CLINICAL STUDY

IGF-I, IGF-I-binding proteins and GH-binding protein in malnourished elderly patients with inflammation receiving refeeding therapy

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Abstract

Objective: To investigate the mechanisms determining the success or failure of refeeding therapy in malnourished elderly patients with inflammation by studying changes in plasma IGF-I, GH-binding protein (GHBP) and IGF-binding protein (IGFBP) levels and IGFBP-3 proteolysis.

Design and methods: We studied 15 severely malnourished hospitalized elderly patients. Weight, food intake, plasma albumin, transthyretin, C-reactive protein (CRP), orosomucoid, interleukin-6 (IL-6), IGF-I, intact and proteolytically degraded IGFBP-3 and GHBP levels were determined on admission and during refeeding therapy designed to increase food intake to 40 kcal/kg body weight per day (15% protein).

Results: Plasma IGF-I, IGFBP-3 and GHBP levels were significantly low for age on admission in all malnourished elderly patients. They increased in nine patients as nutritional status improved (albuminemia > 30 g/l; transthyretinemia > 200 mg/l or weight gain > 5% of initial body weight) and levels of inflammation markers decreased (group 1). In contrast, plasma IGF-I, IGFBP-3 and GHBP levels remained low in six patients in whom nutritional status failed to improve and levels of inflammation markers increased (group 2). IGF-I showed greater variations than IGFBP-3 or GHBP with respect to nutritional status. High plasma CRP and IL-6 levels were associated with high levels of IGFBP-3 proteolysis.

Conclusion: Efficient refeeding therapy was associated with a significant increase in IGF-I plasma levels. In patients with severe and persistent inflammation, high levels of proteolysis of IGFBP-3 may have contributed to the low plasma IGF-I levels, persistence of hypercatabolism and lack of improvement in nutritional status.

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Introduction

Malnutrition may affect up to 50% of elderly patients admitted to hospital (1–3) and is associated with longer stays in hospital (4) and higher mortality, both in hospital (2) and after discharge (5). Impaired nutritional status may be both the cause and consequence of the disease. As a result of tissue damage, which may be caused by trauma, sepsis or malignancies, activated macrophages produce proinflammatory cytokines such as interleukin-1 beta (IL-1 β), tumor necrosis factor alpha (TNF- α), and IL-6, which induce the acute phase response (6). Proinflammatory cytokines trigger hormonal changes and induce a metabolic response dominated by hypercatabolism (7). In the elderly, whose nutritional reserves are low, hypercatabolism is frequently associated with low nutritional intakes due to

the anorexic effect of TNF- α (8), polymedication, immobility and mood disorders, resulting in the rapid development of malnutrition. Malnutrition decreases immune function, leading to a higher risk of infection (9). It also causes a decrease in muscle mass and strength, leading to gait disorders, falls and fractures (10). Low albuminemia is associated with the development of pressure sores and with poor healing (11). Thus, malnutrition may increase morbidity, leading to a life-threatening vicious circle of disease and malnutrition.

The growth hormone (GH)–insulin-like growth factor (IGF) axis undergoes profound changes during malnutrition and catabolic illness (12). IGF-I is a peptide structurally similar to insulin, with effects on growth and metabolism. In particular, IGF-I is a potent stimulator of protein synthesis and an inhibitor of protein catabolism (13). Most of the circulating IGF-I

is produced by the liver (14) in response to the binding of GH to the GH receptor (GH-R). In serum, IGF-I binds to the high-affinity IGF-binding proteins (IGFBPs). Six IGFBPs have been characterized (IGFBP-1 to -6), and four are present at significant concentrations in human serum (IGFBP-1 to -4). Less than 1% of the IGF-I in the bloodstream is free, most (80–90%) being bound in a 150 kDa complex consisting of IGF-I (molecular mass 7.5 kDa), IGFBP-3 (39–42 kDa) and an acid-labile subunit (84–86 kDa) (15). This complex is believed to be the principal carrier of IGF. The rest of the IGF-I in the circulation is bound to IGFBP-1, -2, -3 or -4, each of which circulates as a complex of 30–40 kDa. Partial proteolysis of IGFBP-3 decreases its binding affinity for IGF-I, resulting in increases in IGF-I bioavailability (16) and IGF-I clearance (12). This mechanism may be an important determinant of plasma IGF-I levels.

