Trace element supplementation after major burns increases burned skin trace element concentrations and modulates local protein metabolism but not whole-body substrate metabolism[1–3]

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ABSTRACT

Background: After major burns, patients exhibit an intense catabolism, and the wounds require surgery and grafting for closure. Complications, such as weight loss and delayed wound healing, are worsened by trace element (TE) deficiencies.

Objective: We aimed to assess the effects of TE supplements on systemic substrate turnover and local protein metabolism during wound healing after major burns.

Design: This was a prospective, randomized, placebo-controlled trial in 21 patients aged 35 ± 11 y with burns on 45 ± 16% of their body surface area; 12 had skin biopsies performed on days 3, 10, and 20, and 10 patients underwent a stable-isotope investigation on day 10. Intravenous copper, selenium, and zinc (TE group) or vehicle (V group) was given with a saline solution for 14–21 d. On day 10, [^{13}C]phenylalanine (600-μg/kg bolus followed by 12 $\mu g \cdot kg^{-1} \cdot min^{-1}$) plus 6-[$^{2}H_2$]glucose and [$^{2}H_5$]glycerol were infused for 6 h to determine skin protein turnover. Biopsies were performed 1 and 6 h after the start of infusion to determine [^{13}C]phenylalanine enrichment.

Results: The patients' mean age and burn severity did not differ significantly between the groups nor between the skin investigations subgroups. Plasma TE concentrations were significantly higher in the TE group. In the burned areas, the skin contents of selenium ($P = 0.02$) and zinc ($P = 0.03$) increased by day 20. The supernatant-to-plasma ^{13}C enrichment ratio in burned skin was 0.363 ± 0.094 (TE group) and 0.286 ± 0.130 (V group) after 1 h (NS) and 0.592 ± 0.153 (TE group) and 0.262 ± 0.171 (V group) after 6 h, which reflected lower catabolism in the TE group ($P = 0.03$). No significant differences in whole-body substrate turnover were found between the groups.

Conclusion: TE supplementation was associated with an increased skin tissue content of selenium and zinc and with a reduction in skin protein catabolism. *Am J Clin Nutr* 2007;85:1301–6.

KEY WORDS Critical illness, burns, supplementation, protein turnover, wound healing, trace elements

INTRODUCTION

The skin is the largest organ in humans and accounts for 15% of body weight and 10–25% of whole-body protein turnover in animals (1). Burns involving >20% of the body surface area (BSA) result in extensive inflammatory, endocrine, metabolic, and immune responses. These lead to substantial changes in body composition and tissue function. Tissue repair and wound closure may last for weeks and usually require extensive surgery and skin grafting (2, 3).

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: substrates, vitamins, trace elements (mainly copper, selenium, and zinc) (4), and anabolic factors. Adequate antioxidant defense is also required (5). Micronutrient deficiencies are frequent after major burns (6); burn patients have acute trace element depletion as the result of extensive exudative losses (7, 8). Using trace element supplements, our group showed previously that such interventions are associated with reduced infectious complications and a shorter length of stay in the intensive care unit (8, 9).

The present trial aimed at further investigating the effects of early, large trace element supplementation. Our first report (10) examined the tissue penetration of trace elements and determined whether improved status was associated with beneficial clinical effects, including improved wound healing after major burns. This second report focuses on the effects on local and systemic antioxidant status and substrate turnover.

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 Intensive Care Medicine Department of CHUV in Lausanne, a

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university hospital. Inclusion criteria were admission within 6 h of injury; age 16–65 y; burns covering >20% BSA, including ≥10% BSA assessed as surgical on admission; and written informed consent (from the patients themselves or from their next of kin). Healthy patients undergoing plastic surgery served as controls. For the detailed investigation protocol, inclusion criteria, and clinical management, *see* reference 10.

