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Effect of calcium and magnesium on inflammatory cytokines in accidentally multiple fracture adults

A short-term follow-up

Yongxing Song, MD[a], Long Xu, MD[b], Xin Jin, MD[b], Deqing Chen, MD[b], Xiuhui Jin, BS[c], Guangtao Xu, MD[b,*]

Abstract

Calcium (Ca) and magnesium (Mg), which play an important role in several cellular processes, is essential for normal development of the skeleton and maintenance of tissue homeostasis. Deficiency of these elements might delay bone fracture recovery or accelerates bone loss. We aimed to examine whether supplementation of trace element (TE) promotes fracture healing in accidentally fracturing adults by involvement of inflammatory mechanism.

A short-term follow-up in clinic was performed. Totally, 117 subjects diagnosed with multiple fractures by traffic accidents were recruited in this study. Serum Ca and Mg levels were measured by inductively coupled plasma atomic emission spectrophotometry. Short-term changes such as serum C-reactive protein, interleukin (IL)-1β, IL-6, and tumor necrosis factor alpha in normal treatment and TE supplement groups were detected by enzyme-linked immunosorbent assay. Student *t* test and the Spearman correlation were performed to analyze the data.

Significantly negative correlations between Ca ($r=0.7032$; $P<.001$) and Mg ($r=0.2719$; $P<.05$) and injury severity score were observed. Serum Ca and Mg were significantly increased at Day 5, 7, and 9 following TE supplements. After treatment, serum C-reactive protein, IL-1β, IL-6, and tumor necrosis factor alpha were significantly reduced whereas cytokine levels of the TE supplement group were found to be lower than that of the normal treatment group after Day 3.

These findings suggest that Ca and Mg levels are associated with the injury severity of multiple fractures, and the supplement could reduce the inflammation, which may be beneficial for the bone recovery and disease process.

Abbreviations: Ca = calcium, Cr^{3+} = trivalent chromic ion, CRP = C-reactive protein, Cu = copper, F^- = fluorion, Fe = ferrum, I^- = iodide ion, IL = interleukin, ISS = injury severity score, Mg = magnesium, Mn^{2+} = manganese ion, MoO_4^{2-} = molybdate ion, NT = normal treatment, SeO_3^{2-} = selenite ion, TEs = trace elements, TNF-α = tumor necrosis factor alpha, Zn = zinc.

Keywords: calcium, inflammation cytokines, injury severity score, magnesium, multiple fractures

1. Introduction

Trace elements (TEs) in minute amounts in body tissues have been recognized in relation to human health and are important for optimum human growth and development.[1] A growing body of evidence suggests that deficiency of certain essential TEs is associated with bone disorders including fractures and osteoporosis.[2] Calcium (Ca) is one of the essential TEs in both marine and terrestrial organisms, where it plays a crucial role in the

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The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Human Ethical Committee of Jiaxing University (JUMC-IRB-2018). Written informed consent was obtained from the patients for publication of the studies data. Informed consent was obtained from all subjects involved in the study. Written informed consent has been obtained from the patient(s) to publish this paper.

The authors have no conflicts of interest to disclose.

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

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[a] Department of Orthopedics, China Coast Guard Hospital of the People's Armed Police Force, Jiangnan Hospital Affiliated to Jiaxing University, Jiaxing, ZJ, China, [b] Molecular Pathology Laboratory, Department of Pathology, Institute of Forensic Science, Jiaxing University Medical College, Jiaxing, ZJ, China, [c] Department of Immunology and Human Biology, University of Toronto, Toronto, ON, Canada.

[*] Correspondence: Guangtao Xu, Molecular Pathology Laboratory, Department of Pathology, Institute of Forensic Science, Jiaxing University Medical College, Jiaxing 314001, ZJ, China (e-mail: gtxu@zjxu.edu.cn).

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processes of the formation and maintenance of the skeleton.[3] Homeostasis imbalance of intracellular Ca^{2+} has been demonstrated to be one of the key checkpoints during skeletal muscle injuries, resulting in significant change in the metabolism.[4] Studies have shown that Ca intake is an effective predictor of total bone mass in adults, and its supplement has been considered as a significant source of bone metabolism.[5] Some researchers have also found that the effects of Ca supplement at ion alone on bone status are weaker than when supplements are combined with other minerals.[6] Magnesium (Mg) is an indispensable element and participates in nearly all metabolic processes in the human body.[7] It plays an important role in the regulation of maintaining the structure and function of muscles and bone cells. Mg has reported having a strong protective effect on the skeleton. It is also a cofactor for enzymes that regulate Ca metabolism.[8] Studies have shown that Mg deficiency can lead to increased Ca release from bone.[9] Depletion of Mg is associated with decreased osteoblastic and osteoclastic activity, osteopenia and bone fragility, and is regarded as a modifiable risk factor for fracture and osteoporosis.[10]

