Trace element supplementation after major burns modulates antioxidant status and clinical course by way of increased tissue trace element concentrations[1–3]

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ABSTRACT

Background: After major burns, patients can develop nutritional deficiencies including trace element (TE) deficiencies. Various complications, such as infections and delayed wound healing, influence the clinical course of such patients.

Objectives: We aimed to investigate the effects of large, intravenous doses of TE supplements on circulating and cutaneous TE tissue concentrations, on antioxidant status, and on clinical outcome after major burns.

Design: This was a prospective, randomized, placebo-controlled trial in 21 patients aged 35 ± 11 y ($\bar{x}$ ± SD) with burns on 45 ± 21% of their body surface area. Intravenous copper, selenium, and zinc (TE group) or vehicle (V group) was given with a saline solution for 14–21 d. Blood and urine samples were collected until day 20, and skin biopsy specimens were collected on days 3, 10, and 20.

Results: The age of the patients and the severity of their burns did not differ significantly between the groups. Plasma TE concentrations were significantly higher in the TE group. In burned areas, skin contents of both selenium ($P = 0.05$) and zinc ($P = 0.04$) increased significantly by day 20. Plasma and tissue antioxidant status was improved by supplementation. The number of infections in the first 30 d was significantly lower in the TE group ($P = 0.015$), with a median number of 2 versus 4 infections per patient in the TE and V groups, respectively, as a result of a reduction in pulmonary infections ($P = 0.03$). Wound healing was improved in the TE group, with lower requirements for regrafting ($P = 0.02$).

Conclusions: TE supplementation was associated with higher circulating plasma and skin tissue contents of selenium and zinc and improved antioxidant status. These changes were associated with improved clinical outcome, including fewer pulmonary infections and better wound healing. *Am J Clin Nutr* 2007;85:1293–300.

KEY WORDS Critical illness, burns, supplementation, infection, deficiency, requirements, trace elements

INTRODUCTION

Burns involving >20% of the body surface result in extensive inflammatory, endocrine, metabolic, and immune changes. In the most severe cases, the size of the wounds can reach 1.5–2 m^2. The skin is one of the largest organs in humans and accounts for 15% of body weight and 10–25% of whole-body protein turnover (1). Tissue repair and wound closure may last for weeks after burn injury, and closure usually requires extensive surgery and skin grafting (2). Various complications that are exacerbated or favored by nutritional deficiencies can occur, such as infections, delayed wound healing, and muscle wasting (3); infections remain a leading cause of death after major burns (4–6).

Micronutrient deficiencies are frequent after major burns (7). These patients suffer acute trace element depletion as the result of extensive exudative losses (8, 9): copper, selenium, and zinc are particularly depleted, all of which are involved in wound healing and in various aspects of immune defenses. Locally, wound healing requires a coordinated activation and local penetration of various cell types coming from the circulation and surrounding tissues. The simultaneous presence of numerous nutrients is required (10): amino acids, vitamins, trace elements (mainly copper, manganese, selenium, and zinc) (11, 12), and anabolic factors (13). Adequate antioxidant defense is also required (14). Trace elements, especially selenium, which is essential for the activity of glutathione peroxidase (GSHPx), belong to the body's first line of antioxidant defense in both the intra- and the extracellular compartments.

Using large trace element supplements that were designed to meet the increased losses in burn patients, our group showed in previous trials that such interventions are associated with reduced overall infectious complications and shorter lengths of stay in the intensive care unit (ICU) (9, 15). The present trial aimed at further investigating the tissue penetration of early trace element supplements compared with placebo and determining whether an improved status could be associated with beneficial clinical effects, including improved wound healing and a reduction in specific infectious complications after major burns.

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SUBJECTS AND METHODS

The study was designed as a prospective, randomized, placebo-controlled trial and was approved by the institutional ethics committee. It was conducted in the burns unit of the adult Intensive Care Medicine Department of CHUV in Lausanne, Switzerland, a tertiary university hospital. Inclusion criteria were admission within 6 h of injury; age of 16–65 y; burns on >20% of body surface area (BSA), including ≥10% of the BSA burns assessed as 2nd intermediate to deep or 3rd degree on admission; and informed consent. Written consent was requested from the patients themselves or from their next of kin. Exclusion criteria were foreseen imminent death, chronic cardiac failure grade NYHA 3 or 4, preexisting chronic liver disease, severe renal failure (creatinine clearance < 60 mL/min on admission), obesity [body mass index (in kg/m^2) ≥ 30], or pregnancy. For randomization purposes, the 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). The following variables were recorded: age, weight and height, total burned BSA (in %), and percentage of burns requiring surgery. Six healthy subjects undergoing plastic surgery with skin resection served as controls for skin selenium and zinc concentrations: they were otherwise healthy, aged 43 ± 13 y, with a body mass index of 29.0 ± 6.0, and none was taking vitamin or trace element supplements.

Clinical management

The Parkland formula was used for guidance in fluid resuscitation during the first 24 h, and the patients were further resuscitated according to hemodynamic monitoring by using urinary output (target: 1 mL · kg^{-1} · h^{-1}), pulmonary arterial catheters in patients with burns >40% BSA or severe inhalation injury, and additional fluids and vasoactive drugs as required. All patients received ranitidine 3 × 50 mg/d intravenously for ulcer prevention throughout their stay in the ICU.