On admission, the patients were randomly assigned to receive daily a 250-mL 0.9% saline solution over 12 h intravenously containing either 59 μmol Cu, 4.8 μmol Se, and 574 μmol Zn (TE group) or vehicle (V group). The patients received the intervention solution within 12 h of injury and for 14 d if their burns covered 20–60% BSA (16 patients) or for 21 d if the burns exceeded 60% BSA (5 patients).

Enteral nutrition was to be started within 12 h of admission through a nasoenteral tube according to the unit's protocol. Feeds were a combination of 3 industrial solutions: Isosource energy (60–70% of energy target), Isosource standard, and Isosource fibers (30–40% of target) (Novartis, Basel, Switzerland). Intravenous insulin was delivered continuously to maintain blood glucose between 6 and 10 mmol/L. The energy target was set at 30 kcal · kg^{-1} · d^{-1} until day 3 and was then adapted to 105–110% of measured resting energy expenditure (REE). Both groups received daily vitamin supplements of 1 g ascorbic acid and 100 mg thiamine with a multivitamin preparation delivered intravenously (1 ampoule Soluvit and Vitalipid; Fresenius Kabi, Bad Homburg, Germany) plus 100 mg vitamin E delivered via the feeding tube. Indirect calorimetry was carried out under standardized postabsorptive conditions on days 3 ± 1, 10 ± 1, and 20 ± 1 after admission to determine REE (Deltatrac metabolic monitor, Datex, Finland; 11).

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 1, 5, 10, 15, and 20 for measurement of urea, creatinine, 3-methylhistidine (only on days 10 and 15), and trace elements. Skin biopsy specimens (2 × 1 cm^2) excised during the surgical procedures from burned areas and from healthy skin donor areas were obtained in 12 patients on days 2 ± 1, 10 ± 1, and 20 ± 1 after injury. The biopsy specimens were immediately frozen in liquid nitrogen and kept at −80 °C until analyzed.

Laboratory determinations

Determinations not included below are described in the accompanying article (10). For measurement of skin glutathione peroxidase (GSHPx), glutathione reductase (GR), and glutathione, skin biopsy samples were homogenized in 1 mL cold 0.125 mol phosphate/L, 0.625 mol EDTA/L, pH 7.2, by using a mortar and a pestle. After being centrifuged at 12 000 × *g* for 5 min, the supernatant fluid was collected and stored at −80 °C. Biochemical determinations were performed as previously described (12, 13).

The protein content of the supernatant fluid was determined against a calibration curve of bovine serum albumin. Test mixtures for determination of GR activity contained 0.17 mmol NADPH and 2.2 mmol glutathione disulphide and were incubated at 37 °C. GSHPx was tested at 25 °C in the presence of 0.34 units glutathione reductase/mL from bakers yeast (Sigma, St Louis, MO), 5 mmol EDTA, 3.75 mmol sodium azide, 5 mmol glutathione, 0.28 mmol NADPH, and 75 mmol H_2O_2 (14). One mU of enzyme activity corresponds to 1 nmol NADPH oxidized per minute. Specific activities are expressed as mU/mg protein. Because glutathione is partly oxidized to GSSG during storage, the supernatant fluid samples were reduced with 0.6 mol $NaBH_4$/L (12) for 2 h at room temperature before being assessed by HPLC (Hewlett-Packard Series 1050; Hewlett-Packard, Palo Alto, CA) with electrochemical detection (BAS LC-4C). Glutathione was separated from cysteine by using phase II octadecylsilane of 3 μm (100 × 3.2 mm) and 0.1 mol monochloroacetic acid/L, 3.3 mmol 1-heptanesulphonic acid, pH 2.6. Detection was performed with a Hg-Au electrode (data in nmol/mg protein).

Copper, selenium, and zinc in plasma and selenium and zinc in skin were measured in duplicate by inductively coupled plasma mass spectrometry with aqueous inorganic standards (15). Detailed methods are described in reference 10. Urinary urea was measured by standard laboratory methods, and 3-methylhistidine was measured on a Biochrom 20+ amino acid analyzer by ion-exchange chromatography after sulfosalicylic acid precipitation of proteins.

