender/burn size matched controls). Clear follow-up period and clear points of measurement. Sample sizes given. The numbers of participants at each stage of the study are reported. Baseline trace element levels are comparable between cases and matched controls.
No	No description of how groups were selected.
Unclear	Description of above unclear or unsatisfactory.

Reviewer's response/comment:

4. Are confounding factors identified and strategies to deal with them stated?

Yes	Key confounders (e.g. existing metabolic disease, excessive alcohol use/steroid use/ metabolic agents/self-sabotage of wounds) are recognised and participants excluded if present. Any remaining confounders (e.g. age/gender/smoking status/psychological illness influencing burn injury management) are described and adjusted for, if possible, in the analyses.
No	No mention of confounders, or no attempt to take into account. Participants included despite presence of key confounders without adequate discussion regarding this decision.
Unclear	Description of above unclear or unsatisfactory.

Reviewer's response/comment:

5. Are the outcomes assessed using objective criteria?

Yes	Description of how data were collected. Description of how each outcome was measured (existing definitions or diagnostic criteria/measurement techniques; validated tools): • Length of stay reported in days • Rate of wound healing clearly described in how measured (donor site/ complete healing) and whether measure is objective or subjective (i.e. surgeon/nurse visually assessed subjectively or whether tools/scales were used) • Presence of infection clearly were clearly determined (positive blood/wound cultures/ need for antibiotic intervention/radiologic confirmation of pneumonia, etc.) • Clear objective description of how tissue/plasma levels are sampled and analysed and appropriate for reported results Clear definition of key terms used for each outcome and measurement (e.g. wound healing rate, calculation of TBSA)–either primary or surrogate measures.
No	No or poor description of above outcomes and measurements. Key terms not defined or quantified. Estimates or self reported outcomes reported. Incorrect or non standard methods or instruments used, absence of clear definitions for measurements of outcome measures. No reporting on the reliability and/or validity of the methods used for measuring outcome or training provided for data collectors.
Unclear	Description of above unclear or unsatisfactory.

Reviewer's response/comment:

6. Was follow-up carried out over a sufficient time period?

Yes	Study duration and follow-up defined clearly (including times at which measurements were taken). Follow-up time from commencement of trace element supplementation adequate for each outcome to manifest as a result of supplementation (e.g. wound closure/hospital discharge/mortality >24 hours following commencement of trace elements).
No	Follow-up time too short for surrogate outcomes to manifest (if used or reported).
Unclear	Description of above unclear or unsatisfactory.

Reviewer's response/comment:

7. Were the outcomes of people who withdrew described and included in the analysis?

Yes	Participants analysed in the groups to which they were assigned at baseline. All participants included in final calculations, regardless of whether their outcomes were measured or justification for non-inclusion provided and legitimate. Losses to follow-up/attrition described clearly and outliers accounted for.
No	No explanation of withdrawn patients/loss to follow-up/attrition or the significance of these withdrawals.
Unclear	Description of above unclear or unsatisfactory.

Reviewer's response/comment:

8. Were outcomes measured in a reliable way?

Yes	All outcomes measured using standardised methods or instruments. Authors mention the reliability and/or validity of the measurements they use (including trained data collectors) or piloted within the trial. All participants had outcome measures conducted at the same time points and for the same durations.
No	Estimates or self-reported outcomes reported. Incorrect or non-standard methods or instruments used, absence of clear definitions for measurements of outcome measures. No reporting on the reliability and/or validity of the methods used for measuring outcome or training provided for data collectors. Time points for outcome measure data collection varied between participants.
Unclear	Description of above unclear or unsatisfactory.

Reviewer's response/comment:

9. Was appropriate statistical analysis used?

Yes	Appropriate statistical methods used and described, and methods for addressing confounders included.
No	Statistical methods not described, or inappropriate methods used. Missing data not reported or accounted for.
Unclear	Description of above unclear or unsatisfactory.

Reviewer's response/comment:

JBI critical appraisal tool – descriptive/case series

1. Was the study based on a random or pseudo-random sample?

Yes	Methods section reports how random sampling occurred.
No	No mention of how sampling was performed.
Unclear	Description of above unclear or unsatisfactory.

Reviewer's response/comment:

2. Were criteria for inclusion in the sample clearly defined?

Yes	Inclusion criteria clearly documented and based on pre-defined relevant characteristics.
No	Inclusion criteria not clearly documented or based on pre-defined relevant characteristics.
Unclear	Description of above unclear or unsatisfactory.

Reviewer's response/comment:

3. Were confounding factors identified and strategies to deal with them stated?

Yes	Key confounders (e.g. existing metabolic disease, excessive alcohol use/steroid use/metabolic agents/self-sabotage of wounds) are recognised and participants excluded if present. Any remaining confounders (e.g. age/gender/smoking status/psychological illness influencing burn injury management/variances in baseline nutritional status/intake) are described and adjusted for, if possible, in the analyses.
No	No mention of confounders, or no attempt to take into account. Participants included despite presence of key confounders without adequate discussion regarding this decision.
Unclear	Description of above unclear or unsatisfactory.

