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ZINC INCREASES THE EFFECTS OF ESSENTIAL AMINO ACIDS-WHEY PROTEIN SUPPLEMENTS IN FRAIL ELDERLY

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Abstract: Protein undernutrition is frequent in the elderly. It contributes to the development of osteoporosis, possibly via lower IGF-I. Dietary zinc can influence IGF-I production. *Objectives:* To determine the influence of dietary zinc addition on IGF-I and bone turnover responses to essential amino acids-whey (EAA-W) protein supplements in frail elderly. *Design and setting:* A daily oral protein supplement was given to hospitalized patients for 4 weeks. On a randomized, double-blind basis, patients received either an additional 30 mg/day of zinc or control. *Participants:* Sixty-one hospitalized elderly aged 66.7 to 105.8, with a mini-nutritional assessment score between 17 and 24 were enrolled. *Measurements:* Activities of daily living; dietary intakes; serum IGF-I, IGF-BP3, CrossLaps™, osteocalcin and zinc were measured before and after 1, 2 and 4 weeks of protein supplementation. *Results:* Serum IGF-I rapidly increased in both groups. Zinc accelerated this increase with changes of +48.2±14.3 and +22.4±4.7% ($p<.05$) by 1 week, in the zinc-supplemented and control groups, respectively. Zinc significantly decreased the serum bone resorption marker CrossLaps™ by already 1 week. Activities of daily living improved by +27.0±3.1 and +18.3±4.5% in zinc-supplemented and control groups, respectively.*Conclusion:* In the elderly, zinc supplementation accelerated the serum IGF-I response to EAA-W protein by 1 week and decreased a biochemical marker of bone resorption.

Key words: Osteoporosis, fracture, bone remodeling, nutrition, ageing.

Introduction

Undernutrition, particularly protein deficiency, is often encountered in the elderly population (1, 2). Whilst a reduction in caloric intake may accompany the decrease in energy expenditure in the elderly, it appears that sufficient protein intakes are mandatory for bone health (3). A concurrent reduction of dietary protein with aging could be detrimental for the preservation of bone integrity. A variety of cross-sectional and longitudinal studies have shown a positive association between bone mineral mass and dietary protein intake (4-8). Dietary proteins influence both the production and action of IGF-I and thereby the effect of IGF-I on bone anabolism (3, 9, 10). In preclinical studies, an isocaloric low protein diet decreases areal bone mineral density (BMD) at the levels of skeletal sites formed by trabecular and cortical bone (11, 12), alters trabecular microarchitecture, renders bone cortices thinner (13) and modifies intrinsic bone tissue quality as evaluated by a nanoindentation test (14). These various changes result in a decrease of bone strength. In humans, these alterations could contribute to an increased fracture risk (3, 15).

In terms of molecular mechanisms, an isocaloric low protein diet is associated with increased bone resorption and decreased bone formation, reduced IGF-I production, and an accompanying resistance of bone to the effects of both IGF-I and growth hormone (16, 17). An altered gonadotrop axis in both males and females has also been documented (11, 12). The bone catabolic effects of an isocaloric low protein diet seem to be a TNF-alpha-dependent process (18).

Protein repletion corrects the alterations caused by protein undernutrition. Indeed, in elderly patients with a recent hip fracture, protein supplements decrease the rates of medical complications (19, 20), reduce accelerated bone loss (21, 22), and lower the duration of stay in rehabilitation care facilities by 25% (21). These observations have been associated with an increase in circulating serum IGF-I levels. In preclinical studies, isocaloric essential amino acid supplements correct bone mechanical properties by improving microarchitecture (connectivity and cortical thickness), and intrinsic bone tissue quality (13, 14). The correction of protein deficiency also reverses the alterations in both the somatotrop and gonadotrop axes, and in bone remodeling.