Stable-isotope study

On day 10 ± 1, on the day before or after surgery, while the patient was hemodynamically stable, a 6-h infusion of ring-$^{13}C_6$-phenylalanine ([^{13}C]Phe) was initiated with a 2-μmol/kg bolus (600 μg/kg), followed by a continuous infusion of 0.05 μmol · kg^{-1} · min^{-1} (12 μg · kg^{-1} · min^{-1}) (Cambridge Isotope Laboratory, Cambridge, MA) (1). Infusions of 6,6-[$^2H_2$]glucose (2-mg/kg bolus, 20-μg · kg^{-1} · min^{-1} continuous infusion) and of 1,1,2,3,3-[$^2H_5$]glycerol (9-μg/kg bolus, 0.9-μg · kg^{-1} · min^{-1} continuous infusion) were administered simultaneously. Two skin biopsies were performed after 1 h and after 6 h of isotope infusion to measure intracellular [^{13}C]Phe isotopic enrichment.

Briefly, skin biopsy samples were thawed, weighed, and extracted 3 times with 5% perchloric acid by grinding with a polytetrafluoroethylene pestle in a microcentrifuge tube. Every step was performed at 4 °C. Amino acids from blood were precipitated with perchloric acid, and the supernatant fluid was neutralized. Intracellular free amino acids from the pooled supernatant fluid were recovered and purified by cation-exchange chromatography (Dowex AG, 50W–X8; Bio-Rad laboratories, Richmond, CA). Amino acids were eluted with 2 mL of ammonium hydroxide and 1 mL of water. The eluate was dried under speed vacuum, and the amino acids were derivatized by adding 30 μL of MTBSTFA and 30 μL of acetonitrile (*tert*-butyldimethylsilyl derivative). The isotopic enrichment of [^{13}C]Phe was analyzed by electron-impact ionization–mass spectroscopy (GC 5890/MS 5971; Hewlett-Packard). Mass-to-charge (*m/z*) ratios of 336 and 342 were used for the natural and ^{13}C-enriched phenylalanine, respectively, in agreement with Schwenk et al and Tessari et al (16, 17). Appropriate standard curves were prepared simultaneously for the determination of isotopic enrichment. Amino acids from plasma were precipitated with frozen acetone, and the supernatant fluid was acidified with 3 N HCl and washed 3 times with chloroform. The aqueous phase was used for gas chromatography–mass spectrometry (GC-MS) derivatization and analysis as above.

Phenylalanine extracted from skin samples and blood was analyzed according to Patterson et al (18). ^{13}C enrichments were measured by GC-MS with selective monitoring at *m/z* 336 and 342. Plasma 6,6-$^2H_2$ glucose and 1,1,2,3,3-$^2H_5$ glycerol enrichments were measured on acetylated derivates by GC-MS (GC

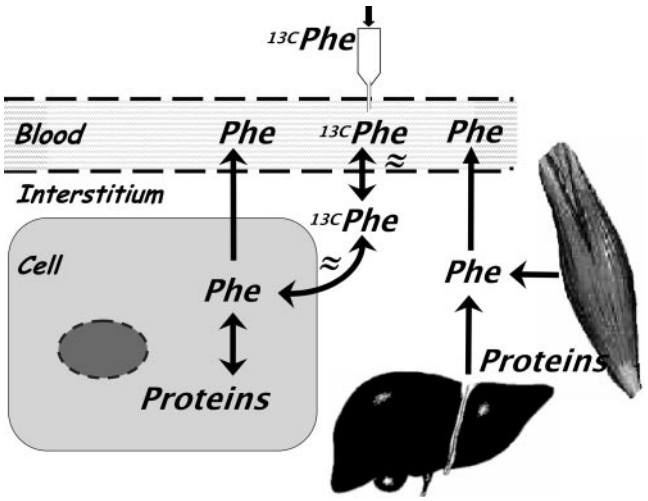

FIGURE 1. Study hypothesis: after injection of ring-$^{13}C_6$-phenylalanine ([^{13}C]Phe) and 6 h of continuous infusion in the blood compartment, [^{13}C]Phe reaches equilibrium in the 3 compartments: blood, interstitial space, and intracellular space. The ratio of intracellular to plasma [^{13}C]Phe enrichment provides an estimate of intracellular protein degradation, assuming that inward transport of Phe from the interstitial space to the cell is not affected.