Fracture in bone is an often-occurring traumatic disease that causes a series of systemic and local physiological and biochemical changes.[11] Inflammation has been identified as a vital biological process for clearance of pathogens and maintenance of tissue homeostasis. During bone injury, various cytokines, chemokines, and growth factors are released to recruit additional inflammatory cells and mediate remodeling. It is well recognized that both the initial fracture hematoma and the subsequent acute inflammation reaction are critical for fracture healing.[12] Thus, fracture repair is a continuous process initiated by a series of cellular and molecular events. Evidence shows that some nutrition supplements with TEs such as Fe, Zn, and Cu, or vitamins are helpful against inflammation whereas their disturbance is associated with increases of inflammatory responses.[13] However, the mechanism of Ca and Mg supplement in multiple fractures has not been fully understood. To improve the speed of fracture healing by TE implement that may change the inflammatory microenvironment of the injury is an important goal of fracture treatment.

Therefore, in this study, we attempted to examine the Ca and Mg levels in accidentally multiple fracture adults and explore the effect of Ca and Mg on inflammatory cytokines in short-term time to illuminate the potential role of the supplement of TE in fracture healing process.

2. Materials and methods

2.1. Patient enrollment

A total of 117 subjects diagnosed with multiple fractures by traffic accidents when coming to clinics were enrolled in this study between January 2018 and March 2019. Their clinic information was obtained from the history database of the hospital, and the general characteristics were listed (Table 1). Inclusion criteria were the cases from severe trauma with traffic accidents without history of bone fractures or other fatal diseases. Patients were in the age of 43 to 52 years and primarily represented 2 or more open or closed fracture in different locations of the body such as skull, spine, pelvis, ribs, and long bone of limbs, accompanied with other visceral trauma including craniocerebral, pulmonary, gut, liver, and kidney injury. Patients usually had a length of hospitalization for 10 days to 1 month with several sever patients having the length of stay for no more than or equal to 7 days in an intensive care unit (ICU). All the injured were randomly divided into 2 groups: the normal treatment (NT group) and TE supplement group (NT + TE group). According to the normal oral requirement of various TEs, the injured in NT + TE group was daily given 250 mL 10% glucose intravenously supplemented with10 mL multi-TEs injection (Addamel, Sino-Swed Pharmaceutical, Beijing, China) which incorporated with Cr^{3+} 0.2 μmol, Cu^{2+} 20 μmol, Fe^{3+} 20 μmol, Mn^{2+} 5 μmol, MoO_4^{2-} 0.2 μmol, SeO_3^{2-} 0.4 μmol, Zn^{2+} 100 μmol, F^- 50 μmol, and I^{-1} μmol. The NT group was given 250 mL of 10% glucose at the second day posttrauma based on conventional therapy. The study protocol was approved by the Human Ethical Committee of our college and the hospital.

Table 1

Characteristics of the subjects.

Characteristics	NT group (n = 62)	NT + TE group (n = 55)
Age (yrs, mean ± SD)	45.8 ± 2.9	46.3 ± 3.7
Sex, n (%)		
Male	38 (61.3)	37 (67.3)
Female	24 (38.7)	18 (32.7)
Fracture locations, n (%)		
Skull	11 (17.7)	7 (12.7)
Spine	7 (11.3)	9 (16.4)
Pelvis	8 (12.9)	6 (10.9)
Ribs	31 (50.0)	27 (49.1)
Long bone of limbs	45 (72.6)	39 (70.9)
Visceral trauma, n (%)		
Craniocerebral trauma	18 (29.0)	13 (23.6)
Pulmonary injury	15 (24.2)	29 (52.7)
Gut injury	24 (38.7)	35 (63.6)
Liver injury	21 (33.9)	14 (25.4)
Kidney injury	6 (9.6)	18 (32.7)
ICU stay (d, mean ± SD)	11.7 ± 0.9	10.3 ± 1.2
Length of hospitalization (d, mean ± SD)	25.6 ± 0.5	23.2 ± 0.8
Surgical operation, n (%)	55 (88.7)	48 (87.3)

NT group: Normal treatment; NT + TE group: Normal treatment + Trace element.