Malnourished adults and children display low plasma levels of IGF-I and IGFBP-3 and high plasma levels of IGFBP-1 (12, 17). Plasma GH levels may be high, as reported in cases of acute malnutrition and in some cases of anorexia nervosa (18), indicating a state of resistance to GH at the level of the GH-R (19). Plasma GH levels may also be low, as reported in cases of prolonged critical illness, thereby contributing to low plasma IGF-I levels (20). Partial proteolysis of IGFBP-3 has been reported to increase during the postoperative phase (21–23) and in critical illness (24, 25), but the mechanisms regulating this process are poorly understood. Changes in the IGF system have not been thoroughly studied in ill, elderly subjects, in whom this system may be affected by both malnutrition and aging. A greater understanding of changes in the IGF system could help unravel the mechanisms involved in the catabolic phase, facilitating the adaptation of refeeding strategies. We therefore performed a pilot study investigating changes in these markers in malnourished elderly patients on refeeding therapy.

Subjects and methods

Elderly controls

Plasma samples from elderly subjects living at home (26)) were analyzed ($n = 244$). The control subjects were aged 65–92 years. They showed no impairment in nutritional status: mean body mass index (BMI) was 25.8 ± 3.4 (mean $\pm$ s.d.) in men, 24.9 ± 4.9 in women; mean albuminemia was 46.2 ± 2.5 g/l and mean transthyretinemia was 280.8 ± 60.2 mg/l. None of these subjects had a BMI below 20, albuminemia below 35 g/l or transthyretinemia below 200 mg/l.

Elderly patients

We studied 15 ill and malnourished patients aged 68–94 years (81.9 ± 8.7 years) admitted to the Unité de

Table 1 Main medical diagnoses of the malnourished elderly patients ($n = 15$), group, and length of stay in the Unit (time to discharge in group 1 and time to death in group 2).

Patient	Main medical diagnoses	Group	Length of stay (days)
1	Severe esophagitis	1	60
2	Rheumatoid arthritis, COPD	1	72
3	Pulmonary edema	1	29
4	Esophageal stenosis	1	65
5	Pleural abscess	2	55
6	Tuberculosis	2	25
7	Fever of unknown origin	1	66
8	Lithiasic pancreatitis	1	31
9	Femoral neck fracture	1	42
10	Cecum cancer	2	45
11	Erysipelas	1	30
12	Paraplegia, pressure sores	1	49
13	Gastric cancer	2	20
14	Kidney cancer	2	33
15	Bladder cancer	2	26

COPD: chronic obstructive pulmonary disease.

Nutrition Gériatrique (Hôpital Charles Foix, Ivry sur Seine, France) for nutritional support. On admission, these subjects were severely malnourished and most had moderate to severe inflammation (Table 1). Explorations were undertaken to determine the cause of the malnutrition and of the inflammation, if any, and patients received specific treatment with regard to their medical diagnosis when possible (antibiotics, diuretics, etc.). Simultaneously, nutritional therapy was initiated, consisting of enriched meals and high-calorie, high-protein supplements to increase energy intake to 40 kcal/kg body weight per day (protein accounted for $15 \pm 2\%$ of energy intake). Tube feeding was used if food intake was insufficient. Secondly, patients were classified into two groups in accordance with the response to refeeding therapy. Nine patients (group 1) showed persistent improvement in nutritional status (albuminemia > 30 g/l; transthyretinemia > 200 mg/l and/or weight gain $> 5\%$ of initial body weight); these patients had stayed 49 ± 17 days in the Unit. Six patients (group 2) failed to improve their nutritional status and their levels of inflammation markers increased. These patients were diagnosed with serious medical conditions that could not be controlled by drug-based or surgical treatment (Table 1). These patients died in the Unit after a stay of 34 ± 13 days.

