

## Growth hormone does not affect albumin synthesis in the critically ill

Hans Barle  
Lena Gamrin  
Pia Essén  
Margaret A. McNurlan  
Peter J. Garlick  
Jan Wernerman

Received: 14 June 2000  
Final revision received: 10 January 2001  
Accepted: 19 February 2001  
Published online: 12 April 2001  
© Springer-Verlag 2001

H. Barle (✉) · L. Gamrin · P. Essén ·  
J. Wernerman  
Department of Anesthesiology  
and Intensive Care, K 32,  
Huddinge University Hospital,  
Karolinska Institute, 141 86 Huddinge,  
Sweden  
E-mail: hans.barle@anaesth.hs.sll.se  
Phone: +46-8-58586390  
Fax: +46-8-7795424

M. A. McNurlan · P. J. Garlick  
Department of Surgery,  
University Medical Center,  
State University of New York at Stony  
Brook, Stony Brook, NY 11794–8191, USA

**Abstract Objective:** To study the effect of growth hormone (GH) on albumin synthesis in critically ill patients.

**Design:** Prospective randomized controlled study.

**Setting:** Two intensive care units, university hospital and county hospital, respectively.

**Patients:** Twenty-two critically ill patients in the intensive care unit.

**Interventions:** Albumin synthesis was measured twice in each patient, with a 5-day interval. The patients in the control group ( $n = 11$ ) received standard intensive care unit (ICU) treatment between measurements, whereas those in the GH group ( $n = 11$ ) also received 0.3 U/kg daily of human recombinant GH.

**Measurements and results:** Albumin synthesis was measured by labeling with L-[ $^2\text{H}_5$ ]phenylalanine. In the control group, the fractional synthe-

sis rate (FSR) of albumin was  $16.3 \pm 4.1\%$ /day (mean and SD) in the first measurement and  $15.7 \pm 4.2\%$ /day 5 days later (NS), whereas in the GH group the corresponding values were  $17.0 \pm 4.7\%$ /day and  $16.7 \pm 5.5\%$ /day (NS). The calculated absolute synthesis rates of albumin, based on FSR and intravascular albumin mass, also showed no effect of GH.

**Conclusion:** Albumin synthesis rates were consistently higher in the two groups of critically ill patients than previously reported values in healthy subjects. However, GH treatment for 5 days neither stimulated nor inhibited albumin synthesis rates in these critically ill patients.

**Key words** Hepatic · Intensive care · Liver · Mass spectrometry · Protein

### Introduction

Critical illness induces profound changes in protein metabolism, changes that are relatively uniform irrespective of the cause of the illness. Among other features, a characteristic severe muscle wasting is observed in these patients, as reflected by an increased amino acid efflux from the periphery [1, 2, 3]. Results from previous studies indicate that this phenomenon may occur in order to maintain an adequately high substrate flow to the splanchnic area for vital processes such as protein synthesis, gluconeogenesis and ureagenesis [4, 5].

Albumin is the individual protein most abundantly synthesized by the liver in healthy subjects, contributing about 15% of total liver protein synthesis in the rat [6]. In the acute-phase response, e.g. in critical illness, the albumin concentration in peripheral blood decreases, albumin being a so-called negative acute-phase reactant. Previous studies, primarily on animals, have shown decreased albumin synthesis rates under these conditions [7]. However, in recent years several reports have been presented that show increased rates of albumin synthesis in states of catabolism, e.g. after stress hormone infusion [8], in conjunction with surgery [9], as well as in critical illness [3, 10, 11].