All the patients were estimated by multiple injury severity score (ISS) when admitted into the hospital. Briefly, the ISS score was the sum of the squares of the highest abbreviated injury score for the 3 most severely injured areas of the body according to the Abbreviated Injury Scale 1990 Revision Update 98. The abbreviated injury score was a means of quantifying the damage of organs and tissues with each injury of the organ scored by 1 to 6 points according to the degree of injury: 1, mild injury; 2, moderate injury; 3, severe injury; 4, critically ill without life-threatening; 5, critically ill with a risk of death; and 6, extremely ill and unable to rescue.

2.2. Sample collection

Peripheral blood samples were collected at the time of 1 hour (D1), D3, D5, D7, and D9 from the beginning of hospitalization when patients were injured by accident. The blood was obtained from the median cubital vein of fasting patients and stored in polypropylene heparinlithium anticoagulation tubes (Guangzhou Saiguo Biotech Co., Ltd, Guangdong, China) that were kept at −25°C until analysis.

2.3. Ca and Mg measurement

All laboratory materials used for the measurement of TEs, including glass pipette tips and autosampler cups, were cleaned thoroughly with abstergent and running water, flushed with distilled water, steeped in dilute nitric acid, and then rinsed thoroughly with deionized distilled water. Serum levels of Ca and Mg were detected by inductively coupled plasma atomic emission spectrophotometry (Bohui Innovation Tech., Beijing, China) previously described.[14] Generally, a volume of 0.2 mL serum was mixed with nitric/sulfuric/perchloric acid (3:1:1, v/v) in Teflon vessel to reduce the disturbance of the organic matrixes. The homogeneous digest was then diluted with water to reduce the nitric acid, and was further nebulized in an argon plasma. During analysis of inductively coupled plasma atomic emission spectrophotometry, the reference wavelength was set at 193.091 nm (the atomic line of carbon emission) for monitoring the remaining undigested organic substance.

2.4. Cytokine expressions

The concentrations of serum interleukin (IL)-1β, IL-6, C-reactive protein (CRP), and tumor necrosis factor alpha (TNF-α) were determined by enzyme-linked immunosorbent assay. Enzyme-linked immunosorbent assay was performed using a kit from Cusabio (Cusabio Biotech Co., Ltd, Wuhan, China) according to the manufacturer's instructions. The sensitivity of the assays was 0.93 pg/mL, 0.78 pg/mL, 1.13 pg/mL, and 1.54 pg/mL for IL-1β, IL-6, CRP, and TNF-α, respectively.

2.5. Statistical analysis

Descriptive parameters were expressed as a percentage (%) or mean ± standard deviation (SD) for subject demographics. The differences of TEs or cytokine levels between groups were analyzed by Student *t* test. The correlation between TEs and ISS score was evaluated using the Spearman rank correlation test. All statistical analyses were performed using SPSS 22.0 software (SPSS Inc., Chicago, IL) and Microsoft Excel 2013 (Microsoft Co.). A P value $< .05$ was considered statistically significant.

3. Results

3.1. The correlation between TEs and ISS score

Elements of Ca and Mg were not significant between genders ($P > .05$, data not shown). At the beginning of hospitalization before the supplement of TEs, significantly negative correlations between serum Ca ($r = -0.7032$, $P < .001$) and Mg ($r = -0.2719$, $P < .05$) concentrations and ISS score were observed, as shown in Figure 1.

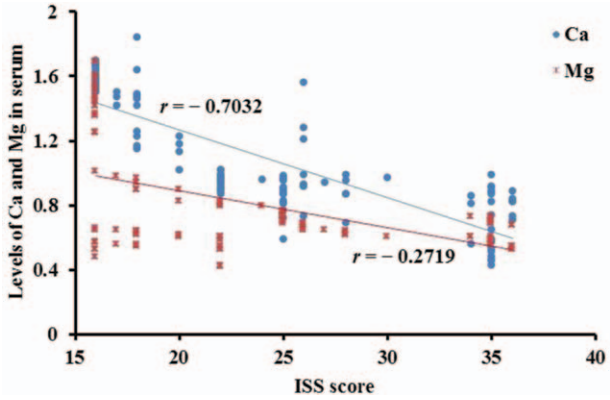

Figure 1. Correlation between serum Ca and Mg and ISS score by the linear fitting scatterplot. Linear correlated coefficients (*r*) were 0.7032 and 0.2719 for Ca and Mg, respectively. Ca = calcium, ISS = injury severity score, Mg = magnesium.