Zinc is known to play a role in IGF-I production (23, 24). Circulating levels of IGF-I increase with dietary zinc supplementation (25, 26). Growth retardation related to zinc deficiency is associated with low serum IGF-I concentrations, inhibition of the anabolic actions of IGF-I (27) and with a decreased expression of the IGF-I and GH receptor genes (27, 28). A meta-analysis of 25 studies investigating the effects of zinc supplementation on growth in prepubertal children showed a highly statistically significant effect on linear growth and weight gain (29). On the other hand, decreased zinc reserves are observed in undernourished subjects (30, 31). The presence of zinc inhibits the stimulatory effect of regucalcin, a regulatory protein involved in intracellular signalling on osteoclast-like cell formation (32). Several studies have shown that zinc has a distinct effect on bone metabolism by inhibiting bone resorption and stimulating bone formation (32-36).

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The relationship between zinc status and IGF-I in the elderly has not been established, although the correlation between serum IGF-I concentrations and zinc intake was significantly better than that with protein intake or age in a group of healthy community-dwelling post-menopausal women (24).

In the present study, we assessed the effect of zinc supplements on circulating IGF-I levels, bone remodeling and physical performance responses to protein repletion in undernourished, elderly patients. The results indicate that zinc supplementation accelerates the serum IGF-I response to essential amino acids-whey protein administration, and is associated with a decrease in the serum levels of a biochemical marker of bone resorption and improved functional performance.

Patients and methods

Patients

Within one week after admission to a tertiary care geriatric hospital, elderly patients of both sexes were recruited between October 1999 and August 2001. Admissions were due to cardiovascular and lung diseases (27%), osteoarticular disorders (16%), infections (13%), neurological diseases (10%), falls, psychiatric disorders or skin diseases (34%). Inclusion criteria consisted of ability to give a written, informed consent and a mini nutritional assessment score between 17 and 24 (corresponding to a failure to thrive category) (37). Exclusion criteria were: severely decompensated cardiac, pulmonary, renal (creatinine clearance <30 ml/min) or hepatic failure; major mental impairment (minimal mental state score <17), urine incontinence, inflammatory syndrom (C-reactive protein >15mg/l), bedridden, corticosteroids at a dose greater than 7.5 mg/day prednisone-equivalent, life expectancy estimated as shorter than one month. All patients were given a daily 20 g oral protein supplement containing 15g whey protein and 5g essential amino acids (in a proportion similar to that found in casein), and 550mg calcium (provided by Novartis Nutrition, Berne, Switzerland). The protein supplement was administered in the evening preferentially, or fractionated throughout the day, for a period of 4 weeks. Using a random number table, the patients were assigned to 30mg/day oral zinc directly added to the protein supplements powder, or no addition, on a strict double-blind basis. Non compliance was defined by consumption of less than 50% of the powder. The patients were stratified into two arms according to baseline serum albumin levels, with a limit between strates of 30 g/l in a fully hydrated state. A group of 8 patients fulfilling the same inclusion and exclusion criteria, but who did not want to participate in an intervention study, were followed at a 4-week interval.

Clinical data

We evaluated clinical status, dietary intakes, activities of daily living (38) and medical treatments at baseline (within one week after admission), and after 1, 2 and 4 weeks of nutritional supplementation. Compliance, incidence of medical complications and side effects of treatment (diarrhea, nausea), and length of hospital stay were recorded.

Biochemical analysis

Fasting venous blood and second voiding urine samples were collected at baseline, and after 1, 2 and 4 weeks of nutritional supplements. Radioimmunoassays, immuno-radiometric assays and enzyme-linked immunoassay were used to measure calcidiol (Incstar Corp, Stillwater, MN), intact parathormone (Nichols institute, San Juan Capistrano, CA), IGF-I after acid-ethanol extraction (Nichols institute, San Juan Capistrano, CA), IGF-binding protein-3 (DSL, Webster, Texas), osteocalcin (Cis-Bio, Gif-sur-Yvette, France), and CrossLaps™ (Roche-diagnostic, Lucerne, Switzerland). Calcitriol was determined using a protein-binding assay (Incstar Corp, Stillwater, MN). Albumin, protein, total calcium, phosphate, creatinine and C-reactive protein were measured using routine laboratory methods. Ionized calcium was measured using a specific electrode. Zinc was determined using the Randox colorimetric method (automate COBAS INTEGRA 700, Roche-diagnostic). Differential white cell counts were determined at weeks 0, 1, 2, and 4. Renal tubular transports of calcium and phosphate were measured with fasting serum and urine values, using nomograms as previously described (39, 40).