5890/MS 5971; Hewlett-Packard), with selective monitoring at *m/z* 331, 333, 159, and 164, respectively. Plasma insulin was determined by radioimmunoassay (kit from Adaltis, Casalecchio di Reno, Italy).

The hypothesis tested in the trial is described in **Figure 1**. Determination of plasma 6,6-$^2H_2$ glucose, 1,1,2,3,3-$^2H_5$ glycerol, and [^{13}C]Phe isotopic enrichments (Masstrace, Worcester, MA) enabled calculation of whole-body phenylalanine, glucose, and glycerol turnover by the use of Steele's equations for steady state conditions (19). The ratio of cell supernatant (ie, free intracellular phenylalanine) to plasma (ie, extracellular phenylalanine) isotopic enrichment was used as an index of intracellular protein catabolism.

Statistical analysis

The results are expressed as means ± SDs and as medians with ranges. Nonparametric tests were used to compare demographic variables and nonparametric variables such as results of surgery. Changes over time were compared by using 2-factor repeated-measures analysis of variance (ANOVA) for the effects of group, time, and intervention. Post hoc comparisons were carried out by Dunnett's or Scheffe's tests where appropriate. Paired *t* tests were used to compare the influence of the trace element supplements on the global turnover of the substrates and on fractional tissue protein synthesis in the wound healing areas. Linear regressions were calculated between urinary variables with adjustment for multiple values per patient. Significance was considered at the level of $P < 0.05$, whereas $P < 0.10$ was considered to indicate a trend. 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; 12 patients underwent skin biopsies (7 in the V group and 5 in the TE group), and 10 patients completed the isotopic study. The latter patients were the most severely burned (**Table 1**).

Indirect calorimetry determinations of REE were compared by use of the Harris and Benedict equation, the mean predicted REE being 1690 ± 336 kcal/d. Measured REE was 2233 ± 532 kcal/d (132% of predicted) on day 2, 2745 ± 601 kcal/d (162% of predicted) on day 10, and 2534 ± 552 kcal/d (149% of predicted) on day 20, with no significant difference between groups.

There was no significant difference in enteral feeding or energy and protein delivery between the groups. The patients were receiving (medians) the following amounts of energy: 2660 kcal/d by day 5, 2754 kcal/d by day 10, and 2900 kcal/d by day 20.

Plasma concentrations of copper, selenium, zinc, and GSHPx were significantly higher after day 5 ($P < 0.05$) in the treatment group (10). CRP values were nonsignificantly lower in the TE group on days 5 and 10 ($P = 0.17$).

The urinary excretion of creatinine, urea, and 3-methylhistidine did not differ significantly between the groups (**Table 2**), nor did the ratio of 3-methylhistidine to creatinine. Creatinine and urea excretion were closely correlated with 3-methylhistidine excretion ($r^2 = 0.678$ and $r^2 = 0.685$ respectively, $P < 0.0001$). Although no significant changes were noted over time in the healthy skin areas, antioxidant enzymes increased in the burned areas over time, with higher concentrations in the TE group (GSHPx, $P = 0.028$; GR, $P = 0.019$; glutathione, $P = 0.0006$; **Table 3**).