3.2. Serum Ca and Mg concentrations

The serum levels of Ca and Mg in all patients were significantly altered after treatment by supplement of TEs (Fig. 2). Compared to the concentration at Day 1 (1.41 ± 0.09 mM), Ca concentration was increased following Day 3 (1.61 ± 0.11 mM), Day 5 (1.72 ± 0.10 mM), Day 7 (1.80 ± 0.08 mM), and Day 9 (1.83 ± 0.06 mM) (Day 3 and 5, both $P < .05$; Day 7 and 9, both $P < .01$). Similarly, Mg concentration was significantly increased after Day 5 (1.51 ± 0.05 mM; $P < .05$), Day 7 (1.60 ± 0.06 mM; $P < .01$), Day 9 (1.66 ± 0.08 mM; $P < .01$), in comparison with Day 1 (1.33 ± 0.10 mM).

3.3. Inflammatory cytokine levels

As shown in Figure 3, CRP (23.26 ± 2.54 mg/L; 18.45 ± 3.12 mg/L), IL-1β (16.50 ± 1.78 ng/mL; 18.16 ± 1.76 ng/mL), and TNF-α (2.01 ± 0.15 ng/mL; 1.67 ± 0.08 ng/mL) were increased remarkably in serum of patients after fracture, and reached the peak at Day 3 in both NT group and NT + TE group. CRP in NT + TE group was significantly lower than that in NT group at Day 3 (18.45 ± 3.12 mg/L vs 23.26 ± 2.54 mg/L), 5 (12.14 ± 2.35 mg/L vs 21.73 ± 2.85 mg/L), 7 (9.54 ± 3.26 mg/L vs 13.16 ± 3.08 mg/L) (all $P < .01$), and Day 9 (7.83 ± 2.15 mg/L vs 9.22 ± 2.68 mg/L) ($P < .05$); IL-1β in NT + TE group was significantly lower than that in NT group at Day 3 (18.16 ± 1.76 ng/mL vs 16.50 ± 1.78 ng/mL) ($P < .05$), Day 7 (10.46 ± 2.33 ng/mL vs 13.84 ± 1.08 ng/mL), and Day 9 (8.25 ng/mL ± 1.88 vs 11.28 ± 2.57 ng/mL) (both $P < .01$); IL-6 (49.14 ± 2.06 μg/mL vs 54.48 ± 2.32 μg/mL) in the NT + TE group was significantly lower than that in NT group at Day 3 ($P < .05$) as well as Day 5 (46.73 ± 2.35 μg/mL vs 48.64 ± 3.01 μg/mL), 7 (40.68 ± 1.87 μg/mL vs 45.46 ± 2.56 μg/mL), and

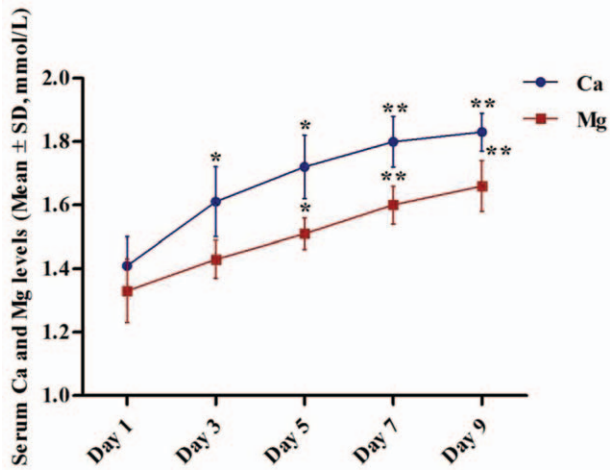

Figure 2. Changes of Ca and Mg levels in serum after treatment by trace elements. Data are shown as mean ± SD. Comparisons are made between Day 1 and Day 3, 5, 7, 9, and significance are marked. $^{*}P < .05$; $^{**}P < .01$. Ca = calcium, Mg = magnesium.