Statistical analysis

Values are means ± SEM. Level of significance was evaluated using an unpaired two-tailed student t-test, when two groups were compared, an ANOVA for repeated measurements was used to evaluate evolution over time and a Wilcoxon rank test was employed as an alternative to the paired student t-test to compare two groups, when the values were not normally distributed.

Results

Trial outline

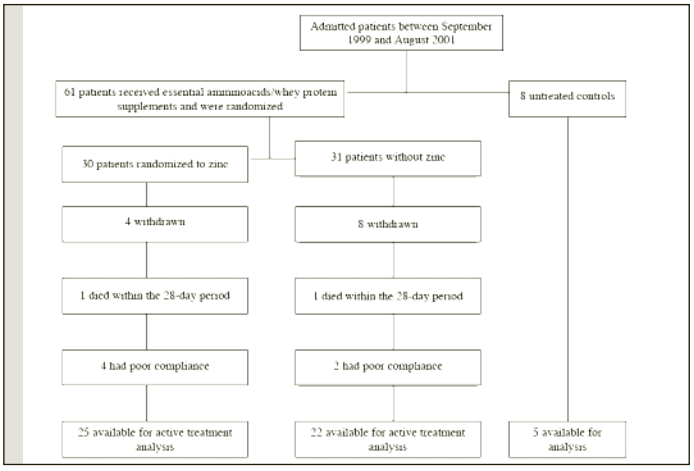
We recruited 61 patients into this randomized, double-blind trial and 8 untreated controls. During the 28-day intervention period, 14 (23%) patients dropped out, 12 due to nausea, low compliance, refusal to pursue the trial at home and 2 deaths (one at home from bronchopneumonia and one in hospital from bronchoaspiration). For the final analysis, 47 patients and 5 untreated controls were available (Fig.1). Twenty-seven patients returned home before the end of the study. Blood and urine samples were collected in their private accommodation.

Baseline characteristics

85% of enrolled patients were women and the mean age was 85.0 ± 7.4 years (mean ± SD, range: 66.7 to 105.8). The different diagnoses were equally distributed in both groups (data not shown). Baseline characteristics were similar in the randomized groups (Table 1). Dietary energy intake was 1228 ± 7.0 Kcal/day (5140 KJ) with 43 ± 3 g/day protein,

representing 0.77 ± 0.03 g/kg BW, with no differences between the two supplemented groups. Body mass index was significantly higher in the untreated controls. Whilst there were no differences in biochemical baseline characteristics, including zinc levels, between the patients in the randomized controlled trial and the untreated controls, the group with zinc supplements had a higher serum level of calcidiol (p=.029) than the group without zinc (Table 2). Except mean serum albumins which were below the lower limit of normal range, confirming the state of malnutrition of the randomized patients, all values were within the reference range.

Figure 1
Trial outline



Among patients admitted into a tertiary care geriatric hospital between September 1999 and August 2001, 69 patients were included in the study (61 in the randomized, double blind trial)