Intervention

On admission to the ICU, the patients were randomly assigned to receive daily 250 mL of a 0.9% saline solution over 12 h containing either 59 μmol Cu, 4.8 μmol Se, and 574 μmol Zn per day intravenously (TE group) or vehicle (V group) (**Table 1**). Trace element supplements were provided as copper gluconate (powder form), sodium selenite solution, and zinc gluconate solution and were mixed with the multitrace element solution (Decan) (all from Laboratoires Aguettant, Lyon, France); the stability and sterility of the supplements and solutions were verified. The patients received the treatment within 12 h of injury and for 14 d if burned on 20–60% BSA (16 patients) or for 21 d if the burns exceeded 60% BSA (5 patients). Intervention infusion was started by 8:20 ± 4:00 h of injury. Infusion was interrupted early on days 6 and 9 in 2 patients in the TE group because they were transferred out of the ICU; these patients are included in the intent-to-treat analysis.

Nutritional support

Enteral nutrition was by protocol of the burn unit to be started within 12 h of admission through a nasogastric or nasojejunal tube. Feeds were a combination of 3 industrial solutions (Novartis, Basel, Switzerland): Isosource energy (60–70% of energy target), Isosource standard, and Isosource fibers (30–40% of target), which contained normal amounts of trace elements. The mean amounts delivered by this route at full strength feeding are indicated in Table 1. In addition, 1.5–3 L/d 5% dextrose was delivered intravenously for free water provision (additional 75–150 g glucose/d). Intravenous insulin was delivered continuously to maintain blood glucose concentrations between 6 and 10 mmol/L. The energy target was set until day 3 at 30 kcal · kg^{-1} · d^{-1} and was then set at 1.1 times measured resting energy expenditure (REE) by using a Deltatrac metabolic monitor (Datex, Kuopio, Finland) (16). Continuous nutrient delivery was escalated to the target over 4 d. Both groups received daily vitamin supplements: 1 g ascorbic acid, 100 mg thiamine, and a multivitamin preparation delivered intravenously (1 ampoule Soluvit and Vitalipid; Fresenius Kabi, Bad Homburg, Germany), plus 100 mg α-tocopherol delivered via the feeding tube.

TABLE 1
Daily intravenous supplements in the trace element (TE) group and mean quantities provided by the enteral feeds between days 5 and 15 in both groups[1]

	TE group	V group	Daily TE content of feeds	Normal enteral absorption
			mg	*%*
Copper		0	2.7 ± 0.7[2]	30–40
(mg)	3.75			
(μmol)	59			
Selenium		0	90 ± 24	80–85
(μg)	375			
(μmol)	4.8			
Zinc		0	21.5 ± 5.6	20–30
(mg)	37.5			
(μmol)	574			
Glu-1-P		0	—	—
(mmol)	12.5			

[1] V, vehicle; Glu-1-P, glucagon-like peptide 1. Except for selenium, the contribution from the feeds was limited because of poor absorption of trace elements.
[2] $\bar{x}$ ± SD (all such values).

Outcome variables

The length of mechanical ventilation, length of ICU and of hospital stay, and outcome were recorded.

Infections

Those occurring during the first 30 d after admission were recorded: their definition was based on the consensus definition criteria for bacteriemia and urine infection (17). Pulmonary infections were defined as the conjunction of a new infiltrate on the chest X-ray, clinical signs of infection, microorganisms in the protected bronchoalveolar lavage fluid, and decision to treat with antibiotics. Skin infection was considered in case of a positive skin biopsy with >1 × 10^5 microorganisms associated with signs of local or systemic infection. To distinguish between contamination and infection with identified microorganisms, only those infections considered to require a new antibiotic were counted; days of antibiotherapy were recorded. Surgical antibiotic prophylaxis was limited to 24 h of either 2 × 1 g/d cefazolin or 3 × 1.2 g/d amoxicillin + clavulanic acid.

Wound management

Early surgical scar excision was considered in all cases. Debridement was initiated between days 2 and 4, starting with the areas that were assessed as third degree burns. Surgery included 5–20% BSA per session and was completed stepwise. The debrided wounds were reconstructed with meshed homografts and with autologous cultured keratinocytes in 7 patients with burns of >50% BSA (cultured at the CHUV dermatology laboratory according to validated procedures; 18). Wounds were covered with paraffin gauze, dry cotton gauze (Kerlix, Appli-set; Applimed, Chatel-St-Denis, Switerland), and elastic bandages. The dressings were kept in place for 5 d; thereafter, daily hydrotherapy and bandaging were performed until complete wound closure. Wound healing was assessed by comparing the total surface requiring surgery and the sum of the surface effectively grafted over the various procedures (grafting index = ratio between the total surface grafted and the total body surface requiring surgery).

Sampling

Blood samples were collected on days 0, 1, 2, 5, 10, 15, and 20. They were separated within 1 h of collection, centrifuged, divided into aliquots, and frozen. Twenty-four–hour urine samples were collected on days 5, 10, and 15 for the determination of urea, creatinine, 3-methylhistidine, and trace elements. Skin biopsy samples (2×1 cm^2 excised during the surgical procedures from burned areas and from healthy skin donor areas) were obtained in 12 patients with the largest burns on days 2 ± 1 (ie, after 2–3 d of supplementation), 10 ± 1, and 20 ± 1 after injury for measurement of tissue selenium, zinc, glutathione, GSHPx, and glutathione reductase (GR). Skin biopsy specimens were immediately frozen in liquid nitrogen. All frozen samples were kept at −80 °C until analyzed.