Growth hormone (GH) has been shown to reduce net protein loss in critical illness, such as burns [12, 13], and trauma and sepsis [14, 15]. Although GH preserves liver protein synthesis in conjunction with elective laparoscopic surgery [16], and also stimulates albumin synthesis in the healthy human [17, 18], GH given to severely traumatized subjects does not reprioritize liver protein synthesis from the acute phase response, as assessed by plasma protein concentrations [19]. As GH treatment has been suggested for the treatment of the critically ill to reduce the effects of catabolism, there is a need to characterize better its effects on human liver protein metabolism in this group of patients. Despite the stimulation of albumin synthesis in healthy subjects, in the critically ill it is possible that positive effects of GH on protein metabolism in the whole body and, in particular, in skeletal muscle, might result in negative effects on liver protein metabolism, e.g. by depriving the liver of substrates for its vital metabolic processes. It may be speculated that such a mechanism may have contributed to the result of a recent study, where GH treatment was shown to be associated with increased mortality and morbidity in critically ill patients [20]. The aim of our study was to investigate the effect of a 5-day GH treatment on albumin synthesis in one group of critically ill patients, and compare with the results from one group not receiving GH.

## Materials and methods

### Materials

L-[<sup>2</sup>H<sub>5</sub>]Phenylalanine, 99 atom percent (Mass Trace, Woburn, Mass., USA) was dissolved in sterile water together with unlabelled phenylalanine (Ajinomoto, Tokyo, Japan) to a concentration of 20 g/l, 15 and 30 mole percent excess (MPE), respectively. The solutions were prepared, heat-sterilized and stored in sterile containers.

### Subjects and treatment

Twenty-two patients admitted to the intensive care units (ICUs) at Huddinge University Hospital and St. Göran's Hospital, Stockholm, Sweden, between the years of 1996 and 1998, were included in the study. The patients were admitted to the ICU following trauma, surgical complications and/or severe infections and had developed multiple organ failure during the course of the illness. The patients were randomized into two comparable groups regarding gender, age, weight, height and body mass index (BMI), and were scored according to acute physiology and chronic health evaluation II (APACHE II) on admission to the ICU (Table 1) [21]. The patients in the two groups were investigated after similar lengths of stay in the ICU, and their outcomes in terms of survival were similar. Prior to each measurement of albumin synthesis, bilateral antecubital venous lines were inserted, one of which was used for blood sampling and the other for injection of L-[<sup>2</sup>H<sub>5</sub>]phenylalanine 45 mg/kg, 15 MPE in the first measurement and 30 MPE in the second, performed 5 days later. During the intervening period, 11 of

the patients (GH group) received 0.3 U/kg each day of growth hormone as a subcutaneous injection. The patients in the control group received no GH treatment. During the treatment period, the patients received all other treatment as required by their medical condition, including mechanical ventilation, sedation, diuretics, and antibiotics. Thus, all patients were ventilator-dependent at the first sampling occasion, except for two patients in the control group and one patient in the GH group. When required, the patients were sedated with midazolam or propofol. All the patients received low molecular weight heparin as prophylaxis against thrombosis. All the patients received nutrition, aiming at a calorie supply of 20–30 kcal/kg/day and a nitrogen supply of 0.1–0.2 g/kg/day. As much as possible of the nutrition was provided enterally, and the remainder parenterally. In the control group, one of the patients at the first sampling occasion, and four patients at the second, received more than 25 % of the nutritional supply enterally. In the GH group, none of the patients received any enteral nutrition at the first sampling occasion, but two patients did at the second (65 and 75 % of the total calorie supply, respectively). The parenteral nutrition, which was provided as continuous intravenous solutions, consisted of a mixture of Intralipid 200 mg/ml, Glukos 100–200 mg/ml and Vamin-Glukos (Pharmacia Upjohn, Stockholm, Sweden), providing non-protein calories in equal amounts. The enteral solution was provided for 10 h (if < 700 ml was provided per 24 h) or 20 h (if > 700 ml/24 h) (Prenutrison/Nutrison, Nutricia, Zoetermeer, Holland). At the time of measurement, the patients were all hemodynamically stable, and were investigated after the initial (fluid) resuscitation had been completed.

The nature, purposes and potential risks of the experimental procedures were explained to the next-of-kin of the patients before we obtained their consent. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and had received a priori approval by the Ethical Committee as well as the Isotope Committee (blood volume measurements) of the Karolinska Institute, Stockholm, Sweden.