Table 1
Patients Baseline Characteristics

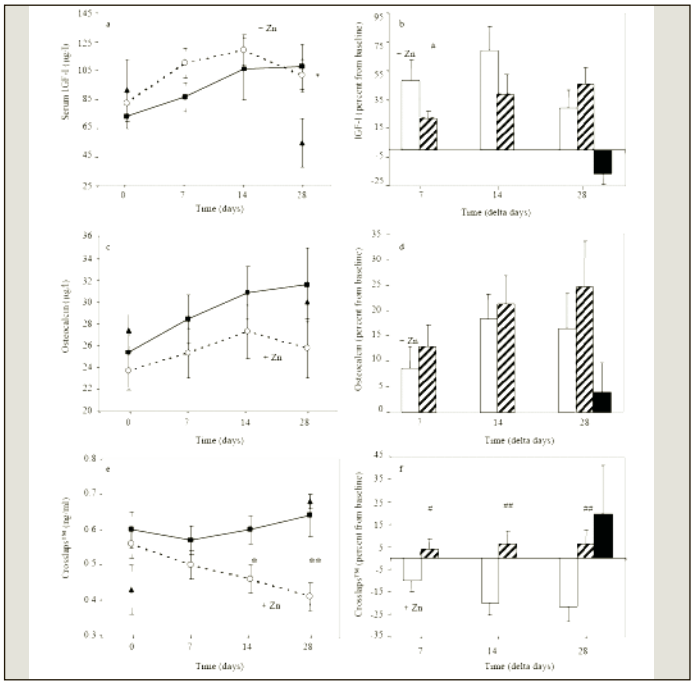
	Normal range	EAA / whey protein with zinc (30mg/d) (n=30)	EAA / whey protein without zinc (n=31)	Untreated controls (n=8)
Age (year)		83.6 (1.3)	86.4 (1.3)	85.0 (2.6)
F/M		25/5	27/4	7/1
Previous accommodation[a]		9 (30%)	13 (42%)	2 (25%)
- private accommodation		21 (70%)	18 (58%)	6 (75%)
- hospital				
Biochemistry				
Protein –adjusted calcium (mmol/l)	2.2-2.6	2.42 (0.02)	2.39 (0.02)	2.32 (0.02)
Ionised calcium (mmol/l)	1.12-1.32	1.20 (0.01)	1.19 (0.01)	1.19 (0.03)
Phosphate (mmol/l)	0.80-1.30	1.02 (0.03)	1.04 (0.03)	1.00 (0.05)
Creatinine (µmol/l)	45.0-98.0	74.9 (4.8)	74.0 (4.2)	72.2 (10.7)
Albumin (g/l)	35.0-52.0	31.0 (0.7)	30.4 (0.8)	33.7 (2.7)
Protein (g/l)	61.0-82.0	64.4 (1.0)	61.9 (0.8)	66.8 (2.5)
Lymphocytes Count (G/l)		1.5 (0.1)	1.5 (0.1)	1.5 (0.2)
CRP (mg/l)	0-10	8.1 (1.6)	11.4 (2.0)	3.5 (1.6)
BRI (mmol/mmol)[b]	0.10-0.50	0.44 (0.07)	0.50 (0.06)	0.42 (0.15)
TmPi/GFR (mmol/l GFR)[c]	0.80-1.35	0.97 (0.04)	0.99 (0.05)	1.03 (0.12)
TRCaI (mmol/l GFR)[d]	2.40-2.90	2.67 (0.03)	2.61 (0.04)	2.60 (0.07)

Values are given as the mean (SEM), except for the previous accommodation. EAA = Essential amino acids. a. Values are given as the absolute number (percentage); b. Bone Resorption Index (BRI) is the fasting urinary calcium-to-creatinine ratio taken as a reflection of net bone resorption; c. Renal tubular reabsorption of phosphate; d. Renal tubular reabsorption of calcium index.

Effects of zinc and / or essential amino acids-whey protein supplements on plasma IGF-I

Zinc levels increased by 57.5% (percent change from baseline) in the zinc-supplemented group after 28 days (Table 2). IGF-I levels decreased in the untreated controls during the 4-week observation period. In contrast, IGF-I rapidly increased in the two groups with essential amino acids-whey protein supplementation (p=.0001) reaching a plateau by only the second week of supplementation (Fig. 2). The addition of 30 mg/day of zinc accelerated the steep elevation of serum IGF-I, compared to the group without zinc, with a response significantly higher at week 1 (p=.027). At 4 weeks, there was no difference with or without zinc addition. IGF binding protein 3 concentrations remained constant, so that IGF-I to IGF binding protein 3 ratio exhibited kinetics similar to that of IGF-I alone which reflects hormone bioactivity (Table 2) (41).