Methods

Blood samples were collected in the morning before breakfast (0700–0800 h), on admission (baseline), then once per week for 4 weeks and once per fortnight thereafter. Blood samples were collected in dry or EDTA-containing tubes, and were analyzed immediately (albumin, transthyretin, C-reactive protein (CRP), orosomucoid) or centrifuged at $+4^\circ\text{C}$, with the resulting

plasma divided into aliquots, frozen immediately and stored at -20°C until use (IGF-I, IGFBP, GH-binding protein (GHBP) and IL-6).

Energy intake Food intake was recorded on 3 consecutive days of each week. Energy intake was determined for each of the 3 days with the Bilnut program from SCDA Nutrisoft (Cereelles, France) and the mean value for these 3 days was calculated.

Albumin, transthyretin, CRP, orosomucoid Plasma albumin, transthyretin, CRP and orosomucoid levels were determined by immunonephelometry with the automated Array Protein System of Beckman (Roissy, France). According to our laboratory data, CRP plasma levels above 6 mg/l and orosomucoid above 1.10 g/l indicate inflammation.

Interleukin-6 Plasma interleukin-6 levels were determined by ELISA using the kit sold by Eurogenetics (Tessenderlo, Belgium).

IGF-I The IGF-I assay was carried out after separating IGF from the binding proteins as previously described (27). Briefly, plasma samples (25 μl) were incubated for 30 min at room temperature in acid (0.01 mol/l HCl) and subjected to ultrafiltration using a Centricon 30 filter (Amicon, Epernon, France). The ultrafiltrate containing IGF was lyophilized, the resulting powder dissolved in 0.1 mol/l phosphate buffer (0.1% BSA, pH 7.4) and assayed as described by Hardouin *et al.* (28), using a rabbit polyclonal IGF-I antiserum (generously provided by Drs Closset, Frankenne and Hennen, Liège, Belgium). The detection threshold was 0.2 ng/ml, the intra-assay coefficient of variation was 3.7% and the inter-assay coefficient of variation was 4.5%.

IGFBP-3 Serum IGFBP-3 was determined with a specific RIA kit purchased from Nichols Institute Diagnostics (San Juan Capistrano, CA, USA). The detection threshold was 0.06 $\mu\text{g/ml}$, the intra-assay coefficient of variation 3.8% and the inter-assay coefficient of variation 6.3%.

IGFBP determination by Western blotting Western ligand blotting was performed as described by Hossenlopp *et al.* (29). Briefly, 3 μl plasma were subjected to SDS-11% PAGE under non-reducing conditions. The proteins were transferred to nitrocellulose and probed with a mixture of ^{125}I -IGF-I and ^{125}I -IGF-II (5×10^5 c.p.m. for each membrane), to detect IGFBP-1 to -4.

For IGFBP-3 Western immunoblotting, nitrocellulose membranes were prepared as for ligand blotting and then incubated for 1 h at 37°C with a rabbit polyclonal human IGFBP-3 antiserum (1/1000; generously provided by Dr Binoux, INSERM U142, Hôpital

Saint-Antoine, Paris, France) to detect intact IGFBP-3 and IGFBP-3 fragments generated by proteolysis. The immunoreactive proteins were detected with the Amersham ECL System (Amersham International, Amersham, Bucks, UK) (30). Two sera from normal individuals were systematically loaded (3 μl) on each gel, to facilitate comparison.

Western ligand blots and immunoblots were analyzed by scanning with a GS700 imaging densitometer, using the Molecular Analyst program (Biorad S.A., Ivry sur Seine, France). The results were expressed in arbitrary units. IGFBP-3 proteolysis was assessed by dividing the density value for the 30 kDa proteolytic IGFBP-3 fragment by the sum of the density values for the 30 kDa (proteolytic fragment) and the 39–42 kDa (intact) IGFBP-3 bands.

GHBP The binding of ^{125}I -human GH to plasma GHBP was measured by gel filtration and HPLC, as previously described (31). Scatchard analysis was performed with the Ligand program (32).

Statistical analysis Correlations between quantitative variables were explored by simple linear regression analysis. Differences between groups in quantitative variables were analyzed by the non-parametric Mann–Whitney test.