Reviewer's response/comment:

4. Were the outcomes assessed using objective criteria?

Yes	Description of how data were collected. Description of how each outcome was measured (existing definitions or diagnostic criteria/measurement techniques; validated tools): • Length of stay reported in days • Rate of wound healing clearly described in how measured (donor site/complete healing) and whether measure is objective or subjective (i.e. surgeon/nurse visually assessed subjectively or whether tools/scales were used) • Presence of infection clearly were clearly determined (positive blood/wound cultures/ need for antibiotic intervention radiologic confirmation of pneumonia, etc.) • Clear objective description of how tissue/plasma levels are sampled and analysed and appropriate for reported results. Clear definition of key terms used for each outcome and measurement (e.g. wound healing rate, calculation of TBSA) – either primary or surrogate measures.
No	No or poor description of outcomes and measurements. Key terms not defined or quantified. Estimates or self-reported outcomes reported. Incorrect or non-standard methods or instruments used, absence of clear definitions for measurements of outcome measures. No reporting on the reliability and/or validity of the methods used for measuring outcome or training provided for data collectors.
Unclear	Description of above unclear or unsatisfactory.

Reviewer's response/comment:

5. If comparisons were being made, was there sufficient description of groups?

Yes	Comparator groups (where applicable) clearly described (e.g. Retrospective/pilot/non-burn injury) and an attempt to identify and measure similarity between groups has been made (i.e. age, gender, body mass, co-morbidities).
No	Comparator groups (where applicable) are not clearly described and no attempt to identify and measure similarity between groups has been made (i.e. age, gender, body mass, co-morbidities).
Unclear	Description of above unclear or unsatisfactory.

Reviewer's response/comment:

6. Was follow-up carried out over a sufficient time period?

Yes	Study duration and follow-up defined clearly (including times at which measurements were taken). Follow-up time from commencement of trace element supplementation adequate for each outcome to manifest as a result of supplementation (e.g. wound closure/hospital discharge/mortality >24 hours following commencement of trace elements).
No	Follow-up time too short for surrogate outcomes to manifest (if used or reported).
Unclear	Description of above unclear or unsatisfactory.

Reviewer's response/comment:

7. Were the outcomes of people who withdrew described and included in the analysis?

Yes	Participants analysed in the groups to which they were assigned at baseline. All participants included in final calculations, regardless of whether their outcomes were measured or justification for non-inclusion provided and legitimate. Losses to follow-up/attrition described clearly and outliers accounted for.
No	No explanation of withdrawn patients/loss to follow-up/attrition or the significance of these withdrawals.
Unclear	Withdrawn patients incompletely described. Numbers of included/withdrawn patients do not match result figures. Description of above unclear or unsatisfactory.

Reviewer's response/comment:

8. Were outcomes measured in a reliable way?

Yes	All outcomes measured using standardised methods or instruments. Authors mention the reliability and/or validity of the measurements they use (including trained data collectors) or piloted within the trial: • All participants had outcome measures conducted at the same time points and for the same durations.
No	Estimates or self-reported outcomes reported. Incorrect or non-standard methods or instruments used. No reporting on the reliability and/or validity of the methods used for measuring outcome or training provided for data collectors. Time points for outcome measure data collection varied between participants.
Unclear	Description of above unclear or unsatisfactory.

Reviewer's response/comment:

9. Was appropriate statistical analysis used and reported?

Yes	Appropriate statistical methods used and described, and methods for addressing confounders included.
No	Statistical methods not described or inappropriate methods used. Missing patient data not reported or accounted for.
Unclear	Description of above unclear or unsatisfactory.

Reviewer's response/comment:

Appendix III. Data extraction instrument

Data extraction tool

Reviewer: Date:

Author: Year:

Journal: Record Number:

Title:

Study

RCT ☐ Quasi-RCT ☐ Longitudinal ☐
Retrospective ☐ Observational ☐ Other ☐

Participants

Setting:

Population:

Sample size

Group A: Group B:

Intervention

Intervention 1:

Intervention 2:

Outcomes

Outcome description	Scale/measurement

Study results

Dichotomous data

Outcome	Intervention ( ) number/total number	Intervention ( ) number/total number

Continuous data

Outcome	Intervention ( ) number/total number	Intervention ( ) number/total number

Author's conclusion:

Reviewer's comments:

Appendix IV. Excluded studies

Excluded following full text retrieval

Andreassi L, Flori L. Pharmacologic treatment of burns. Clinics in Dermatology. 1991 Oct-Dec;9(4):453-8
Reason for exclusion: Not a primary intervention study, just a review article.

Andulics CA, Shapiro M, Shapiro R, Johnson RM. Nutrition in burns: a multicenter study to evaluate the application of evidence-based medicine into practice. Journal of Burn Care and Research. 2008;29(2):S162.
Reason for exclusion: Abstract only, not a primary intervention study, no trace element intervention therefore authors not contacted for further information.

Barbosa E, Faintuch J, Machado Moreira EA. Supplementation of vitamin E, vitamin C, and zinc attenuates oxidative stress in burned children: A randomized, double-blind, placebo-controlled pilot study. Nutrition in Clinical Practice. 2010 April;25(2):216-8.
Reason for exclusion: Commentary of article, not primary article. Primary article also identified and included in critical appraisal process (Barbosa et al., 2009).

Berger M. Acute copper and zinc deficiency due to exudative losses - substitution versus nutritional requirements - Burns 2005;31(6): 711-6. Burns 2006 May;32(3):393.
Reason for exclusion: Letter to editor not a primary study.