TABLE 1
Characteristics of all patients investigated and details for the 10 patients who completed the stable-isotope study[1]

	V group	TE group	V group, isotopes	TE group, isotopes	*P* between isotope patients
n	10	11	5/10	5/11	
Patients with skin biopsies (*n*)	—	—	7/10	5/11	NS
Sex (F/M)	4/6	2/9	3/2	1/4	NS
Age (y)	38 ± 16[2]	46 ± 15	32 ± 9 [26]	46 ± 19 [51]	0.22 (NS)
Burned skin amount (% BSA)	44 ± 20 [41][3]	45 ± 22 [40]	45 ± 26 [36]	62 ± 21 [65]	0.26 (NS)
Surgical burns (% BSA)	34 ± 16 [29]	31 ± 30 [22]	38 ± 19 [30]	56 ± 24 [60]	0.29 (NS)
Regrafting index (%)	18 ± 7 [8]	2 ± 7 [1][4]	27 ± 41 [10]	3 ± 19 [2]	0.32 (NS)

[1] V, vehicle; TE, trace element; BSA, body surface area; regrafting index = difference between surface grafted and that considered surgical. No significant differences were noted between patients involved in the isotope study.
[2] $\bar{x}$ ± SD (all such values).
[3] Median in brackets.
[4] Significantly different from the whole V group, $P = 0.03$ (one-factor ANOVA) (10).

TABLE 2
Urinary urea, creatinine, and 3-methylhistidine (3-MH) concentrations in the 10 patients with complete investigations[1]

	Day 1	Day 5	Day 10	Day 15	Day 20
Urea (mmol/d)					
TE group	277 ± 101	489 ± 186	597 ± 217	489 ± 172	505 ± 204
V group	198 ± 74	443 ± 245	574 ± 234	502 ± 299	484 ± 144
Creatinine (mmol/d)					
TE group	12,68 ± 4.25	12.69 ± 6.05	11.07 ± 3.29	9.06 ± 2.76	8.61 ± 2.69
V group	9.15 ± 3.95	11.69 ± 4.19	11.23 ± 5.30	8.83 ± 5.21	9.65 ± 2.74
3-MH (μmol/d)					
TE group	—	—	510.2 ± 245.1	415.4 ± 89.2	—
V group	—	—	408.5 ± 250.5	315.2 ± 265.5	—
3-MH:creatinine					
TE group	—	—	44.3 ± 14.3	39.0 ± 8.8	—
V group	—	—	39.7 ± 19.7	32.5 ± 13.8	—

[1] All values are $\bar{x}$ ± SD. TE, trace element; V, vehicle. By 2-factor repeated-measures ANOVA, only the change in urea excretion was significant in both groups, $P = 0.038$.

Burned and healthy skin concentrations of trace elements differed in their responses over time. Over the 20-d study period, selenium and zinc concentrations in the healthy donor sites did not change significantly in either the V or the TE group. On day 3, selenium concentrations in healthy and burned areas were higher in the TE group ($P = 0.01$), whereas zinc was not. In the V group, no significant differences in selenium or zinc concentrations were noted between healthy and burned areas. However, concentrations in the V group were significantly lower than in the TE group (selenium, $P = 0.001$; zinc, $P = 0.08$). In the TE group, selenium and zinc concentrations increased in the burned areas, reached significance by day 20, and then reverted to near-normal values (**Figure 2**).

Phenylalanine turnover was 17.86 ± 3.46 mg/min (0.229 ± 0.041 mg · kg^{-1} · min^{-1}) and 16.51 ± 2.41 mg/min (0.231 ± 0.044 mg · kg^{-1} · min^{-1}) in the TE and V groups, respectively (NS). In healthy donor skin, the supernatant:plasma [^{13}C]Phe enrichment ratio was 0.297 ± 0.091 (TE group) and 0.421 ± 0.349 (V group) after 1 h (NS), and 0.388 ± 0.042 (TE group) and 0.432 ± 0.332 (V group) after 6 h, the increase being significant for both groups ($P < 0.001$); the time × group interaction was not significant by 2-factor repeated-measures ANOVA. In burned skin, this ratio was 0.363 ± 0.094 (TE group) and 0.286 ± 0.130 (V group) after 1 h (NS), and 0.592 ± 0.153 (TE group) and