Results

On admission, all patients ($n = 15$) showed severe malnutrition and most had moderate to severe inflammation. Mean plasma levels of albumin were 23 ± 5 g/l and of transthyretin 122 ± 44 mg/l. BMI was 18.5 ± 4.3 (weight 51 ± 12 kg), and food intake 22 ± 14 kcal/kg body weight per day (1071 ± 516 kcal/day). Inflammation markers were high: mean CRP plasma levels were 61 ± 51 mg/l, orosomucoid 2.01 ± 0.60 g/l and IL-6 75 ± 102 pg/ml. Plasma levels of IGF-I, IGFBP-3 and GHBP were significantly lower in our patients than in elderly controls (82 ± 60 vs 179 ± 60 ng/ml, 1.6 ± 0.6 vs 3.3 ± 0.8 $\mu\text{g/ml}$ and 16 ± 5 vs $39 \pm 8\%$ of radioactivity respectively; $P < 0.001$ for all baseline values vs controls). With refeeding therapy, food intake rose to 40 ± 12 kcal/kg body weight/day (1969 ± 587 kcal/day), which was associated only with a significant but moderate increase in plasma transthyretin and IGF-I levels (178 ± 81 mg/l ($P = 0.05$) and 139 ± 68 ng/ml ($P < 0.05$) respectively) and a fall in plasma CRP levels (52 ± 67 mg/l, $P < 0.05$) during hospitalization. Recorded values on the fourth week of hospitalization or last values before death of plasma levels of albumin (27 ± 8 g/l), BMI (19.3 ± 4.1), weight (53 ± 13 kg), orosomucoid (1.65 ± 0.85), IL-6 (72 ± 123 pg/ml), IGFBP-3 (1.9 ± 1.0 $\mu\text{g/ml}$) and GHBP ($19 \pm 13\%$ of

Figure 1 Nutrition and inflammation variables at baseline (B) and after 4 weeks of refeeding therapy or last recorded value before death (R) in elderly patients on refeeding therapy. Group 1: successful refeeding, $n = 9$; group 2: failure of refeeding, $n = 6$.

radioactivity) were not significantly different from baseline values.

However, further analysis of the medical and biological history of each patient showed marked differences in individual responses to nutritional therapy. Nine patients responded well to medical treatment and nutritional therapy (patients 1–4, 7–9, 11, 12; group 1). In contrast, the other six patients showed no improvement in nutritional status in response to nutritional therapy (patients 5, 6, 10, 13–15; group 2). Baseline values, values after 4 weeks of refeeding therapy or last

recorded values before death of variables relating to nutrition and inflammation are reported for each group in Fig. 1, and mean values for each group are reported in Table 2. In group 1, as food intake rose from 21 ± 15 to 40 ± 6 kcal/kg body weight per day, plasma levels of albumin and transthyretin rose significantly ($P < 0.005$ vs baseline values for both parameters) while those of CRP and orosomucoid decreased significantly ($P < 0.05$ vs baseline for both parameters). In group 2, although food intake rose similarly, from 22 ± 12 to 41 ± 19 kcal/kg body weight

Table 2 Mean $\pm$ S.D. values at baseline and after 4 weeks of refeeding therapy or last recorded values before death of variables relating to nutrition and inflammation. Group 1: successful refeeding; group 2: failure of refeeding.

	Group 1 ($n = 9$)		Group 2 ($n = 6$)	
	Value on admission	Value after 4 weeks of refeeding	Value on admission	Last value before death
Albumin (g/l)	25 ± 3	$32 \pm 4^{**}$	$19 \pm 4^{\circ}$	19 ± 5
Transthyretin (mg/l)	153 ± 23	$231 \pm 54^{**}$	$75 \pm 9^{\circ\circ}$	98 ± 27
CRP (mg/l)	36 ± 34	$9 \pm 9^{*}$	$98 \pm 51^{\circ}$	116 ± 65
Orosomucoid (g/l)	1.89 ± 1.21	$1.18 \pm 0.29^{*}$	2.24 ± 0.73	2.37 ± 0.93
IL-6 (pg/ml)	28 ± 20	16 ± 16	$76 \pm 40^{\circ\circ}$	84 ± 71
BMI	19.8 ± 4.7	20.6 ± 4.8	16.5 ± 3.2	17.4 ± 4.0
Weight (Kg)	54 ± 14	56 ± 13	46 ± 9	48 ± 12
Food intake (kcal/day)	1145 ± 574	$2140 \pm 289^{**}$	973 ± 459	$1740 \pm 817^{*}$
Food intake (kcal/kg/body weight/day)	21 ± 15	$40 \pm 6^{*}$	22 ± 12	$41 \pm 19^{*}$

$^{*}P \leq 0.05$ and $^{**}P < 0.005$ from values on admission within each group; $^{\circ}P \leq 0.05$ and $^{\circ\circ}P < 0.005$ from values on admission in group 1 (Mann–Whitney non-parametric test).