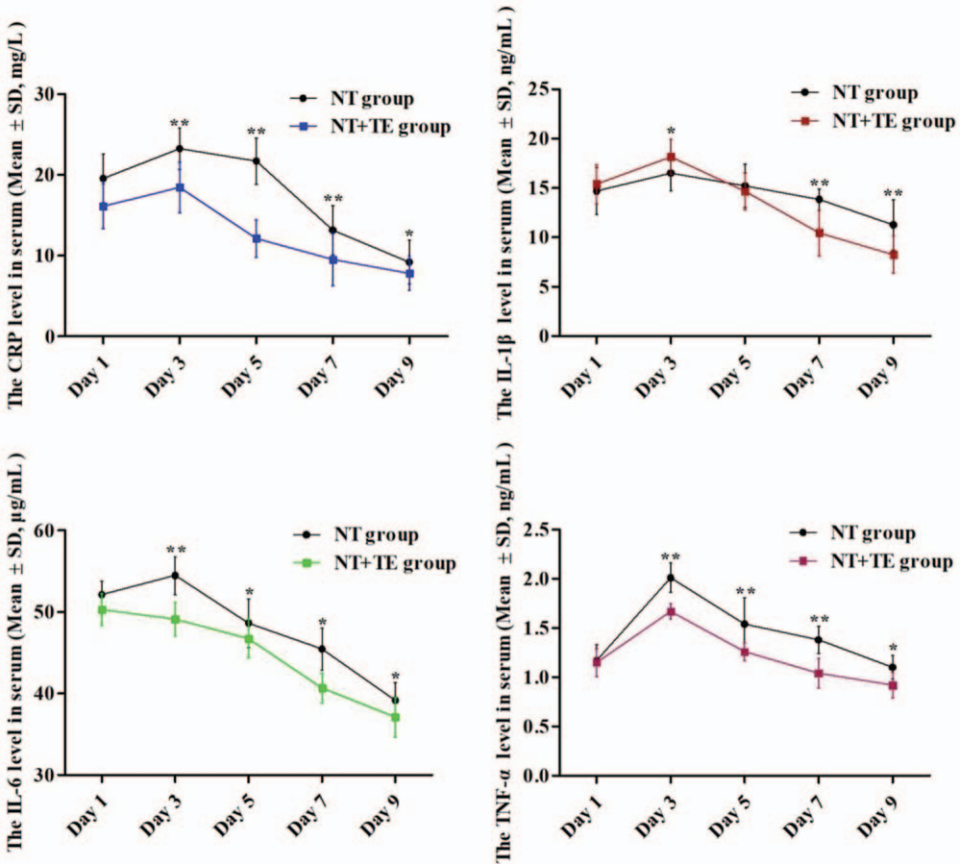

Figure 3. Alterations of serum inflammatory cytokines in the normal treatment and trace element treatment group. NT group: Normal treatment; NT + TE group: Normal treatment + Trace element. Comparisons are made between 2 groups ($^{*}P < .05$; $^{**}P < .01$).

Day 9 (37.12 ± 2.45 μg/mL vs 39.16 ± 2.19 μg/mL) (all $P < .01$); TNF-α in the NT + TE group was significantly lower than that in NT group at Day 3 (1.67 ± 0.08 ng/mL vs 2.01 ± 0.15 ng/mL), 5 (1.26 ± 0.09 ng/mL vs 1.54 ± 0.27 ng/mL), 7 (1.04 ± 0.15 ng/mL vs 1.38 ± 0.14 ng/mL) (all $P < .01$), and Day 9 (0.92 ± 0.13 ng/mL vs 1.10 ± 0.12 ng/mL) ($P < .05$).

Further we analyzed gender-specific changes in inflammatory cytokines, but without significance (data not shown). The correlation between inflammatory cytokines and ISS score was performed to find out whether these indices changes were associated with the injury degree (Table 2). Alterations of IL-6 in Day 3, 5, 7, and 9 were significantly positively with ISS score, indicating an elevating anti-inflammation response. Levels of CRP, IL-1β, and TNF-α were negatively with ISS score, suggesting sever injury correlates with pro-inflammatory environment.

4. Discussion

Fractures occurs commonly worldwide, and the cause of this problem is mostly unknown, possibly associated with age, nutrition, hormonal status, inadequate reduction, accompanying diseases, infections, drugs, and traumas.[15] Traffic accidents contribute to most common injuries and mainly found in joints and bones, which is complex and multiple.[16] Among them, the most serious injury is multiple fractures, which have the characteristics of large-scale, complexity and severity. In the present study, we found significantly negative correlations

Table 2

Spearman correlations between ISS score and inflammatory cytokines (n = 117).

	ISS score									
	Day 1		Day 3		Day 5		Day 7		Day 9	
Inflammatory indices	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
IL-6	−0.47	<.001	−0.41	<.001	−0.33	.004	−0.24	.059	−0.16	.073
CRP	−0.51	<.001	−0.35	<.001	−0.39	<.001	−0.15	.073	−0.04	.962
IL-β	−0.31	<.001	−0.24	.016	−0.18	.047	−0.11	.191	−0.13	.663
TNF-α	−0.27	<.001	−0.49	<.001	−0.26	.018	−0.18	.039	−0.12	.174

CRP = C-reactive protein, IL = interleukin, ISS = injury severity score, TNF-α = tumor necrosis factor alpha.

between serum Ca and Mg concentrations and ISS score at the beginning of hospitalization. It suggests that serious injury could bring large loss of TEs including Ca and Mg due to the blood bleeding or fluid imbalances, which may not be conducive to the body healing. Our result has a similar trend compared to other studies reporting that circulating TEs such as Cu, Se, and Fe are dramatically decreased in response to trauma in the early stage.[17]