Figure 2



a) b) Effects of zinc addition on the IGF-I response to essential amino acids-whey protein supplements. The results are means SEM [absolute values a) and percent from baseline b)] and were obtained after 1, 2 and 4 weeks of nutritional supplements containing 20 g of protein (15g whey protein and 5 g of essential amino acids) without zinc (a: closed squares; b: stippled columns) or with zinc (a: open squares; b: open columns). Untreated controls are represented by triangles (a) and filled columns (b). # p = 0.0001: ANOVA for repeated measures; there is a significant increase overtime in both groups receiving amino acids-whey protein; * p < 0.05 vs controls without zinc.
c) d) Effects of zinc addition on the serum osteocalcin response to essential amino acids-whey protein supplements. The results are means SEM [absolute values c) and percent from baseline d)] and were obtained after 1, 2 and 4 weeks of nutritional supplements containing 20 g of protein (15 g whey protein and 5 g of essential amino acids) without zinc (c: closed squares; d: stippled columns) or with zinc (c: open squares; d: open columns). Untreated controls are represented by triangles (c) and filled columns (d).
e) f) Effects of zinc addition on the serum CrossLaps™ response to essential amino acids-whey protein supplements. The results are means SEM [absolute values e) and percent from baseline f)] and were obtained after 1, 2 and 4 weeks of nutritional supplements containing 20 g of protein (15g whey protein and 5 g of essential amino acids) without zinc (e: closed squares; f: stippled columns) or with zinc (e: open squares; f: open columns). Untreated controls are represented by triangles (e) and filled columns (f). * p < 0.05, ** p < 0.005: ANOVA for repeated measures; it showed a significant difference between zinc supplemented and the unsupplemented group. * p < 0.05, ** p < 0.01.

Table 2
Changes in Biochemical Variables

	Normal Range	Group	D0	D7	D14	D28
Zinc (µmol/l)	10.0-23.0	prot+zn[1]	12.9 ± 0.8	13.5 ± 0.4 (+29.0%)	14.5 ± 1.2 (+38.4%)	16.0 ± 0.9 (+57.5%)
		prot[2]	14.1 ± 2.7	13.4 ± 2.4 (-4.4%)	13.3 ± 2.5 (-6.5%)	14.4 ± 3.1 (+3.6%)
		untreated[3]	NA	NA	NA	NA
IGF-I (µg/l)	41.0-213.0	prot+zn	82.6 ± 9.0	110.3 ± 10.3 (+48.2%)#	119.3 ± 10.7 (+69.2%)	101.5## ± 11.2 (+29.2%)
		prot	73.3 ± 8.3	86.6 ± 10.1 (+22.4%)	106.1 ± 21.5 (+38.7%)	107.8## ± 15.5 (+45.8%)
		untreated	91.4 ± 21.5	NA	NA	54.8 ± 16.8 (-16.8%)
IGF-BP3 (µg/l)		prot+zn	2.2 ± 0.1	2.3 ± 0.1 (+6.5%)	2.3 ± 0.1 (+7.0%)	2.3 ± 0.1 (+7.1%)
		prot	2.1 ± 0.1	2.2 ± 0.1 (+7.2)	2.3 ± 0.1 (+12.4%)	2.4 ± 0.1 (+14.6%)
		untreated	1.9 ± 0.2	NA	NA	1.5 ± 0.2 (-11.2%)
IGF-I / IGF-BP3		prot+zn	35.6 ± 2.5	45.9 ± 3.3 (+37.3%)	50.2 ± 4.0 (+53.4%)	41.7 ± 3.2 (+18.2%)
		prot	33.6 ± 2.7	36.9 ± 3.3 (+13.1%)	41.7 ± 4.5 (+23.0%)	41.6 ±4.2 (+21.6%)
		untreated	45.4 ± 8.0	NA	NA	26.8 ±7.2 (-8.3%)
Osteocalcin (µg/l)	8.0-40.0	prot+zn	23.7 ± 1.7	25.3 ± 2.2 (+12.8%)	27.3 ± 2.4 (+18.3%)	25.8 ± 2.7 (+16.4%)
		prot	25.4 ± 1.7	28.4 ± 2.2 (+6.0%)	30.8 ± 2.4 (+21.4%)	31.6 ± 3.4 (+21.5%)
		untreated	27.4 ± 1.6	NA	NA	30.0 ± 2.3 (+3.8%)
Crosslaps ™ (ng/ml)	0.50-0.92	prot+zn	0.56 ± 0.04	0.5 ± 0.04 (-10.3%) ‡	0.46* ± 0.04 (-20.1%) ‡‡	0.41** ± 0.04 (-21.5%) ‡‡
		prot	0.60 ± 0.05	0.57 ± 0.04 (+3.9%)	0.60 ± 0.04 (+6.29%)	0.64 ± 0.06 (+6.1%)
		untreated	0.43 ± 0.07	NA	NA	0.68 ± 0.02 (+74.7%)
Intact PTH (pmol/l)	1.0-6.0	prot+zn	5.2 ± 0.4	NA	NA	5.0 ± 0.5 (+0.4%)
		prot	5.7 ± 0.7	NA	NA	4.2 ± 0.4 (-14.8%)
		untreated	4.3 ± 0.9	NA	NA	5.3 ± 1.7 (+18.5%)
Calcidiol (nmol/l)[a]	20.-95.0	prot+zn	55.9 ± 5.4	NA	NA	70.4 ± 6.7 (+39.1%)
		prot	39.7 ± 4.9 §	NA	NA	56.2 ± 5.9 (+68.8%)
		untreated	67.6 ± 12.4	NA	NA	70.0 ± 11.5 (+20.5%)
Calcitriol (pmol/l)[b]	40.0-140.0	prot+zn	90.4 ± 9.7	NA	NA	92.1 ± 6.6 (+17.4%)
		prot	75.0 ± 8.1	NA	NA	88.1 ± 6.8 (+25.5%)
		untreated	NA	NA	NA	NA