TABLE 3
Antioxidant enzyme concentrations in skin biopsy samples from both the nonburned healthy skin areas that served for grafting and the burned areas

	Day 3	Day 10	Day 20
Healthy skin (donor)			
GSH (mU/mg protein)			
V group	15.2 ± 8.9	15.6 ± 6.3	19.1 ± 5.0
TE group	13.7 ± 13.9	7.4 ± 6.8	11.5 ± 5.5
GR (mU/mg protein)			
V group	35.9 ± 16.1	29.0 ± 14.4	31.6 ± 10.1
TE group	22.8 ± 12.3	22.1 ± 4.7	22.3 ± 10.4
GSHPx (mU/mg protein)			
V group	38.9 ± 10.0	38.9 ± 9.7	41.4 ± 8.1
TE group	37.3 ± 18.1	39.4 ± 24.3	28.3 ± 27.3
Burned skin			
GSH			
V group	5.5 ± 4.3	7.0 ± 6.5[2]	11.8 ± 7.5[2]
TE group	2.2 ± 2.3[3]	3.2 ± 3.5[3]	16.8 ± 4.5[2]
GR			
V group	14.0 ± 5.5	26.9 ± 11.1	21.6 ± 7.9
TE group	4.5 ± 4.2	18.1 ± 8.7[3]	27.5 ± 14.8[2]
GSHPx			
V group	16.3 ± 11.2	28.3 ± 15.8[2]	40.8 ± 29.0[2]
TE group	18.0 ± 10.3	33.3 ± 15.7[2]	51.5 ± 29.2[2]

[1] All values are $\bar{x}$ ± SD; $n = 7$ in the V group and 6 in the TE group. V, vehicle; TE, trace element; GSH, glutathione; GR, glutathione reductase; GSHPx, glutathione peroxidase. By 2-factor repeated-measures ANOVA, no significant differences were observed in the healthy skin areas. By contrast, in burned skin, the time × group interaction was significant for GSH (P=0.006), GR (P=0.019), and GSHP (P=0.028).

[2] Significantly different from baseline, $P < 0.05$ (Dunnett's test).

[3] Significantly different from the V group at the same time, $P < 0.05$ (Scheffe's test).

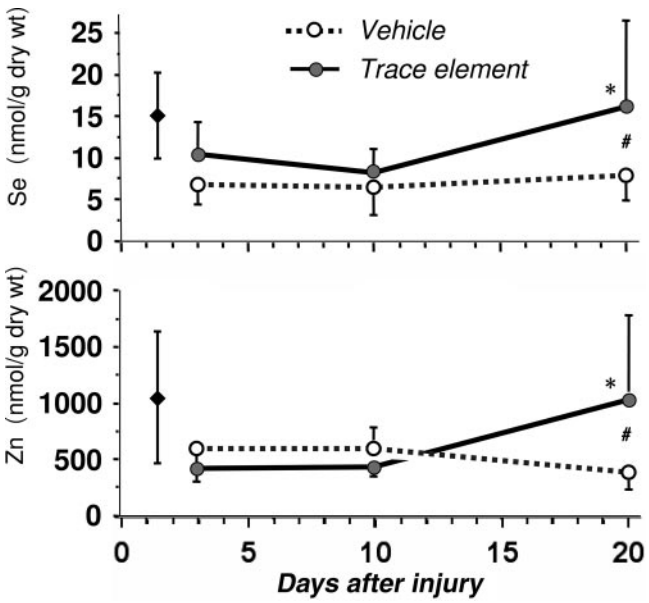

FIGURE 2. Mean (±SD) selenium and zinc contents increased significantly in burned skin in the patients in the trace element group ($n = 5$) but not in the vehicle group ($n = 7$). The 2-factor repeated-measures ANOVA showed significant interactions (time × group) for both elements: selenium ($P = 0.05$), zinc ($P = 0.004$). *Significantly different from day 3 (Dunnett's test). #Significantly different between groups at the same time (Scheffe's test). The isolated values (♦) represent the mean concentrations in healthy volunteers.