Laboratory determinations

Copper, zinc, and selenium in plasma and zinc and selenium in skin were measured in duplicate by inductively coupled plasma mass spectrometry (Plasmaquad 3 ICP-MS; VG Elemental, Winsford, Cheshire, United Kingdom) with aqueous inorganic standards (19). All plasma specimens were diluted in 1% nitric acid/0.2% *n*-butanol/0.2% *n*-propanol and 10 ppb indium as an internal standard. Skin samples were dried, weighed, digested for 2 h at 90 °C in 70% concentrated nitric acid/30% hydrogen peroxide, and made up to 5 mL with ultrapure water before analysis as for plasma. Iron status was determined once on day 20. Serum iron was measured spectrophotometrically by using ferrozine; transferrin and ferritin were measured by immunoturbidimetry. Plasma GSHPx was measured by the RANSEL method (Randox Laboratories, Belfast, United Kingdom). Erythrocyte superoxide dismutase was measured by the RANSOD method (Randox Laboratories; reference range: 1025–1525 U/g hemoglobin), and erythrocyte GSHPx was determined by the RANSEL method (Randox Laboratories; reference range: 24–86 U/g hemoglobin); both were measured on a Falcor 300 analyzer (A Menarini, Florence, Italy). Total antioxidant status was measured on a RA100 analyzer (Bayer UK, Berks, United Kingdom), by the degree of suppression of the stable radical 2,2'-azinodi-3-ethylbenzothiazoline sulphonate (ATBS; Randox Laboratories) (20). Interleukin (IL)-6 and IL-10 were measured in plasma samples by using ELISA kits from R&D Systems (Abingdon, United Kingdom). α-Tocopherol and retinol were measured by reversed-phase HPLC after hexane extraction with the use of retinol acetate as an internal standard. Plasma C-reactive protein, albumin, uric acid, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, and leukocyte counts were carried out by using standard methods. Skin GSHPx, GR, and glutathione methods are described in detail elsewhere (21).

Statistical analysis

The results are expressed as means ± SDs or as medians and ranges. Baseline variables in the 2 groups were compared by using one-way ANOVA. Changes over time were compared by using 2-factor repeated-measures ANOVA for the effects of group (vehicle or trace element), time, and their interaction (group × time). Post hoc comparisons were carried out by Dunnet's (effect of time versus baseline in each group), or Scheffe's test (between-group comparisons at the same time point), where appropriate. Chi-square tests were used for nonparametric variables. Significance was considered at the level of $P < 0.05$, whereas trends were considered up to $P = 0.10$. The statistical package used was JMP version 5.5 (SAS Institute Inc, Cary, NC).

RESULTS

A total of 21 patients were enrolled in the study (**Table 2**); the characteristics of the patients did not differ significantly between the groups. Skin biopsy specimens were available for 12 of the 21 patients (7 in the V group, 5 in the TE group). Only the most severely burned patients who were considered to require surgery on repeated occasions beyond day 14 were enrolled in this part of the study; they suffered burns affecting 51 ± 23% BSA, of which 43 ± 22% BSA required surgical treatment; 6 of these (3 per group) had inhalation injury.

Indirect calorimetry determinations of REE were compared with the Harris & Benedict equation; the mean predicted REE was 1690 ± 336 kcal/d. Measured REE did not differ significantly between the groups and was 2233 ± 532 kcal/d on day 2 (132% of predicted), 2745 ± 601 kcal/d on day 10 (162% of predicted), and 2534 ± 552 kcal/d on day 20 (149% of predicted). This corresponded to 28 ± 6, 35 ± 8, and 34 ± 6 kcal · kg^{-1} · d^{-1}, respectively.

Enteral feeding was started by protocol within 16 h of admission in all but one patient in the TE group who was fed orally. The patients received 2130 ± 910 kcal/d by day 5, 2475 ± 1000 kcal/d by day 10, and 2685 ± 1000 kcal/d by day 20 with no significant difference between the groups.

Plasma copper, selenium, zinc, and GSHPx concentrations were significantly higher after day 5 in the treatment group; the time × group interaction was highly significant, except for zinc. The results of post hoc comparisons are depicted in **Figure 1**. There were no significant differences in plasma retinol and tocopherol, C-reactive protein, IL-6, IL-10, total antioxidant status, albumin (low from day 1), liver test results, or erythrocyte SOD and GSHPx between the groups (**Table 3**). In contrast with plasma GSHPx, which increased, erythrocyte GSHPx did not increase in response to supplementation. Iron biochemistry on day 20 did not differ significantly between the groups (**Table 4**).

Concerning skin trace elements, selenium and zinc concentrations in the V group in both burned and healthy skin remained stable over time and did not differ significantly in healthy and burned areas but and were significantly lower than in the TE

TABLE 2
Patient characteristics in the vehicle (V) and trace element (TE) groups[1]

	V group (n = 10)	TE group (n = 11)	P
Sex (F/M)	4/6	2/9	NS
Age (y)	38 ± 16[2]	46 ± 15	NS
Burned BSA (%)	44 ± 20 (20–85)[3]	45 ± 22 (16–92)	NS
Surgical BSA (%)	34 ± 16 (14–65)	31 ± 30 (0–90)	NS
Inhalation (n)	4	5	NS
Burn index (age + BSA)	83 ± 21	91 ± 22	NS
ABSI	9 (6–13)[4]	9 (4–15)	NS
Ryan score (median)	1	1	NS
SAPS II	29 (21–45)	35 (22–62)	NS
Patients with skin biopsy samples (n)	7/10	5/11	NS
Grafting index (% BSA operated per % BSA requiring surgery)	144 ± 45	94 ± 41	0.02
Length of mechanical ventilation (d)	12 ± 6	7 ± 6	NS (0.12)
Length of ICU stay (d)	47 ± 37	35 ± 27	NS
Median (range)	38 (16–145)	23 (6–91)	
Length of ICU stay/% burned BSA	1.2 ± 0.7	0.8 ± 0.4	NS (0.11)
Deaths (n)	1/10	1/11	NS

[1] BSA, body surface area; ABSI, abbreviated burn severity index; SAPS, simplified acute physiology score; ICU, intensive care unit. Analysis by one-way ANOVA.
[2] $\bar{x}$ ± SD (all such values).
[3] Range in parentheses (all such values).
[4] Median; range in parentheses (all such values).

group (Se: $P = 0.001$, Zn: $P = 0.08$; **Table 5**). Comparison of concentrations on day 3 in burned and healthy skin combined showed significantly higher selenium concentrations in the TE group ($P = 0.01$), whereas zinc content did not differ significantly between groups. In the TE group, selenium and zinc concentrations increased in the burned areas, and the change was significant by day 20 (Se: $P = 0.05$; Zn: $P = 0.004$), corresponding with a normalization of skin content.