Berger M, Binnert C, Baines M, Raffoul W, Cayeux M, Chiolero R, et al. Trace element supplements influence protein metabolism and tissue levels after major burns. Intensive care medicine. 2004 Sep 2004;30(Supplement):S61.
Reason for exclusion: Conference abstract for Berger et al., 2007 article (excluded due to outcome measures not complying with protocol and duplicate data from another publication from the same group)

Berger MM. Antioxidant micronutrients in major trauma and burns: evidence and practice. Nutrition in Clinical Practice. 2006 Oct;21(5):438-49.
Reason for exclusion: Not a primary intervention study, just a review article.

Berger MM, Binnert C, Chiolero RL, Taylor W, Raffoul W, Cayeux MC, et al. Trace element supplementation after major burns increases burned skin trace element concentrations and modulates local protein metabolism but not whole-body substrate metabolism. The American Journal of Clinical Nutrition. 2007 May;85(5):1301-6.
Reason for exclusion: Outcome measures not complying with protocol and duplicate data from another publication published by the same research group (Berger et al., 2007: included in critical appraisal).

Berger MM, Cavadini C, Bart A, Blondel A, Bartholdi I, Vandervale A, et al. Selenium losses in 10 burned patients. Clinical Nutrition. 1992 Apr;11(2):75-82.
Reason for exclusion: Selenium losses studied only, no trace element supplementation intervention.

Berger MM, Cavadini C, Guinchard S, Krupp S, Dirren H. Effect of greatly increased Cu, Zn and Se intakes on trace element status and clinical evolution in major burns. Clinical Nutrition. 1992;11, Supplement(0):22.
Reason for exclusion: Conference abstract for Berger et al., 1994 article (included in critical appraisal process).

Berger MM, Chiolero R. Relations between copper, zinc and selenium intakes and malondialdehyde excretion after major burns. Burns. 1995 Nov;21(7):507-12.
Reason for exclusion: Testing effect of TE supplementation on MDA levels, not outcomes as per pre-specified protocol.

Berger MM, Eggimann P, Heyland DK, Chiolero RL, Revelly JP, Day A, et al. Reduction of nosocomial pneumonia after major burns by trace element supplementation: aggregation of two randomised trials. Critical Care. 2006;10(6):R153.
Reason for exclusion: Aggregation study of data from Berger et al., 1998 and Berger et al., 2007 (included in critical appraisal process).

Berger MM, Shenkin A. Trace element requirements in critically ill burned patients. Journal of Trace Elements in Medicine and Biology. 2007;21 Suppl 1:44-8.
Reason for exclusion: Not a primary intervention study, just a review article.

Berger MM, Spertini F, Baines M, Schindler C, Wiesner L, Shenkin A, et al. O.6 Trace element supplements have pharmacological effects and reduce infectious complications in burns-a randomized trial. Clinical Nutrition. 1997;16,Supplement(2):2
Reason for exclusion: Conference abstract for Berger et al., 1998 article (included in critical appraisal process).

Bernal ME, Varon J, Acosta P, Montagnier L. Oxidative stress in critical care medicine. International Journal of Clinical Practice. 2010 Oct;64(11):1480-8.
Reason for exclusion: Not a primary intervention study, just a review article.

Bing-guo L, Wei-shia H, Tsi-siang S. Causes of death in aged burn patients: Analysis of 36 cases. Burns. 1990;16(3):207-10.
Reason for exclusion: No trace element supplementation/intervention (i.e. not a study of effectiveness).

Boosalis M, Solem L, McCall J, McClain CJ. Serum copper, zinc, silver and selenium concentrations in burn patients. Journal of Parenteral and Enteral Nutrition. 1984;8(1):102.
Reason for exclusion: No trace element supplementation/intervention (i.e. not a study of effectiveness).

Brandt RB, Riggle MA, Haynes BW, Chan WM. Vitamin-A, zinc and retinol-binding protein in human burn patients. Federation Proceedings. 1982;41(3):388.
Reason for exclusion: No trace element supplementation/intervention (i.e. not a study of effectiveness).

Cunningham J, Lydon M, Decheke M. Parenteral-nutrition for severely burned children – assessment of the AMA guidelines for zinc, copper and manganese. Journal of the American College of Nutrition. 1988 Oct;7(5):423.
Reason for exclusion: Patients age < 2 and no trace element intervention/supplementation.

Cunningham JJ, Harris LJ, Briggs SE. Nutritional support of the severely burned infant. Nutrition in Clinical Practice. 1988 Apr;3(2):69-73.
Reason for exclusion: No trace element supplementation/intervention, outcome measures not pre-defined as per protocol.

de Haan KE, de Goeij JJ, van den Hamer CJ, Boxma H, de Groot CJ. Changes in zinc metabolism after burns: observations, explanations, clinical implications. Journal of Trace Elements and Electrolytes in Health and Disease. 1992 Sep;6(3):195-201.
Reason for exclusion: No trace element supplementation/intervention (i.e. not a study of effectiveness).

Dickerson RN. Metabolic support of the thermally injured patient. Hospital Pharmacy. 2006 February;41(2):186-94.
Reason for exclusion: Not a primary intervention study, just a review article.

Forceville X, Laviolle B, Annane D, Vitoux D, Bleichner G, Korach JM, et al. Effects of high doses of selenium, as sodium selenite, in septic shock: A placebo-controlled, randomized, double-blind, phase II study. Critical Care. 2007;11(4).
Reason for exclusion: No burns patients included in patient cohort (critical care only).