0.262 ± 0.171 (V group) after 6 h, the increase in the TE group being significantly larger ($P = 0.002$), with a significant time × group interaction ($P = 0.029$). This higher ratio in the TE group reflected the decreased protein catabolism in burned skin.

Mean concentrations of plasma glucose and insulin did not differ significantly throughout the stable-isotope study. Glucose turnover also did not differ significantly between groups, being 357 ± 67 mg/min (4.67 ± 1.36 mg · kg^{-1} · min^{-1}) in the TE group and 304 ± 83 mg/min (4.26 ± 1.27 mg · kg^{-1} · min^{-1}) in the V group. Glycerol turnover was 14.6 ± 3.8 mg/min (0.18 ± 0.023 mg · kg^{-1} · min^{-1}) and 22.0 ± 13.4 mg/min (0.30 ± 0.176 mg · kg^{-1} · min^{-1}) in the TE and V groups, respectively (nonsignificantly lower values in the TE group: $P = 0.17$).

Wound healing was improved in the TE group as a whole as shown by the lower regrafting index ($P = 0.013$). Wound healing was also nonsignificantly lower in the 10 patients with detailed skin investigations.

DISCUSSION

Wound healing is a major issue in burn patients, because delayed healing with skin graft failure prolongs hospital stay. Trace elements are essential for anabolic pathways and therefore can be expected to play a key role in wound healing. Copper is essential for wound repair through its role in the lysyl oxidases, copper enzymes that depend on copper status for their activity and that initiate the cross-linking of collagen and elastin (20, 21). Zinc is required for all anabolic pathways and is an essential cofactor for many of the enzymes involved in protein synthesis. Zinc deficiency has been shown to negatively affect wound healing in nonburn conditions (22).

In the present study of the effect of high-dose, intravenous zinc, copper, and selenium supplements in burned patients, supplementation increased tissue selenium and zinc concentrations (10): higher selenium concentrations were observed in the skin donor areas after only 3 d of supplementation. Compared with skin concentrations in healthy volunteers, concentrations in the TE group were nearly normalized by day 20, whereas they remained very low in the V group. These changes were associated with earlier normalization of antioxidant enzymes and glutathione in the skin, with a reduction in surgical grafting requirements and with a significantly better index of skin graft take. The better wound healing may well be due to a reinforcement of local antioxidant defenses, as well as to more specific improvement in protein dynamics.

Endocrine stress hormones and the inflammatory cytokines are the most important of the many triggers of catabolism (23). Having observed shorter wound healing times in our 2 previous trials (8, 9), we hypothesized that trace element depletion might be a further trigger. Those patients receiving large doses of trace elements had higher tissue concentrations of selenium and zinc. We found no significant effect of the supplements on global protein, glucose, or glycerol turnover. Glucose turnover values were elevated (>4 mg · kg^{-1} · min^{-1}, compared with 1.5–2 mg · kg^{-1} · min^{-1} in healthy subjects), but were typical of those in stressed patients (24). Urinary urea and 3-methylhistidine excretions did not differ significantly between groups, which agrees with the measured phenylalanine turnover.

The results of the present study confirm the results of our previous trials (8, 9) that supplementation with copper, selenium, and zinc is associated with a beneficial effect on wound healing and a reduction in skin grafting requirements. Why should such patients have a better graft take? Different mechanisms may be advocated: *1*) better nutrition could be proposed, but both groups were fed identically; *2*) less wound infections might have occurred, but the frequency of skin infections was unaffected by the supplements (10); *3*) local antioxidant status may have been improved (25)—this is a likely mechanism and indeed along with increased trace element concentrations we observed higher activities of antioxidant enzymes and glutathione; *4*) the trace elements may stimulate some local wound effects—the migration of keratinocytes plays an important role in the re-epithelialization of cutaneous wounds [it has been shown in vitro that zinc, copper, and manganese enhance keratinocyte migration, and one of the mechanisms was through a modulation of integrin functions (26)]; and *5*) the trace elements may lead to a decrease in protein catabolism. The present study of systemic and skin phenylalanine turnover was specifically designed to address this possibility.