Concentrations of skin glutathione, GSHPx, and GR differed in burned and healthy skin areas. On day 3, the healthy donor area content was significantly higher than that of the burned areas (**Figure 2**): glutathione, 14.6 ± 10.3 compared with 4.2 ± 3.8 mU/mg protein ($P = 0.007$); GSHPx, 36.2 ± 12.6 compared with 16.9 ± 10.3 mU/mg protein ($P = 0.0008$); and GR, 31.1 ± 15.6 compared with 10.6 ± 6.8 mU/mg protein ($P = 0.0007$). Changes over time were not significant. In the healthy donor skin area, there was no significant difference at day 3 between the V and TE groups; in the burned area, GR was significantly lower at day 3 ($P = 0.016$), whereas glutathione and GSHPx did not differ significantly. By contrast with healthy donor skin, in the burned areas, the increase over time was significant for both glutathione ($P = 0.0006$), GR ($P = 0.019$), and GSHPx $P = 0.028$. In the TE groups, all 3 enzymes increased significantly more.

The length of mechanical ventilation, of ICU stay, and of hospital stay, as well as the length of stay in the ICU per %BSA did not differ significantly (Table 2). There were 2 deaths, one in each group.

Wound healing was better in the TE group as shown by the lower grafting requirements (Table 2). Seven patients with burns covering >50% BSA (range: 50–92%) received keratinocyte cultures (mean: 7337 cm^2; range: 1140 to 22 020 cm^2). Two patients in the TE group required less surgery than expected according to the admission assessment, which was carried out by the same surgeon (WR). The first patient did not require any

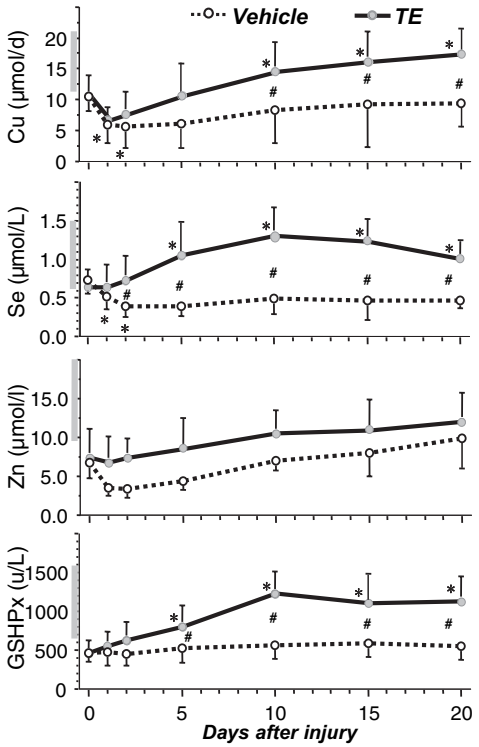

FIGURE 1. Mean (±SD) plasma copper, selenium, zinc, and glutathione peroxidase (GSHPx) concentrations in the vehicle (n = 10) and trace element (TE; n = 11) groups. The thick gray vertical bar on the left of each graph shows the reference ranges. By 2-factor repeated-measures ANOVA, the changes over time were significantly different between groups, the interaction (time × group) being significant except for zinc, as follows: copper ($P = 0.013$), selenium ($P < 0.0001$), and GSHPx ($P < 0.0001$). Post hoc comparisons: *significantly different from baseline within groups (Dunnett's test); #significantly different between groups at the same time (Scheffe's test).

TABLE 3
Circulating plasma vitamins, inflammatory indicators, and erythrocyte antioxidant enzymes in the vehicle (V) and trace element (TE) groups[1]