All values are given as the mean ± SEM. Values ( ): percent change from baseline within the group. NA = not assessed. 1) prot +zn: Essential amino acids / whey protein with zinc (30mg/d), n=30. 2) prot: Essential amino acids / whey protein without zinc, n=31. 3) untreated: Untreated control, n=8. a) 25-hydroxyvitamin D. b) 1, 25-dihydroxyvitamin D; # p <.05: unpaired two tailed student t-test; as compared the zinc supplemented group with the unsupplemented subjects; ## p=.0001: ANOVA for repeated measures; there is a significant increase overtime in both groups receiving amino acids-whey protein. * p<.05, ** p<.005: ANOVA for repeated measures; it showed a significant difference between zinc supplemented and the unsupplemented group. ‡ p<.05, ‡‡ p<.005: unpaired two tailed student t-test; as compared the zinc supplemented group with the unsupplemented subjects. § p < .05 zinc supplemented vs unsupplemented.

Effects of zinc and / or essential amino acids-whey protein supplements on biochemical markers of bone remodeling and on calcium-phosphate metabolism

In response to essential amino acids-whey protein supplements, the bone formation marker osteocalcin increased in the two supplemented groups, reaching a maximum by the second week of supplementation (Fig. 2). There was no change in untreated controls. Zinc did not affect the osteocalcin response to protein repletion.

The bone resorption marker CrossLaps™ increased in the untreated controls, remained stable in the essential amino acids-whey protein group, and significantly decreased upon zinc supplementation (Fig. 2), with significant differences between the two supplemented groups, detectable at just one week of supplementation.

Although the increase of dietary protein intake may improve calcium intestinal absorption (42), plasma levels of parathyroid hormone, 25-hydroxyvitamin D3 and 1,25-dihydroxyvitamin D3 (Table 2), calcium (total, ionised and protein-adjusted), phosphate, proteins and creatinine (data not shown) did not change during the study and did not differ between the two groups who received similar amount of protein and calcium. The renal handling of calcium or of phosphate was also not influenced.