Figure 2 Plasma IGF-I, IGFBP-3 and GHBP levels at baseline (B) and after 4 weeks of refeeding therapy or last recorded values before death (R) in malnourished elderly patients on refeeding therapy are compared with those obtained in healthy elderly subjects (65–92 years; control). Group 1: successful refeeding, $n = 9$; group 2: failure of refeeding, $n = 6$.

per day, plasma levels of albumin and transthyretin remained very low. Plasma CRP, orosomucoid and IL-6 levels, which were high at baseline, increased further, albeit not significantly, in relation to severe diseases (e.g. cancer, tuberculosis, profound abscess; Table 1). Baseline plasma levels of albumin and transthyretin were significantly lower and those of CRP and IL-6 were significantly higher in group 2 than in group 1 (Table 2).

Baseline values of plasma levels of IGF-I, IGFBP-3 and GHBP, values after 4 weeks of refeeding therapy or last recorded values before death for each group and values for elderly controls are reported in Fig. 2, and mean values are reported in Table 3. At baseline, plasma IGF-I, IGFBP-3 and GHBP levels were significantly lower in group 1 and in group 2 than in healthy controls aged 65–92 years ($P < 0.001$ for baseline values vs normal values). At baseline, the concentrations of IGF-I, IGFBP-3 and GHBP did not differ significantly between groups 1 and 2, although the difference was almost significant for plasma IGF-I levels ($P = 0.06$). In group 1, successful nutritional therapy was associated with a significant rise in plasma IGF-I levels, which reached normal values after 4 weeks (181 ± 52 ng/ml, $P < 0.002$ vs values

at baseline). IGF-I levels were more responsive ($+121 \pm 125\%$) to nutritional therapy than albumin ($+29 \pm 23\%$) or transthyretin ($+52 \pm 34\%$) levels. In group 1, the plasma concentrations of IGFBP-3 and GHBP both increased, although not significantly, in response to nutritional therapy. In group 2, the lack of improvement in variables relating to nutrition and inflammation was associated with a lack of significant change in plasma IGF-I, IGFBP-3 and GHBP concentrations.

Western ligand blotting showed very low quantities of IGFBP-3 in plasma in our malnourished elderly patients at baseline (patients 4, 9, 12 in Fig. 3; patients 1, 2 in Figs 4 and 5). Plasma IGFBP-3 levels then increased markedly as nutritional status improved, but did not reach normal levels for all patients before the end of hospitalization.

Western immunoblotting (Fig. 4) showed that, in patient 1, the level of IGFBP-3 proteolysis was higher than that in young controls at baseline. We observed a strong increase in IGFBP-3 proteolysis on day 10 (Fig. 4, lane 2), when this patient presented a severe inflammation, as shown by high CRP levels, caused by a pulmonary infection. Plasma IL-6 levels also increased simultaneously. By day 27 (Fig. 4, lane 3)

Table 3 Mean $\pm$ s.d. values for plasma levels of IGF-I, IGFBP-3 and GHBP in elderly controls, at baseline and after 4 weeks of refeeding therapy or last recorded values before death in each group. Group 1: successful refeeding; group 2: failure of refeeding.