Variable and group	Day 0	Day 1	Day 2	Day 5	Day 10	Day 15	Day 20	Reference range	*P* for time effect
Retinol								1.5–4.3 μmol/L	<0.0001
V group	1.75 ± 0.62	0.88 ± 0.49	0.46 ± 0.31	0.61 ± 0.40	1.04 ± 0.55	1.84 ± 1.10	2.30 ± 0.93		
TE group	1.64 ± 0.86	0.93 ± 0.53	0.44 ± 0.28	0.62 ± 0.51	1.01 ± 0.72	1.34 ± 0.91	1.71 ± 0.66		
α-Tocopherol								12–46 μmol/L	<0.0001
V group	23.5 ± 8.7	18.3 ± 7.8	17.4 ± 9.1	26.2 ± 9.1	32.1 ± 13.1	32.4 ± 11.3	34.8 ± 7.4		
TE group	20.3 ± 11.6	16.5 ± 8.2	1.38 ± 7.9	27.3 ± 11.7	35.3 ± 16.8	29.0 ± 8.7	35.3 ± 9.4		
TAS								1117–1855 U/L	0.047
V group	1128 ± 200	10 390 ± 286	925 ± 314	949 ± 305	1054 ± 298	1006 ± 246	1130 ± 229		
TE group	1128 ± 200	876 ± 361	811 ± 254	994 ± 321	926 ± 432	986 ± 448	1002 ± 243		
CRP								<10 mg/L	<0.0001
V group	2 ± 3	87 ± 59	172 ± 59	204 ± 66	265 ± 96	167 ± 90	112 ± 51		
TE group	2 ± 2	81 ± 65	177 ± 51	172 ± 59	183 ± 54	180 ± 67	161 ± 95		
IL-6								<10 pg/mL	NS
V group	122 ± 99	353 ± 338	585 ± 908	178 ± 133	937 ± 2770	171 ± 379	56 ± 41		
TE group	227 ± 280	568 ± 487	696 ± 807	206 ± 184	126 ± 66	124 ± 204	112 ± 171		
IL-10								<8 pg/mL	NS
V group	39.5 ± 41.8	28.2 ± 37.8	25.0 ± 19.2	15.9 ± 11.2	56.9 ± 143.3	44.8 ± 72.8	17.0 ± 21.5		
TE group	79.1 ± 108.1	60.6 ± 58.9	54.6 ± 115.6	20.7 ± 13.2	22.5 ± 30.2	12.5 ± 5.6	22.2 ± 24.4		
AP								11–62 IU/L	<0.0001
V group	78 ± 42	56 ± 43	52 ± 28	68 ± 19	137 ± 61	171 ± 145	164 ± 48		
TE group	50 ± 19	31 ± 13	42 ± 11	94 ± 46	117 ± 42	121 ± 61	154 ± 40		
E-GSHPX								24–86 U/g Hb	<0.0001
V group	70.7 ± 25.9	73.3 ± 25.5	70.7 ± 26.3	59.7 ± 13.5	62.7 ± 14.0	59.2 ± 14.3	56.1 ± 10.2		
TE group	59.6 ± 9.7	60.7 ± 6.9	60.6 ± 6.9	55.3 ± 7.1	53.0 ± 7.5	52.5 ± 9.0	56.2 ± 4.5		
E-SOD								1025–1525 U/g Hb	NS
V group	1437 ± 290	1524 ± 329	1505 ± 358	1386 ± 215	1378 ± 219	1482 ± 303	1325 ± 284		
TE group	1573 ± 254	1577 ± 210	1486 ± 234	1392 ± 195	1480 ± 232	1480 ± 232	1527 ± 460		

[1] All values are $\bar{x}$ ± SD. TAS, total antioxidant status; CRP, C-reactive protein; IL, interleukin; AP, alkaline phosphatase; E-GSHPX, erythrocyte glutathione peroxidase; E-SOD, erythrocyte superoxide dismutase. Two-factor repeated-measures ANOVA for the effect of group, time, and time × group interaction was applied; only the time effect was significant in both groups for the variables indicated on the left.

surgery (spontaneous wound healing) and the second required only 2% grafting (included in intent-to-treat analysis).

Compared with that in the V group, there were significantly fewer total infectious complications in the TE group in the first 30 d, with a median of 2 versus 4 infections per patient. This was primarily related to a lower number of pulmonary infections (**Table 6**).

In the TE group, urinary excretion was significantly higher than in the V group for selenium and zinc ($P < 0.0001$) through days 5, 10, and 15, whereas copper was not ($P = 0.066$). The mean daily excretion in groups TE and V was as follows: selenium, 2.72 ± 1.33 compared with 0.84 ± 0.25 μmol/d; zinc, 87.1 ± 26.3 compared with 20.6 ± 11.6 μmol/d; copper, 4.38 ± 7.11 compared with 0.85 ± 0.45 μmol/d. In the TE group, urinary excretions were 56% (Se), 15% (Zn), and 7.5% (Cu) of the supplementation doses.

DISCUSSION

The results of the present randomized controlled trial confirm and expand on the main results of our previous 2 studies (9, 15). Trace element supplements were associated with early normalization of the low plasma trace element concentrations usually observed in burned patients during the first weeks and improved antioxidant status as shown by the normalization of plasma GSHPx. These changes were associated with a significantly improved clinical course (better graft take, fewer infectious complications), which was reflected by a nonsignificantly shorter ICU stay.

Infectious complications are the main cause of death in patients with major burns (4), cutaneous and pulmonary complications being the most prominent. Supplementation was associated with a significant reduction in infectious complications,

TABLE 4
Circulating plasma indicators of iron status in the vehicle (V) and trace element (TE) groups[1]

Day 20	Iron	Ferritin	Transferrin	Iron-binding capacity
	μmol/L	*μg/L*	*μmol/L*	*%*
Reference value range	12.5–25.1	30–300	23–43	20–40
Mean of V + TE groups[2]	4.6 ± 2.6	478 ± 347	18 ± 6	13 ± 7

[1] No significant differences between groups were found.
[2] $\bar{x}$ ± SD; $n = 21$.