The bone is the main storage site for Ca and Mg ions in the mammalian body. Bone fractures, especially when treated surgically, are associated with changes in the homeostasis of TEs.[18] Experimental studies have shown that TEs, a part of supplementing nutrition, have a positive effect on the healing of bone fractures.[19] However, the clinical application of supplementation of TEs in fracture patients to promote fracture healing and the associated molecular mechanisms remain to be elucidated. In this study, a short-term increase of Ca and Mg in the serum of fracture patients was observed after treatment by supplement of TEs. In comparison with day 1, we found increased Ca level at Day 3, 5, 7, and Day 9. A variety of studies have explored Ca supplementation as a population-based public health intervention to prevent fractures, especially in elderly populations.[20] A prior study investigating the serum Ca level by dietary intake among elderly Chinese above the age of 65 finds that a higher intake of Ca is significantly associated with higher serum Ca levels,[21] and serum Ca level has inverse association with risk of ischemic injury.[22] Supplementation with 1.2 g of Ca daily for 18 months in a population of very old women reduces the incidence of non-vertebral fractures by 30% and of hip fractures by 41%.[23] Mg is a major component of bone that plays an important role in several cellular processes. Evidence shows that insufficient serum Mg is independently associated with an increased risk of fractures,[24] and a combination of Mg with other nutritional chemicals induces more bone formation for fracture healing.[25] Oral Mg supplement may increase the bone mineral density and bone size to promote fracture healing.[26] In the present study, increased Mg concentration was detected in fracture patients after Day 5, 7, and Day 9 by TE supplement. This result has a similar trend with previous reports demonstrating that Mg supplementation within 30 days or above increases serum concentrations and bone Mg content.[27] It suggests that Mg supplement is associated with the circulating Mg balance that plays a pivotal role in the process of bone healing for fracture patients.

Fracture healing involves a complex interaction of cells and molecules, during which inflammatory response is a key factor for bone recovery, and interference with any of this process either promotes or more likely retards fracture healing.[28] There are increasing studies investigating the relationship between TE and inflammation because cellular responses of inflammation are mostly mediated by chemical factors derived from the action of the inflammatory stimulus on plasma or cells.[29] A previous study using an animal trauma model to assess the impact of TE supplementation on the inflammatory response shows that TE supplementation can improve the TE status and stabilize IL-6 and IL-10 against inflammatory response.[30] Epidemiology studies also have found that patients receiving TE supplementation could reduce markers of burn stress-induced inflammation such as IL-1β, INF-γ, and IL-6 with a result of shortening the length of stay in clinics, which indicated that TE supplementation may alleviate inflammation to prompt recovery.[31] Our results found that after 3 days of TE supplementation, inflammatory cytokines of CRP, IL-1β, IL-6, and TNF-α among surgical patients were significantly lower that of the NT. The correlation between inflammatory cytokines and ISS score was performed to find out whether these indices changes were associated with the injury degree. Levels of IL-6, CRP, IL-β, and TNF-α were statistically negatively with ISS score in the first few days, suggesting sever injury correlates with pro-inflammatory environment. Cytokines of CRP, IL-1β, IL-6, and TNF-α served as pro-inflammatory role are essential mediators of immune response and inflammatory reaction.[32] Currently, although there is insufficient evidence in effects of TE supplementation on fracture patients, existed research has discovered that TE supplementation is helpful for disease recovery such as obstructive pulmonary disease,[33] autistic spectrum disorder,[34] bowel disease,[35] and even cancers.[36] Disordered expressions in IL-1β, IL-6, and TNF-α have been deemed as effective inflammatory biomarkers linking with the development and process of fractures.[37] Results in our study demonstrate that TE supplementation is associated with the inflammatory changes in of CRP, IL-1β, IL-6, and TNF-α in fracture patients.

There are also some limitations in this study. First, subjects in this study are limited within certain age, resulting in a failure of comparison in different population as injury recovery may vary in different ages. Second, we are unable to eliminate the disruption of food intake during the supplement of TE. Third, we do not recruit enough subjects characterized by their fracture locations, so we cannot compare the surgical effect between different sources of fractures after TEs supplementation. In addition, sample size is not sufficient, and we only observed in short time. Even though there might be some biases, we still find the protective tendency of TE supplement on bone fractures by mediating the inflammatory cytokines. In total, we find that decreased Ca and Mg is related to injury degree in accidentally fracturing patients. Elevated Ca and Mg and reduced inflammatory cytokines in peripheral circulation are observed after a short-term treatment by TEs. These changes suggest that TEs supplementation may be an effective way against inflammation, which is conducive to the recovery of bone fractures.