Forceville X, Vitoux D, Gauzit R, Combes A, Lahilaire P, Chappuis P. Selenium, systemic immune response syndrome, sepsis, and outcome in critically ill patients. Critical Care Medicine. 1998 Sep;26(9):1536-44.
Reason for exclusion: No burns patients included in patient cohort (critical care only) and no trace element supplementation as a measure of effectiveness.

Gartner R, Albrich W, Angstwurm MWA. The effect of a selenium supplementation on the outcome of patients with severe systemic inflammation, burn and trauma. BioFactors. 2001;14(1-4):199-204.
Reason for exclusion: Not a primary intervention study, just a review article.

Giladi AM, Dossett LA, Fleming SB, Abumrad NN, Cotton BA. High-dose antioxidant administration is associated with a reduction in post-injury complications in critically ill trauma patients. Injury-International Journal of the Care of the Injured. 2011 Jan;42(1):78-82.
Reason for exclusion: On contact with author, no burns patients included in patient cohort (critical care only).

Goodwin CW. Metabolism and nutrition in the thermally injured patient. Critical Care Clinics. 1985;1(1):97-118.
Reason for exclusion: Not a primary intervention study, just a review article.

Gottschlich MM, Jenkins M, Warden GD, Baumer T, Havens P, Snook JT, et al. Differential effects of three enteral dietary regimens on selected outcome variables in burn patients. Journal of Parenteral and Enteral Nutrition. 1990;14(3):225-36.
Reason for exclusion: Does not fit trace element intervention study inclusion criteria as compared three experimental enteral feeds rather than trace element suppplementation/intervention effectiveness, no difference in Zn intake between groups (except week 2) all patients received Zn supplementation on top of enteral diet, no comparison of Se intake.

Hunt D, Lane H, Beesinger D, Gallagher K, Rowlands BJ, Johnston D. Selenium and glutathione-peroxidase levels in hospitalized burn patients. Federation Proceedings. 1983;42(4):927.
Reason for exclusion: No trace element supplementation/intervention (i.e. not a study of effectiveness).

Hunt DR, Lane HW, Beesinger D, Gallagher K, Halligan R, Johnston D, et al. Selenium depletion in burn patients. Journal of Parenteral and Enteral Nutrition. 1984;8(6):695-9.
Reason for exclusion: No trace element supplementation/intervention (i.e. not a study of effectiveness).

Ireton CS, Hunt JL, Lang ED. Nutritional management of a thermally injured patient. Nutritional Support Services. 1984;4(2):64.
Reason for exclusion: No trace element supplementation/intervention (i.e. not a study of effectiveness).

Mayes T, Gottschlich MM, Kagan RJ. An evaluation of the safety and efficacy of an anti-inflammatory, pulmonary enteral formula in the treatment of pediatric burn patients with respiratory failure. Journal of Burn Care & Research. 2008 Jan-Feb;29(1):82-8.
Reason for exclusion: Respiratory outcome measures only (not of interest to the review), no true trace element intervention as testing safety of pulmonary enteral nutrition formula.

Mendez C, Jurkovich GJ, Wener MH, Garcia I, Mays M, Maier RV. Effects of supplemental dietary arginine, canola oil, and trace elements on cellular immune function in critically injured patients. Shock. 1996 Jul;6(1):7-12.
Reason for exclusion: No burns patients included in patient cohort (critical care only).

Rollins C, Moore A, Neumeister M, Cooney C, Dodson S. Burn Micronutrient Pilot Repletion Study: Characterizing relationships between vitamin and mineral supplementation and health outcomes of adult burn patients. Journal of the American Dietetic Association. 2010;110(9, Supplement):A34.
Reason for exclusion: Poster published only, authors contacted and unable to provide data on patients that fitted inclusion criteria (burns patients meeting inclusion criteria per protocol) as cohort mostly out of pre-defined patient inclusion characteristics.

Stucki P, Perez M-H, Cotting J, Shenkin A, Berger MM. Substitution of exudative trace element losses in burned children. Critical Care. 2010;14(1):439.
Reason for exclusion: Letter to the editor and case series only (i.e. not a study of effectiveness), no comparitor. Author contacted for further data, however unable to provide within a reasonable time frame for completion of systematic review.

Zoch G, Meissl G, Bayer S, Kyral E. Reduction of the mortality rate in aged burn patients. Burns. 1992;18(2):153-6.
Reason for exclusion: No trace element supplementation/intervention (i.e. not a study of effectiveness).

Excluded following critical appraisal

Al-Jawad FH, Sahib AS, Al-Kaisy AA. Role of antioxidants in the treatment of burn lesions. Annals of Burns and Fire Disasters. 2008 Dec 31;21(4):186-91.
Reason for exclusion: ≤ 4 criteria, no detail on recruitment, allocation to groups, comparison of baseline demographics between groups, or care given, high risk of allocation and selection bias, no attempt to take counfounding factors into account; unclear measures of outcomes; unclear statistical analysis used.

Barbosa E, Faintuch J, MacHado Moreira EA, Gonalves Da Silva VR, Lopes Pereima MJ, Martins Fagundes RL, et al. Supplementation of vitamin E, vitamin C, and zinc attenuates oxidative stress in burned children: A randomized, double-blind, placebo-controlled pilot study. Journal of Burn Care and Research. 2009 September-October;30(5):859-66.
Reason for exclusion: ≤ 4 criteria, unclear methodology throughout study. Letter sent to corresponding author address provided for clarrification of methodology, without reply.