TABLE 5
Trace element concentration in skin biopsy samples from patients and controls[1]

	Day 3	Day 10	Day 20
Healthy skin (donor)			
Selenium (nmol/g dry wt)			
V group ($n = 7$)	6.36 ± 2.17	6.90 ± 3.13	7.04 ± 5.60
TE group ($n = 5$)	10.67 ± 6.78	11.95 ± 8.63	9.55 ± 2.42
Control group ($n = 6$)			15.14 ± 4.95
Zinc (nmol/g dry wt)			
V group	577.7 ± 164.9	472.2 ± 251.6	672.5 ± 554.8
TE group	961.8 ± 1075.4	622.9 ± 1028.0	948.8 ± 581.7
Control group	—	—	1040.8 ± 690.3
Burned skin			
Selenium			
V group ($n = 7$)	6.70 ± 2.34[2]	6.47 ± 3.35	8.05 ± 2.67
TE group ($n = 5$)	10.8 ± 3.70[2]	7.50 ± 3.07	18.68 ± 12.37[3]
Zinc			
V group	591.1 ± 288.3	592.7 ± 240.8	416.7 ± 190.3
TE group	406.7 ± 157.9	408.2 ± 327.0	1180.4 ± 774.3

[1] All values are $\bar{x}$ ± SD. The values on day 3 are not true baseline values and probably already reflect the effect of supplements: pooled healthy and burned skin selenium content was already higher in the TE group (10.77 ± 5.21 compared with 6.52 ± 2.17 nmol/g dry wt, $P = 0.010$), whereas pooled zinc content values were not (684.1 ± 788.1 compared with 584.4 ± 225.8 nmol/g dry wt). Two-factor repeated-measures ANOVA in burned skin: selenium ($P = 0.05$), zinc ($P = 0.004$).
[2] Significant difference between groups at the same time (Scheffe test).
[3] Significantly different from baseline value (Dunnett test).

especially bronchopneumonia, as in our previous trials (9, 15). In the 1998 trial, the main criticism regarding the effect of supplements on pulmonary infections was that the TE group suffered more inhalation injuries than did the control group, which might have favored early bronchopneumonia. The present trial was stratified for inhalation injury and confirmed the beneficial effect of the trace element supplements. The number of skin infections was unchanged as in the previous trial. Why should the trace elements reduce the pulmonary infections more than the other types of infection? One hypothesis is that because the trace elements were delivered intravenously by a central venous catheter, the first organ to receive them was the lung. The trace element dose might have been just sufficient to improve the antioxidant and immune defense for the lung, but insufficient to achieve a cutaneous immune effect. Furthermore, the skin is exposed to continuous microorganism contamination, which is not the case in the lung.

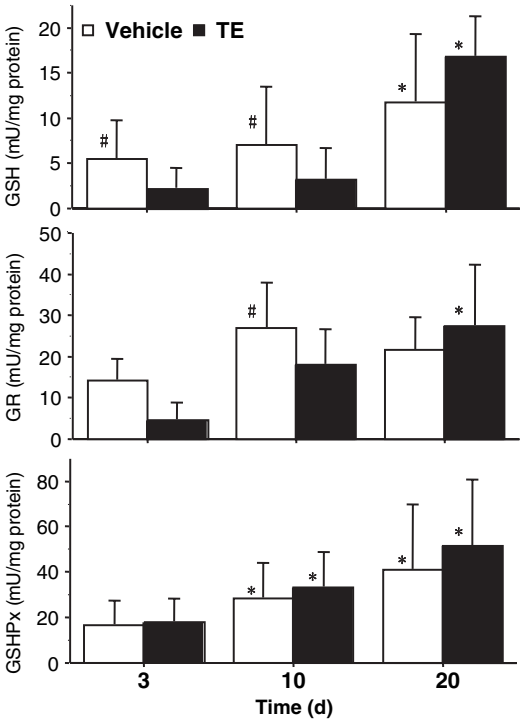

FIGURE 2. Mean (±SD) changes in glutathione (GSH), glutathione reductase (GR), and glutathione peroxidase (GSHPx) in burned skin in the vehicle ($n = 7$) and trace element (TE; $n = 7$) groups. By 2-factor repeated measures ANOVA, the interaction (time × group) was significant for all variables: glutathione ($P = 0.006$), GR ($P = 0.019$), and GSHPx ($P = 0.028$). *Significantly different from baseline within groups. #Significantly different between groups at the same time.

Wound healing is a major issue in burns, and delayed healing with graft failure is a serious problem. Trace elements directly affect most metabolic pathways. Copper is essential for wound repair; indeed, lysyl oxidases are extracellular copper enzymes that initiate the cross-linking of collagen and elastin, and their activities decline with inadequate copper status (22, 23). Zinc is required for most anabolic pathways, and deficiency has negative effect on wound healing in nonburn conditions (24). In one of our previous small trials, there was already a suggestion that copper, selenium, and zinc supplements were associated with a beneficial effect on wound healing (9), with a reduction of regrafting requirements. In the present study, higher tissue selenium concentrations were observed in both healthy and burned skin areas after 3 d of supplementation, the concentrations being stable or further increasing thereafter. The higher skin selenium concentration was correlated with the dose delivered during the first 2–3 d. We lacked a basal sample on day 0, because no biopsy could be performed for ethical reasons at that time point. If such a sample had been available, the differences between groups over time would probably have been even more significant. Compared with skin concentrations in healthy volunteers, trace

TABLE 6
Number and location of infectious episodes in the vehicle (V) and trace element (TE) groups[1]

Infection (no. and location)	V group (n = 10)	TE group (n = 11)	P value
Infection episodes in 30 d (n = 59)	36 (1–5)[2]	23 (1–4)	0.015
Infections (mean per patient) (n = 2.8)	3.6 ± 1.3 (4)[3]	2.1 ± 1.0 (2)	0.01
Pulmonary (n = 27)	18 (0–3)	9 (0–2)	0.03
Cutaneous (n = 19)	10 (0–2)	9 (0–3)	NS
Urinary (n = 4)	2 (0–1)	2 (0–1)	NS
Bacteremia (n = 5)	3 (0–2)	2 (0–1)	NS
Other (n = 3)	3 (0–1)	1 (0–1)	NS
Days on antibiotics in 30 d	17.1 ± 5.9 (18)	13.5 ± 8.1 (12)	NS

[1] The total infection rate was lower in the TE group, mainly because of a reduction in the number of pulmonary infections. The lower number of days on antibiotics was not significant. Analysis by chi-square test.
[2] n; range in parentheses (all such values).
[3] $\bar{x}$ ± SD; median in parentheses (all such values).

element concentrations nearly normalized in the patients in the TE group by day 20, whereas they remained very low in the vehicle group. Similar tissue results were observed for zinc: selenium and zinc concentrations increased significantly in the TE group. Of major potential importance was the observation that confirmed our previous result; namely, that intravenous trace element supplements improved wound healing after major burns, the increased uptake into the burned area being associated with a reduction in skin grafting requirements and a significantly lower regrafting index.