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Author contributions

Conceptualization, G.X. and Y.S.; methodology, Y.S., L.X., X.J., D.C., and X.J.; data analysis, L.X. and G.X.; writing-original draft preparation, review and editing, L.X. and G.X.; project administration, G.X., L.X., and Y.S.; funding acquisition, G.X. All authors have read, and confirm that they meet, ICMJE criteria for authorship.

Conceptualization: Yongxing Song, Guangtao Xu.
Data curation: Yongxing Song, Deqing Chen.
Investigation: Guangtao Xu.
Methodology: Yongxing Song, Long Xu, Xin Jin, Deqing Chen, Xiuhui Jin, Guangtao Xu.
Resources: Yongxing Song.
Software: Long Xu, Xin Jin.
Visualization: Guangtao Xu.

Writing – original draft: Long Xu, Deqing Chen, Xiuhui Jin, Guangtao Xu.
Writing – review & editing: Long Xu, Guangtao Xu.

References

[1] Weber RA, Yen FS, Nicholson SPV, et al. Maintaining iron homeostasis is the key role of lysosomal acidity for cell proliferation. Mol Cell 2020;77:645–55.e7.
[2] He S, Zhuo L, Cao Y, et al. Effect of cadmium on osteoclast differentiation during bone injury in female mice. Environ Toxicol 2020;35:487–94.
[3] Weivoda MM, Chew CK, Monroe DG, et al. Identification of osteoclast-osteoblast coupling factors in humans reveals links between bone and energy metabolism. Nat Commun 2020;11:87.
[4] Morton AB, Norton CE, Jacobsen NL, Fernando CA, Cornelison DDW, Segal SS. Barium chloride injures myofibers through calcium-induced proteolysis with fragmentation of motor nerves and microvessels. Skelet Muscle 2019;9:27.
[5] Datta M, Schwartz GG. Calcium and vitamin D supplementation and loss of bone mineral density in women undergoing breast cancer therapy. Crit Rev Oncol Hematol 2013;88:613–24.
[6] Xing T, Hu Y, Wang B, Zhu J. Role of oral calcium supplementation alone or with vitamin D in preventing post-thyroidectomy hypocalcaemia: a meta-analysis. Medicine (Baltimore) 2019;98:e14455.
[7] de Baaij JH, Hoenderop JG, Bindels RJ. Magnesium in man: implications for health and disease. Physiol Rev 2015;95:1–46.
[8] van Dronkelaar C, van Velzen A, Abdelrazek M, van der Steen A, Weijs PJM, Tieland M. Minerals and sarcopenia; the role of calcium, iron, magnesium, phosphorus, potassium, selenium, sodium, and zinc on muscle mass, muscle strength, and physical performance in older adults: a systematic review. J Am Med Dir Assoc 2018;19:6–11.e3.
[9] Hu B, Wu YH, Tong F, et al. Apocynin alleviates renal ischemia/reperfusion injury through regulating the level of zinc and metallothionen. Biol Trace Elem Res 2017;178:71–8.
[10] Orchard TS, Larson JC, Alghothani N, et al. Magnesium intake, bone mineral density, and fractures: results from the Women's Health Initiative Observational Study. Am J Clin Nutr 2014;99:926–33.
[11] Toosi S, Behravan J. Osteogenesis and bone remodeling: a focus on growth factors and bioactive peptides. Biofactors 2020;46:326–40.
[12] Glass GE, Chan JK, Freidin A, Feldmann M, Horwood NJ, Nanchahal J. TNF-alpha promotes fracture repair by augmenting the recruitment and differentiation of muscle-derived stromal cells. Proc Natl Acad Sci U S A 2011;108:1585–90.
[13] Jamilian M, Mirhosseini N, Eslahi M, et al. The effects of magnesium-zinc-calcium-vitamin D co-supplementation on biomarkers of inflammation, oxidative stress and pregnancy outcomes in gestational diabetes. BMC Pregnancy Childbirth 2019;19:107.
[14] Xu G, Su R, Li B, et al. Trace element concentrations in human tissues of death cases associated with secondary infection and MOF after severe trauma. Biol Trace Elem Res 2015;168:335–9.
[15] Serbest S, Tiftikçi U, Tosun HB, Kısa Ü. The irisin hormone profile and expression in human bone tissue in the bone healing process in patients. Med Sci Monit 2017;23:4278–83.
[16] Serbest S, Tiftikci U, Tosun HB, Gumustas SA, Uludag A. Is there a relationship between fracture healing and mean platelet volume? Ther Clin Risk Manag 2016;12:1095–9.