Caldis-Coutris N, Gawaziuk JP, Logsetty S. Zinc supplementation in burn patients. Journal of Burn Care and Research. 2012 Sep-Oct;33(5):678-82.
Reason for exclusion: < 4 criteria, no detail on recruitment, allocation to groups, comparison of baseline demographics between groups, or care given, high risk of allocation and selection bias, no attempt to take counfounding factors into account; unclear measures of outcomes.

Cunningham JJ, Lydon MK, Briggs SE, DeCheke M. Zinc and copper status of severely burned children during TPN. Journal of the American College of Nutrition. 1991 Feb;10(1):57-62.
Reason for exclusion: < 4 criteria, no detail on recruitment, allocation to groups, comparison of baseline demographics between groups, high risk of allocation and selection bias, no attempt to take counfounding factors into account; unclear measures of outcomes; unclear statistical analysis used.

Cunningham JJ, Leffell M, Harmatz P. Burn severity, copper dose, and plasma ceruloplasmin in burned children during total parenteral-nutrition. Nutrition. 1993 Jul-Aug;9(4):329-32.
Reason for exclusion: < 4 criteria, no detail on recruitment, allocation to groups, comparison of baseline demographics between groups, high risk of allocation and selection bias, no attempt to take counfounding factors into account; unclear measures of outcomes; unclear statistical analysis used.

McClain CJ, McClain ML, Boosalis MG, Hennig B. Zinc and the stress response. Scandinavian Journal of Work, Environment and Health. 1993;19 Suppl 1:132-3.
Reason for exclusion: < 4 criteria, no detail on recruitment, allocation to groups, comparison of baseline demographics between groups, high risk of allocation and selection bias, no attempt to take counfounding factors into account; unclear measures of outcomes; unclear statistical analysis used. Extended abstract publication with note that “full report to be published elsewhere”. Corresponding author contacted for full report, reference for prior work investigating zinc serum levels following injury without intervention were provided (Boosalis et al., Serum zinc response in thermal injury, J Am Coll Nutr, 1988) however not references for effectiveness of supplementation studies.

Pochon JP. Zinc-and copper-replacement therapy-a must in burns and scalds in children? Progress in Pediatric Surgery. 1981;14:151-72.

Reason for exclusion: ≤ 4 criteria, no detail on recruitment, allocation to groups, comparison of baseline demographics between groups, no attempt to take counfounding factors into account; unclear statistical methods used.

Appendix V. Statements of authorship

Statement of Authorship

Title of Paper	The effectiveness of trace element supplementation following severe burn injury: A systematic review protocol
Publication Status	⦿ Published, ○ Accepted for Publication, ○ Submitted for Publication, ○ Publication style
Publication Details	Kurmis, R., Aromataris, E., & Greenwood, J. The effectiveness of trace element supplementation following severe burn injury: A systematic review protocol. JBI Database of Systematic Reviews & Implementation Reports 2013;11(11) 44 - 53

Author Contributions

By signing the Statement of Authorship, each author certifies that their stated contribution to the publication is accurate and that permission is granted for the publication to be included in the candidate's thesis.

Name of Principal Author (Candidate)	Rochelle Kurmis		
Contribution to the Paper	Conceptualised topic and developed background, PICO question. Wrote manuscript and acted as corresponding author.		
Signature		Date	26/2/15

Name of Co-Author	Edoardo Aromataris		
Contribution to the Paper	Supervised development of work, assisted in development of search strategy and manuscript evaluation.		
Signature		Date	26/3/15

Name of Co-Author	John Greenwood		
Contribution to the Paper	Helped to evaluate and edit the review question and the manuscript.		
Signature		Date	25/02/2015

Name of Co-Author			
Contribution to the Paper			
Signature		Date	

Statement of Authorship

Title of Paper	Trace element supplementation following severe burn injury: A systematic review and meta-analysis
Publication Status	○ Published, ◉ Accepted for Publication, ○ Submitted for Publication, ○ Publication style
Publication Details	Kurmis, R., Greenwood, J., & Aromataris, E. Trace element supplementation following severe burn injury: A systematic review and meta-analysis. Journal of Burn Care and Research. Status: accepted for publication (in press)

Author Contributions

By signing the Statement of Authorship, each author certifies that their stated contribution to the publication is accurate and that permission is granted for the publication to be included in the candidate's thesis.

Name of Principal Author (Candidate)	Rochelle Kurmis		
Contribution to the Paper	Developed and performed comprehensive systematic review search and eligibility screening, critical appraisal of included studies, metaanalysis and data synthesis. Wrote manuscript and acted as corresponding author.		
Signature		Date	25/2/15

Name of Co-Author	John Greenwood		
Contribution to the Paper	Helped to evaluate and edit the rmanuscript.		
Signature		Date	25/2/15

Name of Co-Author	Edoardo Aromataris		
Contribution to the Paper	Supervised development of work, assisted in development of search strategy, assisted in data interpretation and manuscript evaluation.		
Signature		Date	26/3/15