The evolution over time of the skin's antioxidant enzymes in the burned areas differed from that in the healthy donor skin. The concentrations and activities of glutathione reductase, GSHPx, and glutathione increased significantly in the burned areas in the supplemented group by day 20, which contrasted with the lack of such an effect in the healthy donor areas.

The skin trace element and enzymatic data presented here are totally new in burns, and no other trials are available for comparison. We observed a different trace element and enzymatic behavior in burned and nonburned healthy skin sites (donor sites). The concentrations in the TE group were significantly higher by day 20 in areas that healed better as reflected by lower regrafting requirements: this supports the concept that reinforcing antioxidant defenses, or restoring them in the case of acute depletion, is beneficial for wound healing.

Lipid peroxidation in the wounds is considered partly responsible for secondary damage to distant tissues after burn injuries (25). Early wound excision has been advocated on this basis to reduce peroxide delivery. We previously showed that urinary malondialdehyde excretion was lower in trace element–supplemented patients than in controls (26), reflecting lower lipid peroxidation. This hypothesis is supported by evidence from animal trials. In selenium-depleted rats, oxidative stress after injury is increased (27), and selenium supplements only partially reverse the condition. Trace elements may therefore have a favorable effect on wound healing through improved antioxidant mechanisms as well as through effects on anabolic pathways. In addition, by reinforcing systemic antioxidant defenses and improving trace element status, trace elements improve immune function and reduce exposure to microorganisms.

The amounts of copper, selenium, and zinc delivered by the intravenous route to the TE group were significantly larger than those recommended by the American Burn Association (28) and those recommended during parenteral nutrition (29); the amounts used aimed at substituting for the acute exudative losses. They correspond to 2.5, 5, and 7.5 times the parenteral nutrition doses for copper, selenium, and zinc, respectively, being much higher than the small standard amounts delivered by identical enteral feeding in both groups. Our supplements were designed to prevent the depletion that is observed after major burns by substituting for losses, thereby leaving the body able to activate normal defense mechanisms. No toxicity was observed.

Determining the trace element requirements in patients with major burns is difficult (30). The quantities used in the present trial were based on balance studies carried out in 10 patients with burns covering 33% BSA (8, 9), whereas the present patients had larger burns (45% BSA). These needs are not nutritional requirements but purely substitution requirements (31). Interpretation of plasma concentrations in the post-trauma period is difficult (32), but nonetheless, the absence of any supernormal copper, selenium, or zinc plasma concentration; the normalization of plasma GSHPx activity; and satisfactory clinical progress are arguments in favor of safety. It is even possible that the substitution doses were too low in the patients with larger burns. Nevertheless, clinicians should be cautious about further increasing the doses; trace element may be toxic in some situations as shown by studies of home total parenteral nutrition and in cholestasis (33). In the present case, the risk of toxicity was limited by the short duration of the supplementation, which corresponded roughly to the duration of absence of a skin barrier, ie, to the period of massive exudative losses.

The present study was not designed as a balance study, but we have indirect evidence of retention of the supplements. Selenium is normally largely excreted in the urine: in the TE group, a mean of 2.7 μmol was lost per day (ie, 3 times more than in the control group), but this was much less than the 4.8 μmol that was delivered in the supplements. Copper and zinc are mainly excreted in the feces, and only a small part is excreted in the urine. Indeed, urinary losses were much smaller than the doses delivered. Regarding safety, copper delivery was monitored through liver function tests, and the results of these did not differ significantly between the groups, as shown in Table 3.

Concerning study limitations, the small number of patients was of course the most important limitation and is typical of most burn studies. Nevertheless, the clinical results observed confirm our 2 previous trials. This limitation is particularly relevant for

the skin biopsy samples, which were only available for only 12 of 21 patients, thus limiting the study's power. Despite the small numbers of subjects, however, both trends toward consistent changes and significant differences in clinical endpoints were observed. All data converged toward trace element–mediated restoration of antioxidant defenses and the improved trace element status being beneficial; no result contradicted the hypothesis.

We conclude that large, early trace element supplementation combining copper, selenium, and zinc is safe and beneficial after major burns. Clinical benefits were a reduction in the number of infectious complications (mainly pneumonia), better wound healing as shown by the lower skin grafting requirements, and a nonsignificantly shorter treatment time in the ICU. Large multicenter trials are required to confirm these encouraging data.

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The study was done on the institutional time of the investigators. The authors performed the analysis of the samples, the statistical evaluation of the data, and prepared the manuscript independently. The contributions of the authors were as follows—MMB: study design, clinical supervision, data collection, statistical outwork, data interpretation, and redaction of manuscript; M Baines: laboratory method development, data interpretation, and redaction of manuscript; WR, study design, clinical supervision, data collection, data interpretation, and redaction of manuscript; M Benathan, laboratory method development, data interpretation, and redaction of manuscript; RLC, study design, data interpretation, and redaction of manuscript; CR, laboratory method development, data interpretation, and redaction of manuscript; J-PR, study design, statistical outwork, data interpretation, and redaction of manuscript; M-CC, study design, clinical supervision, data collection, and data interpretation; IS, laboratory method development and data interpretation; AS, study design, data interpretation, and redaction of manuscript. None of the authors had any conflicts of interest (eg, bonds, economical implication in the industry).