We used [^{13}C]Phe, which was previously shown to reflect skin protein metabolism in animal models (1) and in humans (27). Our data indicate that [^{13}C]Phe infusion led to a rapid labeling of intracellular [^{13}C]Phe both in healthy and in burned skin, which essentially reached a steady state in most patients as soon as 1 h after infusion. The ratio of cell supernatant (ie, free intracellular Phe) to plasma (ie, extracellular Phe) isotopic enrichment was used as an index of intracellular protein catabolism. This interpretation is based on the fact that intracellular Phe originates from either intracellular protein catabolism, which releases unlabeled Phe, or from inward transport of extracellular Phe with an isotopic enrichment equal to plasma Phe. Under such conditions, the ratio of supernatant-to-plasma Phe isotopic enrichment reflects intracellular protein catabolism, provided that Phe transport from extracellular fluid is not altered. The present results at least partly confirm our hypothesis: higher plasma and skin selenium concentrations were associated with an increased ratio of intracellular to plasma [^{13}C]Phe, which strongly suggests that supplementation reduced protein catabolism. The ratio can increase as a result of either reduced intracellular protein catabolism or increased transport of extracellular Phe. We favor the first hypothesis, because numerous reports indicate increased protein catabolism in burn and injury, whereas alterations of amino acid transport have to our knowledge not been described. Whatever the mechanism underlying this change in the [^{13}C]Phe ratio, it can be held to reflect beneficial effects on intracellular net protein synthesis. The skin accounts for 10–25% of whole-body protein turnover. Total Phe turnover was unaffected, however, so the findings of reduced catabolism are probably limited to the skin. The better skin graft take is clinical evidence of a beneficial effect, especially in this group of patients that tended to have more severe injuries.

The small number of patients was the most important limitation of our study, particularly for the skin biopsy and protein turnover studies, which reduced the power of the results. The very small differences in detectable [^{13}C]Phe at the skin level, despite the application of the skin protocol described by Zhang et al (1), probably indicates that the doses required for skin studies should be increased in the future to produce higher skin incorporation of ^{13}C. Nevertheless, the changes were large enough to result in significant differences between the groups. Moreover, the clinical results observed in the current study confirm the 2 prior trials (8, 9). Finally, we still lack a dose-finding study in

patients with major burns: trace elements have a dose response curve, with potential toxicity. This series of studies should not favor the conclusion that "more is better," but should stimulate research to find the appropriate doses and durations of treatment in burns as well as in other critical illness.

We conclude that large early supplementation combining copper, selenium, and zinc is safe and beneficial after major burns. Clinical benefits include better wound healing as shown by the lower skin grafting requirements, and a nonsignificantly shorter duration of intensive care unit treatment normalized per % BSA. In addition, trace elements appear to be preferentially taken up by skin from the burned area, and although trace element repletion does not alter whole-body protein catabolism, it does appear to reduce protein catabolism in the skin.

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The study was done on the institutional time of the investigators. The authors performed the analysis of the samples and 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; CB: study design, laboratory method development, data interpretation, and redaction of manuscript; RLC: study design, data interpretation, and redaction of manuscript; WT: laboratory method development, data interpretation, and redaction of manuscript; WR: study design, clinical supervision, data collection, data interpretation, and redaction of manuscript; M-CC: study design, clinical supervision, data collection, and data interpretation; MB: laboratory method development, data interpretation, and redaction of manuscript; AS: study design, data interpretation, and redaction of manuscript; LT: study design, laboratory method development, data interpretation, and redaction of manuscript. None of the authors had any conflicts of interest (eg, bonds, economical implication in the industry).

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