Effects of zinc and/or essential amino acids-whey protein supplements on functional performances

There was no difference in the dietary intakes recorded by the dietician between the two groups and at the end of the study. The number of kilocalories was, in the supplemented group with additional zinc, 1237±126 at the beginning and 1096±133 at the end, and in the other protein supplemented group 1219±280 and 1292±350, respectively. There was a small but not significant decrease in spontaneous protein intake in the supplemented group with additional zinc at the end of the trial but without any difference comparing the two supplemented groups.

Functional performances were evaluated by recording the Activity of Daily Living score (38). This index slightly improved upon essential amino acids-whey protein supplements, irrespective of zinc addition (Table 3). However, in a prespecified subgroup with baseline serum albumin lower than 30g/l, zinc significantly increased this response. Hospital stay averaged 42.5±3.5 days and was not different among the groups (Table 3).

Table 3
Changes at 28 Days in Functional Performances Variables

		EAA / whey protein with zinc (30mg/d)			EAA / whey protein without zinc			Untreated controls
		All n = 30	alb<30g/l n=10	alb>30g/l n=20	All n=31	alb<30g/l n=13	alb>30g/l n=18	n=8
ADL score[a]	D0	10.6 (0.5)	11.6 (1.0)	10.1 (0.6)	10.5 (0.5)	10.2 (0.7)	10.8 (0.7)	NA
	D28	7.7 (0.3)	7.6 (0.6)	7.8 (0.4)	8.4 (0.6)	8.6 (0.9)	8.1 (0.8)	NA
	Percent change	-27.0 (3.1)**	-25.7 (8.1)*	-27.4 (3.2)	-18.3 (4.5)**	-11.9 (6.5)	-24.7 (5.5)	NA
BMI[b]	D0	22.1 (1.1)	21.8 (1.3)	22.2 (1.5)	21.7 (0.7)	21.7 (1.4)	21.8 (0.9)	26.2 (1.0) #
	D28	22.7 (2.1)	22.5 (2.1)	22.8 (3.2)	21.4 (1.2)	21.4 (1.9)	21.5 (1.5)	25.7 (1.0)
	Percent change	-0.5 (0.7)	-0.8 (1.3)	-0.4 (0.9)	-1.3 (0.8)	-1.5 (0.8)	1.1 (1.6)	-1.9 (1.5)
Hospital stay (day)		45.4 (5.7)	52.1 (7.2)	42.1 (7.8)	39.7 (4.3)	48.2 (9.0)	33.7 (3.4)	39.3 (8.8)

Values are given as the mean (SEM); EAA = Essential amino acids; NA= not assessed; alb= albumin. a. Activity of daily living according to Katz (38). This score estimates the degree of dependency for bathing, dressing, going to the toilet, transferring from bed, continence and feeding according to a scale from 6 to 18 (with 6 as complete independence). b. Body Mass Index [weight (kg) / height2 (m2)]. ** p=.001 (ANOVA for repeated measurement) in the two supplemented groups (with and without zinc); there is a significant increase overtime in both groups. * p=.006 (Wilcoxon rank test), as compared to the group without zinc supplement and with an albumin < 30g/l. # p < .05 comparing the untreated group vs the whole supplemented group

Discussion

In the present study, we investigated whether the addition of zinc, which is known to influence IGF-I production, is able to modify the IGF-I response to essential amino acids-whey protein supplements in undernourished elderly patients. The results indicate that a zinc supplement significantly accelerated IGF-I recovery and reduced the serum levels of a biochemical marker of bone resorption. Activities of daily living were significantly improved by zinc supplementation in the sub-group of patients with lower serum albumin.

Protein malnutrition is often observed in the elderly and is associated with a large series of morbidities, including osteoporosis and impaired post-fracture recovery (3, 8). Previous randomized controlled trials in recent hip fracture patients have demonstrated that a simple oral casein protein supplement given for periods between 5 weeks and 6 months, that just corrected protein undernutrition, not only improved fracture outcome (19-21, 43), but also attenuated proximal femur bone loss (21). These findings were associated with increased serum IGF-I levels (21). Our study confirms the beneficial effects of oral protein supplements on IGF-I activity in the non fractured frail elderly, with a rapid response detectable by just one week of supplementation. This is in agreement with previous studies that showed 5 weeks of protein supplementation was able to significantly improve the outcome after hip fracture (19, 20). The type of supplements we used in the present study were based on observations of animal experiments, in which essential amino acid supplements were able to increase bone strength, to improve microarchitecture and intrinsic bone tissue quality (13). This was observed in adult rats receiving an isocaloric low protein diet, a situation reminiscent of the conditions of the frail elderly investigated in the current trial.