REFERENCES

1. Zhang XJ, Sakurai Y, Wolfe RR. An animal model for measurement of protein metabolism in the skin. Surgery 1996;119:326–32.
2. Sheridan RL. Burn care: results of technical and organizational progress. JAMA 2003;290:719–22.
3. Herndon DN, Wolf SE, Chinkes DL, Wolfe RR. Reversal of catabolism by beta-blockade after sever burns. N Engl J Med 2001;345:1223–9.
4. Murphy KD, Lee JO, Herndon DN. Current pharmacotherapy for the treatment of severe burns. Expert Opin Pharmacother 2003;4:369–84.
5. Peck MD, Weber JM, McManus A, Sheridan RL, Heimbach D. Surveillance of burn wound infections: a proposal for definitions. J Burn Care Rehabil 1998;19:386–9.
6. Wilkinson RA, Fishman JA. Effect of thermal injury with *Pseudomonas aeruginosa* infection on pulmonary and systemic bacterial clearance. J Trauma 1999;47:912–7.
7. Berger MM, Shenkin A. Trace elements in trauma and burns. Curr Opin Clin Nutr Metab Care 1998;1:513–7.
8. Berger MM, Cavadini C, Bart A, et al. Cutaneous zinc and copper losses in burns. Burns 1992;18:373–80.
9. Berger MM, Cavadini C, Chioléro R, Guinchard S, Krupp S, Dirren H. Influence of large intakes of trace elements on recovery after major burns. Nutrition 1994;10:327–34.
10. Peck MD, Chang Y. Nutritional support for burn injuries. J Nutr Bioch 1999;10:380–96.
11. Meyer NA, Muller MJ, Herndon DN. Nutrient support of the healing wound. New Horizons 1994;2:202–14.
12. Tenaud I, Sainte-Marie I, Jumbou O, Litoux P, Dréno B. In vitro modulation of keratinocyte wound healing integrins by zinc, copper and manganese. Br J Dermatol 1999;140:26–34.
13. Martin P. Wound healing—aiming for perfect skin regeneration. Science 1997;276:75–81.
14. Åsman B, Wijkander P, Hjerpe A. Reduction of collagen degradation in experimental granulation tissue by vitamin E and selenium. J Clin Periodontol 1994;21:45–7.
15. 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 1998;68:365–71.
16. Breitenstein E, Chioléro RL, Jéquier E, Dayer P, Krupp S, Schutz Y. Effects of beta-blockade on energy metabolism following burns. Burns 1990;16:259–64.
17. American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference: definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. Crit Care Med 1992;20:864–74.
18. Vernez M, Raffoul W, Gailloud-Matthieu MC, et al. Quantitative assessment of cell viability and apoptosis in cultured epidermal autografts: application to burn therapy. Int J Artif Org 2003;26:793–803.
19. Forrer R, Gautschi K, Stroh A, Lutz H. Direct determination of selenium and other trace elements in serum samples by ICP-MS. J Trace Elem Med Biol 1999;12:240–7.
20. Miller NJ, Rice-Evans C, Davies MJ, Gopinathan V, Milner A. A novel method for measuring antioxidant capacity and its application to monitoring antioxidant status in premature neonates. Clin Sci 1993;84:407–12.
21. 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. Am J Clin Nutr 2007;85:1301–6.
22. Jonas J, Burns J, Abel EW, Cresswell MJ, Strain JJ, Paterson CR. Impaired mechanical strength of bone in experimental copper deficiency. Ann Nutr Metab 1993;37:245–52.
23. Rucker RB, Kosonen T, Clegg MS, et al. Copper, lysyl oxidase, and extracellular matrix protein cross-linking. Am J Clin Nutr 1998;67 (suppl):S996–1002.
24. Schwartz JR, Marsh RG, Draelos ZD. Zinc and skin health: overview of physiology and pharmacology. Dermatol Surg 2005;31:837–47.
25. Demling RH, Picard L, Campbell C, Lalonde C. Relationship of burn-induced lung lipid peroxidation on the degree of injury after smoke inhalation and body burn. Crit Care Med 1993;21:1935–43.
26. Berger MM, Chioléro R. Relations between copper, zinc and selenium intakes and malondialdehyde excretion after major burns. Burns 1995; 21:507–12.
27. Agay D, Sandre C, Ducros V, et al. Optimization of selenium status by a single intraperitoneal injection of Se in Se-deficient rat: possible application to burned patient treatment. Free Radic Biol Med 2005;39: 762–8.
28. Peck M. Initial nutritional support in burn patients. American Burn Association Guidelines. J Burn Care Rehabil 2001;22:595–665.
29. Shenkin A. Micronutrients and antioxidants in home parenteral nutrition. Clin Nutr 2001;20(suppl):47–50.
30. Voruganti VS, Klein GL, Lu HX, Thomas S, Freeland-Graves JH, Herndon DN. Impaired zinc and copper status in children with burn injuries: need to reassess nutritional requirements. Burns 2005;31: 711–6.
31. Berger MM. Acute copper and zinc deficiency due to exudative losses—substitution versus nutritional requirements. Burns 2005;31:711–6.
32. Shenkin A. Trace elements and inflammatory response: implications for nutritional support. Nutrition 1995;11:100–5.
33. Reynolds N, Blumsohn A, Baxter JP, Houston G, Pennington CR. Manganese requirement and toxicity in patients on home parenteral nutrition. Clin Nutr 1998;17:227–30.