[17] Chen K, Lv J, Wang G, et al. Changes of serum trace elements in early stage trauma and its correlation with injury severity score. Medicine (Baltimore) 2018;97:e10077.
[18] Galusha AL, Howard LJ, Kruger PC, Marks T, Parsons PJ. Bone mineral composition among long-term parenteral nutrition patients: postmortem assessment of calcium, phosphorus, magnesium, and select trace elements. JPEN J Parenter Enteral Nutr 2020;45:175–82.
[19] Notomi T, Kuno M, Hiyama A, et al. Role of lysosomal channel protein TPC2 in osteoclast differentiation and bone remodeling under normal and low-magnesium conditions. J Biol Chem 2017;292:20998–1010.
[20] Kahwati LC, Weber RP, Pan H, et al. Vitamin D, calcium, or combined supplementation for the primary prevention of fractures in community-dwelling adults: evidence report and systematic review for the US Preventive Services Task Force. JAMA 2018;319:1600–12.
[21] Lee WP, Lin LW, Yeh SH, Liu RH, Tseng CF. Correlations among serum calcium, vitamin D and parathyroid hormone levels in the elderly in southern Taiwan. J Nurs Res 2002;10:65–72.
[22] Dibaba DT, Xun P, Fly AD, et al. Calcium intake and serum calcium level in relation to the risk of ischemic stroke: findings from the REGARDS study. J Stroke 2019;21:312–23.
[23] Listed N. Supplementation with vitamin D3 and calcium prevents hip fractures in elderly women. Nutr Rev 1993;51:183–5.
[24] Venkatraman SK, Swamiappan S. Review on calcium- and magnesium-based silicates for bone tissue engineering applications. J Biomed Mater Res A 2020;108:1546–62.
[25] Wang Z, Wang X, Tian Y, et al. Degradation and osteogenic induction of a SrHPO4-coated Mg-Nd-Zn-Zr alloy intramedullary nail in a rat femoral shaft fracture model. Biomaterials 2020;247:119962.
[26] Duale C, Cardot JM, Joanny F, et al. An advanced formulation of a magnesium dietary supplement adapted for a long-term use supplementation improves magnesium bioavailability: in vitro and clinical comparative studies. Biol Trace Elem Res 2018;186:1–8.
[27] Matsuzaki H, Fuchigami M, Miwa M. Dietary magnesium supplementation suppresses bone resorption via inhibition of parathyroid hormone secretion in rats fed a high-phosphorus diet. Magnes Res 2010;23:126–30.
[28] Ragipoglu D, Dudeck A, Haffner-Luntzer M, et al. The role of mast cells in bone metabolism and bone disorders. Front Immunol 2020;11:163.
[29] van Swelm RPL, Wetzels JFM, Swinkels DW. The multifaceted role of iron in renal health and disease. Front Immunol 2020;11:163.
[30] Yuan WA, Yu XJ, Liu FQ, Wang HP, Wang D, Lai XP. Effects of trace element supplementation on the inflammatory response in a rabbit model of major trauma. J Trace Elem Med Biol 2010;24:36–41.
[31] Rehou S, Shahrokhi S, Natanson R, Stanojcic M, Jeschke MG. Antioxidant and trace element supplementation reduce the inflammatory response in critically ill burn patients. J Burn Care Res 2018;39:1–9.
[32] Gupta J, Mitra N, Kanetsky PA, et al. Association between albuminuria, kidney function, and inflammatory biomarker profile in CKD in CRIC. Clin J Am Soc Nephrol 2012;7:1938–46.
[33] Chang HH, Chen PS. Inflammatory biomarkers for mood disorders - a brief narrative review. Curr Pharm Des 2020;26:236–43.
[34] Skalny AV, Simashkova NV, Skalnaya AA, et al. Trace element levels are associated with neuroinflammatory markers in children with autistic spectrum disorder. J Trace Elem Med Biol 2018;50:622–8.
[35] Mohammadi E, Qujeq D, Taheri H, Hajian-Tilaki K. Evaluation of serum trace element levels and superoxide dismutase activity in patients with inflammatory bowel disease: translating basic research into clinical application. Biol Trace Elem Res 2017;177:235–40.
[36] Lin Y, Wu C, Yan W, Guo S, Liu B. T five serum trace elements associated with risk of cardia and noncardia gastric cancer in a matched case-control study. Cancer Manag Res 2020;12:4441–51.
[37] Che J, Yang J, Zhao B, et al. The effect of abnormal iron metabolism on osteoporosis. Biol Trace Elem Res 2020;195:353–65.