Zinc insufficiency is highly common in the elderly and may contribute to a frailty syndrome, possibly through an effect on IGF-I production and/or action (24, 27, 28). We thus performed the present study in order to assess the hypothesis that zinc could magnify the circulating levels and actions of IGF-I and the functional response to protein repletion. Plasma zinc measurements may not be the reflection of body stores because most of the body zinc stores are intracellular. Hence, mild zinc insufficiency can occur with low but still in the normal range plasma levels. At baseline, both groups had a zinc serum concentration at the lower limit of normal range. After four weeks, it remained stable in the non supplemented group, whilst an increase of close to 60% was observed in the group receiving zinc (Table 2). Our results indicate that the increase in serum IGF-I levels induced by protein supplements was accelerated, with a significant difference detectable after only one week of zinc addition. This could represent a clinically relevant advantage, since the potential benefits of correcting low IGF-I concentration could be achieved at a very early stage of intervention. A rapid improvement would be particularly desirable in the phase of recovery after surgery or in the rehabilitation phase of undernourished elderly. By 4 weeks of protein supplements, however, there was no further advantage of zinc supplementation.

Previous studies in humans and in animals have demonstrated that protein deficiency is associated with an increased bone loss and decreased bone strength (3, 12). These modifications of bone strength are the results of a markedly increased bone resorption and decreased bone formation. In the present study, untreated controls showed an increase in the bone resorption marker, CrossLaps™, during the 4-week observation period, whereas the values remained stable in the group receiving the essential amino acids-whey protein supplements. Zinc addition was associated with a significant reduction of the bone resorption marker. At the present time, we have no argument in favor of a direct or indirect effect of zinc on bone turnover. In vitro experiments have shown some inhibition of bone resorption by zinc (32, 34). However, our experimental design does not allow one to selectively assess the effects of zinc supplements on bone remodeling, since there

was an interaction with protein repletion. Additional studies are still required to confirm this hypothesis. In preclinical studies, essential amino acids supplements decreased bone resorption in rats fed an isocaloric low protein diet (13). The mechanisms of this protein effect on bone resorption have not yet been elucidated. IGF-I is unlikely to be the only factor involved since this growth factor acts to increase bone remodeling, as demonstrated by significant increases in osteocalcin, and is involved in osteoclastogenesis regulation (44). The patients enrolled in the study were clearly undernourished as indicated by the mini nutritional assessment score (37) and the low values of serum albumin and IGF-I. Despite a strict randomization procedure, there was a small difference in vitamin D status at baseline between the zinc group and the controls. There is likely to be a link between vitamin D and IGF-I (45, 46); however, in our study we didn't find any correlation between IGF-I level and vitamin D (data not shown). This is very unlikely to explain the IGF-I response to zinc. Although the present study was underpowered to detect significant changes in functional performances, we were able to observe that in the prespecified subgroup with baseline albumin below 30g/l, zinc addition was associated with a more favorable evolution of ADL score.

In conclusion, the present study has shown that protein supplements (including whey protein and essential amino acids) increase serum IGF-I and a bone formation marker in undernourished elderly patients. Furthermore, we have demonstrated that the addition of zinc to the protein supplements accelerates the IGF-I response, and this is accompanied by an inhibition of bone resorption. This nutritional combination could be beneficial in the frail elderly at risk for osteoporotic fracture.

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Author Contributions:

- AR was in charge of recruiting the patients, collecting, analyzing and interpreting the data, and of manuscript preparation
- PA contributed to study concept and design, data collection, analysis and interpretation, and to manuscript preparation
- SGB participated in patient recruitment and data collection
- RR contributed to study concept and design, data collection, analysis and interpretation, and to manuscript preparation

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