	Elderly controls	Elderly malnourished patients			
		Group 1 ($n = 9$)		Group 2 ($n = 6$)	
		Value on admission	Value after 4 weeks of refeeding	Value on admission	Last value before death
IGF-I (ng/ml)	179 ± 60	$92 \pm 28^{\#}$	$181 \pm 52^{**}$	$68 \pm 13^{\#}$	$77 \pm 31^{\#}$
IGFBP-3 (ng/ml)	3.3 ± 0.8	$1.7 \pm 0.7^{\#}$	2.5 ± 1.1	$1.3 \pm 0.3^{\#}$	$1.1 \pm 0.5^{\#}$
GHBP (% of radioactivity)	38.5 ± 8.0	$17.3 \pm 5.4^{\#}$	24.2 ± 7.5	$13.3 \pm 4.9^{\#}$	$12.5 \pm 4.7^{\#}$

$^{\#}P < 0.005$ from values in elderly controls; $^{**}P < 0.005$ from values on admission (Mann–Whitney non-parametric test).

Figure 3 Western ligand blotting experiment. Serum samples from malnourished elderly patients (patients 4, 9, 12) at baseline (lanes 5, 7, 9) and after successful refeeding therapy (lanes 6, 8, 10) are compared with those of elderly (lanes 3, 4) and young (lanes 1, 2) controls.

after treatment for sepsis, CRP and IL-6 levels had normalized, and the level of IGFBP-3 proteolysis had markedly decreased. CRP and IL-6 levels and IGFBP-3 proteolysis levels displayed no detected change thereafter (Fig. 4, lanes 4–7). Plasma IGF-I levels increased rapidly when the inflammation and IGFBP-3 proteolysis subsided. In patient 2, Western immunoblotting (Fig. 5) showed high levels of IGFBP-3 proteolysis on admission. This patient presented with active rheumatoid polyarthritis and pulmonary infection resulting in a strong inflammation on admission, which partly subsided during hospitalization. As in patient 1, high levels of IGFBP-3 proteolysis were associated with high plasma CRP and IL-6 levels (Fig. 5, lane 1). IGFBP-3 proteolysis and inflammation decreased thereafter, and plasma IGF-I levels normalized as the patient gained 5 kg in body weight, reflecting efficient refeeding (Fig. 5, lanes 2–5).

Discussion

In our elderly subjects with malnutrition and inflammation, IGF-I, IGFBP-3 and GHBP levels were lower on admission than those in elderly controls. Although food intake was increased similarly in all patients, patients with receding inflammation showed a marked improvement in nutritional markers and an increase in IGF-I, IGFBP-3 and GHBP levels ($n = 9$; group 1), whereas patients with sustained inflammation showed no improvement in nutritional markers and no increase in IGF-I, IGFBP-3 and GHBP levels ($n = 6$; group 2). High plasma CRP and IL-6 levels were associated with high level of IGFBP-3 proteolysis.

Commonly used nutritional markers lack sensitivity and specificity in assessing nutritional status. Although nutrition and inflammation markers may be used as predictors of outcome (in our work, values of

Figure 4 Western ligand blotting and Western immunoblotting experiments. (A) Serum samples from patient 1 at baseline (lane 1) and during refeeding therapy (lanes 2–7) are compared with those of young (lanes 8, 9) and elderly (lanes 10–12) controls. Western ligand blotting (upper panel) shows low plasma IGFBP-3 levels at baseline, which subsequently increase during nutritional therapy. Plasma IGFBP-2 levels are higher at baseline than that in young and elderly controls, and subsequently decrease during nutritional therapy. Western immunoblotting (lower panel) shows a marked increase in the levels of IGFBP-3 proteolysis on day 14 (lane 2), when a pulmonary infection led to an increase in plasma CRP and IL-6 levels. (B) The changes in plasma CRP and IL-6 levels are compared with those of IGF-I and to the level of IGFBP-3 proteolysis.

Figure 5 Western ligand blotting and Western immunoblotting experiments. (A) Serum samples from patient 2 at baseline (lane 1) and during refeeding therapy (lanes 2–5) are compared with those of young (lanes 6, 7) and elderly (lane 8) controls. Western ligand blotting (upper panel) shows low plasma IGFBP-3 levels at baseline, which subsequently increase moderately during nutritional therapy. Plasma IGFBP-2 levels are higher in patient 2 at baseline than in young and elderly controls and subsequently decrease moderately during refeeding therapy. Western immunoblotting (lower panel) shows strong IGFBP-3 proteolysis at baseline (lane 1), which subsequently decreases (lanes 2–5) together with plasma CRP and IL-6 levels. (B) The changes in plasma CRP and IL-6 levels compared with those of IGF-I and to the level of IGFBP-3 proteolysis.