Name of Co-Author			
Contribution to the Paper			
Signature		Date	

Reference list

1. Deitch EA. Nutritional support of the burn patient. Crit Care Clin. Jul 1995;11(3):735-750.
2. Berger MM, Baines M, Raffoul W, et al. Trace element supplementation after major burns modulates antioxidant status and clinical course by way of increased tissue trace element concentrations. Am J Clin Nutr. May 2007;85(5):1293-1300.
3. Klein GL, Przkora R, Herndon DN. Vitamin and trace element homeostasis following severe burn injury. In: Total Burn Care. 3rd ed: Elsevier Inc, Philadelphia, USA; 2007:387-390.
4. Gamliel Z, DeBiasse MA, Demling RH. Essential microminerals and their response to burn injury. JBCR. May/June 1996;17(3):264-272.
5. Kurmis R, Aromataris E, Greenwood JE. The effectiveness of trace element supplementation following severe burn injury: A systematic review protocol. JBI DB Syst Rev Impl Reps. Dec 2013;11(11):44-53.
6. Kurmis R, Greenwood J, Aromataris E. Trace element supplementation following severe burn injury: A systematic review and meta-analysis. J Burn Care Res. In Press.
7. American Burn Association. Burn incidence and treatment in the United States: 2012 fact sheet. [Internet]. Available at: http://www.ameriburn.org/resources_factsheet.php. Accessed 29th August 2014, 2013.
8. Burns Registry os Australia and New Zealand. Burns Registry of Australia and New Zealand Annual Report 1 July 2012 to 30 June 2013. [Internet]. Available at: http://anzba.org.au/assets/BRANZ_AnnualReportYear4_Final.pdf. Accessed 20 February, 2015.
9. American Burn Association, National Burn Repository. 2014 National Burn Repository report of data from 2004-2013. [Internet]. Available at: http://www.ameriburn.org/2014NBRAnnualReport.pdf. Accessed 11th December, 2014.
10. World Health Organization. Burns. Fact Sheet No365: WHO; 2014. Available at: http://www.who.int/mediacentre/factsheets/fs365/en/. Accessed 14 November, 2014.
11. Wolf SE, Arnoldo BD. The year in burns 2011. Burns. 2012;38:1096-1108.
12. World Health Organization. A WHO Plan for Burn Prevention and Care. WHO Press. [Internet]. Available at: http://whqlibdoc.who.int/publications/2008/9789241596299_eng.pdf?ua=1. Accessed 14 November, 2014.
13. Cubison TC, Pape SA, Parkhouse N. Evidence for the link between healing time and the development of hypertrophic scars (HTS) in paediatric burns due to scald injury. Burns. Dec 2006;32(8):992-999.
14. Hassan S, Reynolds G, Clarkson J, et al. Challenging the dogma: relationship between time to healing and formation of hypertrophic scars after burn injuries. J Burn Care Res. Mar-Apr 2014;35(2):e118-124.
15. Herndon D., ed. Total Burn Care. 3rd ed. Philadelphia: Saunders, Elsevier Inc; 2007.
16. The Australian Lung Foundation. Respiratory Infectious Disease Burden in Australia; 2007. [Internet]. Available from: http://lungfoundation.com.au/wp-content/uploads/2014/03/2007-RID-Case-Statement.pdf. Accessed 11 December, 2014.
17. Osler T, Glance LG, Hosmer DW. Simplified estimates of the probability of death after burn injuries: extending and updating the baux score. J Trauma. Mar 2010;68(3):690-697.
18. Heyland DK, Dhaliwal R, Suchner U, et al. Antioxidant nutrients: a systematic review of trace elements and vitamins in the critically ill patient. Intensive Care Med. Mar 2005;31(3):327-337.
19. National Health and Medical Research Council. Nutrient Reference Values for Australia and New Zealand. Baghurst. K, ed. Canberra: National Health and Medical Research Council.; 2006. [Internet]. Available from: http://www.nhmrc.gov.au/_files_nhmrc/publications/attachments/n35.pdf. Accessed 14 November, 2014.