transthyretin and IL-6 especially could distinguish the two groups without overlap), the roles of malnutrition or inflammation related to the disease cannot be told apart. In particular, albumin and transthyretin plasma levels decline in the acute phase response. Moreover, these biological markers and weight are influenced by changes in fluid balance, which are common in severe illness. However, more specific techniques have documented the failure of nutritional support to prevent protein loss in septic or injured patients (33–35). The effects of stressors (trauma, sepsis or multiple organ failure) are thought to make improvement in nutritional status or increase in lean body mass by nutritional support alone very difficult, if not impossible (36). Cytokine production and endocrine changes are thought to be responsible for most of the metabolic drift that characterizes severe illness, but the precise mechanisms involved are unclear.

The IGF system is thought to play a major role in catabolism (37). IGF-I has strong insulin-like metabolic effects (13). In particular, IGF-I stimulates protein synthesis (38) and inhibits protein breakdown (39); it is an anabolic hormone. Plasma IGF-I levels are regulated by nutrition, via plasma insulin levels and protein and calorie intake (12). Thus, in malnutrition, low food intake leads to a fall in plasma IGF-I levels, which contributes to protein wasting.

In our malnourished elderly patients, malnutrition was often associated with inflammation. Both nutritional and inflammation processes may be involved in the changes in the IGF variables that we observed. In humans, nutrition has been shown to influence IGF-I

levels in clinical situations that do not involve an inflammation, such as fasting (40), anorexia nervosa (18, 41) and diabetes (42). In these clinical situations, the decline in IGF-I plasma levels is thought to be mainly due to a decrease in the number of hepatic GH-binding sites and to post-receptor defects (19, 43), conferring resistance to the action of GH. Our results also show that GHBP levels, which are thought to reflect the number of hepatic GH-Rs, are low in elderly malnourished patients, increase in response to successful refeeding, but remain low in cases in which refeeding therapy fails, suggesting persistent GH resistance. However, inflammation *per se* may also modulate plasma IGF-I levels. First, the proinflammatory cytokines IL-1 β , IL-6 and TNF- α inhibit expression of the IGF-I gene (44, 45), and low circulating IGF-I concentration may be caused by a direct effect of cytokines in the liver. This effect may involve suppression of cytokine signaling inhibition of GH signaling by proinflammatory cytokines (46, 47). Secondly, plasma IGF-I levels may be affected by proteolysis of IGFBP-3, the principal carrier protein of IGF-I. It has been suggested that the proteolysis of IGFBP-3 modulates IGF-I activity by increasing the bioavailability of IGF-I to the tissues (16, 48). However, the partial proteolysis of IGFBP-3 also increases IGF-I clearance (12), decreasing the bioavailability of IGF-I, and may therefore be involved in the development of catabolic conditions. The protease involved seems to be a calcium-dependent serine protease (49); its site of production has not been determined. Little is known about the regulation of IGFBP-3 proteolysis. The partial proteolysis of IGFBP-3 is

detectable in normal serum (30), but has been reported to increase considerably in the postoperative phase (21–23) and during critical illness (24, 25). In critically ill patients who had recently undergone surgery, Davies *et al.* (25) reported this proteolytic activity to be strongly regulated by parenteral nutrition. However, in fasted healthy controls (age- and sex-matched), no IGFBP-3 proteolysis was observed, suggesting that other specific factors related to surgery are involved. We suggest that these specific factors probably include factors related to the inflammation. To the best of our knowledge, ours is the first report to show that changes in the level of partial proteolysis of IGFBP-3 are closely associated with changes in plasma CRP and IL-6 levels in malnourished, ill patients. Our results suggest that proinflammatory cytokines may be involved in the regulation of the partial proteolysis of IGFBP-3. Thus, in malnourished patients with inflammation, we hypothesize that IGFBP-3 proteolysis may favor the maintenance of low IGF-I plasma levels and the failure of refeeding therapy. However, this hypothesis is supported by the study of the changes in CRP, IL-6 and proteolyzed IGFBP-3 in two patients only, and requires confirmation by larger studies.

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