20. Combs GF, Jr. Selenium in global food systems. Br J Nutr. May 2001;85(5):517-547.
21. Lyons GH, Judson GJ, Stangoulis JCR, et al. Trends in selenium status of South Australians. MJA. 2004;180:383-386.
22. Rayman MP. Dietary selenium: time to act. BMJ. Feb 8 1997;314(7078):387-388.
23. Food and Agriculture Organization of the United Nations., World Health Organization. Chapter 15: Selenium. In: Human Vitamin and Mineral Requirements. Report of a joint FAO/WHO expert consultation. Bangkok, Thailand; 2001. [Internet]. Available from: http://www.fao.org/3/a-y2809e.pdf. Accessed 31 October, 2014.
24. Tan JC, Burns DL, Jones HR. Severe ataxia, myelopathy, and peripheral neuropathy due to acquired copper deficiency in a patient with history of gastrectomy. JPEN. Sep-Oct 2006;30(5):446-450.
25. Australian Institute of Health and Welfare. Weight loss surgery in Australia. Canberra 2010. [Internet]. Available from: http://www.aihw.gov.au/WorkArea/DownloadAsset.aspx?id=6442472773. Accessed 31 October, 2014.
26. Ma J, Betts NM. Zinc and copper intakes and their major food sources for older adults in the 1994-96 continuing survey of food intakes by individuals (CSFII). J Nutr. Nov 2000;130(11):2838-2843.
27. Berger MM, Cavadini C, Bart A, et al. Cutaneous copper and zinc losses in burns. Burns. Oct 1992;18(5):373-380.
28. Berger MM, Cavadini C, Bart A, et al. Selenium losses in 10 burned patients. Clin Nutr. Apr 1992;11(2):75-82.
29. Berger MM, Shenkin A. Vitamins and trace elements: Practical aspects of supplementation. Nutrition 2006;22:952-955.
30. Shenkin A. Trace elements and inflammatory response: Implications for nutritional support. Nutrition. Jan-Feb 1995;11(1 Suppl):100-105.
31. Berger M. Basics in clinical nutrition: Nutritional support in burn patients. e-SPEN. December 2009;4(6):e308-e312.
32. Galloway P, McMillan DC, Sattar N. Effect of the inflammatory response on trace element and vitamin status. Ann Clin Biochem. May 2000;37 (Pt 3):289-297.
33. Nordlund MJ, Pham TN, Gibran NS. Micronutrients after burn injury: A review. J Burn Care Res. Mar-Apr 2014;35(2):121-133.
34. Caldis-Coutris N, Gawaziuk JP, Logsetty S. Zinc supplementation in burn patients. J Burn Care Res. Sep-Oct 2012;33(5):678-682.
35. Wallach J. Interpretation of Diagnostic Tests. 8th Edition ed. Philadelphia: Lippincott Williams & Wilkins; 2007.
36. Masters B. Nutrition support in burns-Is there consistency in practice. J Burn Care Res. July-August 2008;29(4):561-571.
37. Graves C, Saffle J, Cochran A. Actual burn nutrition care practices: An Update. J Burn Care Res. Jan-Feb 2009;30(1):77-82.
38. Rousseau A-F, Losser M-R, Berger MM, et al. ESPEN endorsed recommendations: Nutritional therapy in major burns. Clinl Nutr. 2013;32(4):497-502.
39. Berger MM, Cavadini C, Chiolero R, et al. Influence of large intakes of trace elements on recovery after major burns. Nutrition. 1994;10(4):327-334+352.
40. Berger MM, Spertini F, Shenkin A, et al. Trace element supplementation modulates pulmonary infection rates after major burns: A double-blind, placebo-controlled trial. Am J Clin Nutr. Aug 1998;68(2):365-371.
41. Heyland DK, Dhaliwal R, Drover JW, et al. Clinical practice guideline for nutrition support in the mechanically ventilated, critically illl adult patient; June 2013. [Internet]. Available from: www.criticalcarenutrition.com. Accessed 27 July, 2013.
42. McClave SA, Martindale RG, Vanek VW, et al. Guidelines for the provision and assessment of nutrition support therapy in the adult critically ill patient: Society of Critical Care Medicine

(SCCM) and American Society for Parenteral and Enteral Nutrition (A.S.P.E.N.). JPEN. May 2009;33(3):277-316.
43. Kreymann KG, Berger MM, Deutz NEP, et al. ESPEN guidelines on enteral nutrition: Intensive care. Clin Nutr. 2006;25(2):210-223.
44. Landucci F, Mancinelli P, De Gaudio AR, et al. Selenium supplementation in critically ill patients: A systematic review and meta-analysis. J Crit Care. Feb 2014;29(1):150-156.
45. Manzanares W, Dhaliwal R, Jiang X, et al. Antioxidant micronutrients in the critically ill: A systematic review and meta-analysis. Crit Care. 2012;16(2):R66.
46. Gagnon G, Voirol P, Soguel L, et al. Trace element monitoring in the ICU: Quality and economic impact of a change in sampling practice. Clin Nutr. Jun 2015;34(3):422-427.
47. The Joanna Briggs Institute. Joanna Briggs Institute Reviewers' Manual: 2014 edition. Adelaide, Australia: The Joanna Briggs Institute; 2014.
48. Higgins JPT, Green S, eds. Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 [updated March 2011]. The Cochrane Collaboration; 2011.
49. Heyland D, Muscedere J, Wischmeyer PE, et al. A randomized trial of glutamine and antioxidants in critically ill patients. N Engl J Med. Apr 18 2013;368(16):1489-1497.
50. Kavanagh BP. The GRADE system for rating clinical guidelines. PLoS Med. Sep 2009;6(9):e1000094.
51. Guyatt G, Gutterman D, Baumann MH, et al. Grading strength of recommendations and quality of evidence in clinical guidelines: Report from an American College of Chest Physicians task force. Chest. Jan 2006;129(1):174-181.
52. Prelack K, Dylewski M, Sheridan RL. Practical guidelines for nutritional management of burn injury and recovery. Burns. Feb 2007;33(1):14-24.
53. Berger MM, Raffoul W, Shenkin A. 'Practical guidelines for nutritional management of burn injury and recovery'-a guideline based on expert opinion but not including RCTs. Burns. Feb 2008;34(1):141-143.
54. Straus S, Tetroe J, Graham ID, eds. Knowledge translation in health care: Moving from evidence to practice. 2nd ed. Chichester, West Sussex, PO19 8SQ, UK: John Wiley & Sons; 2013.
55. Lavrentieva A, Kontakiotis T, Bitzani M. Enteral nutrition intolerance in critically ill septic burn patients. J Burn Care Res. Jul-Aug 2014;35(4):313-318.
56. Kurmis R, Heath K, Ooi S, et al. A prospective multi-center audit of nutrition support parameters following burn injury. J BURN CARE RES. Oct 13 2014.
57. Sudenis T, Hall K, Cartotto R. Enteral nutrition: What the dietitian prescribes is not what the burn patient gets! J Burn Care Res. Apr 9 2014.
58. Berger MM, Spertini F, Shenkin A, et al. Clinical, immune and metabolic effects of trace element supplements in burns: A double-blind placebo-controlled trial. Clin Nutr. Apr 1996;15(2):94-96.
59. Berger MM, Shenkin A, Spertini F, et al. Large trace element supplements reduce the number of infectious episodes after burns. Trace Elements in Man and Animals - 9. 1997;433-435.
60. Berger MM, Baines M, Raffoul W, et al. Trace element supplementation after major burns modulates antioxidant status and clinical course by way of increased tissue trace element concentrations. Am J Clin Nutr. 2007;85(5):1293-1300.
61. Al-Kaisy AA, Sahib AS, Al-Biati HA. Effect of zinc supplement in the prognosis of burn patients in iraq. Ann Burns Fire Disasters. Sep 30 2006;19(3):115-122.
62. Sahib AS, Al-Jawad FH, Alkaisy AA. Effect of antioxidants on the incidence of wound infection in burn patients. Ann Burns Fire Disasters. Dec 31 2010;23(4):199-205.
63. Nordlund M, Pham TN, Honari SE, et al. Dual enteral and intravenous zinc supplementation in severe burns. J Burn Care Res. 2014;35(Suppl 1)S143.
64. Smith DJ. Use of Biobrane in wound management. J Burn Care Rehabil. 1995;16(3):317-320.
65. The Joanna Briggs Institute. Joanna Briggs Institute Reviewers' Manual: 2014 edition/supplement: Summary of findings tables for Joanna Briggs Institute Systematic Reviews: Adelaide, Australia: The Joanna Briggs Institute; 2014.

66. Langendam MW, Akl EA, Dahm P, et al. Assessing and presenting summaries of evidence in Cochrane Reviews. Syst Rev. 2013;2:81.
67. Guyatt G, Oxman AD, Akl EA, et al. GRADE guidelines: 1. Introduction-GRADE evidence profiles and summary of findings tables. J Clin Epidemiol. Apr 2011;64(4):383-394.
68. Brozek J, Oxman AD, Schunemann H. GRADEprofiler [computer program]. Version 3.6.1; GRADE Working Group. 2011. The Nordic Cochrane Centre, The Cochrane Collaboration, Copenhagen, Denmark, 2014.
69. Huang TS, Shyu YC, Chen HY, et al. Effect of parenteral selenium supplementation in critically ill patients: a systematic review and meta-analysis. PLoS One. 2013;8(1):e54431.
70. Young B, Ott L, Kasarskis E, et al. Zinc supplementation is associated with improved neurologic recovery rate and visceral protein levels of patients with severe closed head injury. J Neurotrauma. Jan 1996;13(1):25-34.
71. Heyland DK, Jones N, Cvijanovich NZ, et al. Zinc supplementation in critically ill patients: A key pharmaconutrient? JPEN. Sep-Oct 2008;32(5):509-519.
72. Berger MM, Shenkin A. Role of trace elements and other antioxidants in the critically ill. Care of the Critically Ill. 2003;19(4):120.
73. Pearson A, Wiechula R, Court A, et al. The JBI model of evidence-based healthcare. Int J Evidence-Based Healthcare. 2005;3(8):207-215.
74. Rechner IJ, Lipman J. The costs of caring for patients in a tertiary referral Australian Intensive Care Unit. Anaesth Intensive Care. Aug 2005;33(4):477-482.
75. Vanderheyden S, Casaer MP, Kesteloot K, et al. Early versus late parenteral nutrition in ICU patients: Cost analysis of the EPaNIC trial. Crit Care. 2012;16(3):R96.
76. Ahuja RB, Goswami P. Cost of providing inpatient burn care in a tertiary, teaching, hospital of North India. Burns. Jun 2013;39(4):558-564.
77. Heyland DK, Dhaliwalm R, Day A, et al. Optimizing the dose of glutamine dipeptides and antioxidants in critically ill patients: A phase I dose-finding study. JPEN. Mar-Apr 2007;31(2):109-118.
78. Committee on Trauma American College of Surgeons. Chapter 14: Guidelines for Trauma Centers Caring for Burn Patients. In: Rotondo MF, Cribari C, Smith RS, eds. Resources for Optimal Care of the Injured Patient; 2014. [Internet]. Available from: https://www.facs.org/~/media/files/quality%20programs/trauma/vrcresources.ashx. Accessed 23 April, 2015.
79. Splett PL. Phase II: Recommendations from experts in health care financing. JADA. 1991;91:S36+.
80. Ivy ME, Possenti PP, Kepros J, et al. Abdominal compartment syndrome in patients with burns. J Burn Care Rehabil. Sep-Oct 1999;20(5):351-353.
81. Oda J, Yamashita K, Inoue T, et al. Resuscitation fluid volume and abdominal compartment syndrome in patients with major burns. Burns. Mar 2006;32(2):151-154.
82. Deitch EA. Role of the gut lymphatic system in multiple organ failure. Curr Opin Crit Care. Apr 2001;7(2):92-98.
83. Heyland DK, Dhaliwal R, Day AG, et al. REducing Deaths due to OXidative Stress (The REDOXS Study): Rationale and study design for a randomized trial of glutamine and antioxidant supplementation in critically-ill patients. Proc Nutr Soc. Aug 2006;65(3):250-263.