### Blood samples

During measurements, venous blood samples were drawn at the following intervals: 0, 5, 10, 15, 30, 50, 70 and 90 min after the injection of phenylalanine, for the determination of isotopic enrichment of phenylalanine in plasma (free) and albumin, respectively. Samples at 0 min were also used, to determine the concentrations of plasma albumin and the serum concentrations of insulin-like growth factor I (IGF-I), insulin, cortisol and glucagon, as well as for liver function tests (alanine aminotransferase, alkaline phosphatase,  $\gamma$ -glutamyltransferase, bilirubin and prothrombin complex). The samples were stored at –80 °C prior to being analyzed.

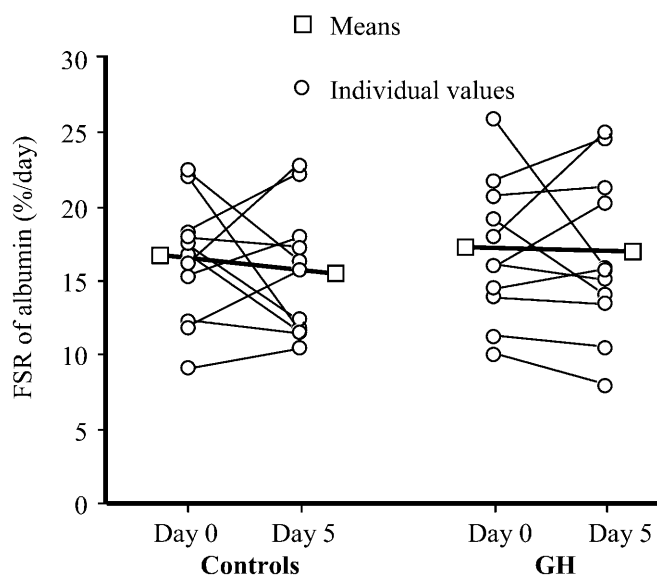
Measurements of plasma volume were taken, beginning 30 min after the injection of phenylalanine, with <sup>131</sup>I-labeled albumin (100 kBq, Institutt for Energiteknikk, Kjeller, Norway). Blood samples were taken at 0, 20, 30, 40 and 45 min to assess isotope dilution.

### Sample preparation

The details of the preparation and analysis of plasma albumin and plasma for the enrichment of L-[<sup>2</sup>H<sub>5</sub>]phenylalanine have been extensively described elsewhere [22, 23]. We previously demonstrated that plasma phenylalanine enrichment closely approximates the enrichment within the liver in healthy humans when a flooding amount of L-[<sup>2</sup>H<sub>5</sub>]phenylalanine is given [16]. In our present study, albumin in plasma samples was extracted from 9 % trichloroacetic

**Table 1** Patients' characteristics (*AAA* aortic abdominal aneurysm, *APACHE* acute physiology and chronic health evaluation, *COPD* chronic obstructive pulmonary disease, *GI* gastrointestinal, *ICU* intensive care unit, *SD* standard deviation, *surg.* surgery performed), *ARDS* adult respiratory distress syndrome, *BMI* body mass index,

Diagnosis		Gender (F/M)	Age (years)	Weight (kg)	Height (cm)	BMI (kg/m <sup>2</sup> )	APACHE II at Admission	Days in ICU at start of study	Outcome 6 Months
Controls	1 COPD, pneumonia	F	69	47	168	16.7	19	6	Survived
	2 GI-bleeding, sepsis	F	73	63	175	20.6	22	19	Died
	3 Esophageal cancer (surg.)	M	81	73	185	21.3	18	15	Died
	4 Gastrectomy, ARDS	M	75	75	169	26.3	13	6	Died
	5 Testis cancer, (surg.)	M	54	89	179	27.8	8	7	Survived
	6 Alcoholism, cardiogenic shock	M	44	74	182	22.3	18	6	Survived
	7 Sigmoid cancer (surg.)	M	55	76	183	22.7	8	6	Survived
	8 COPD, status: post asystole	M	45	115	186	33.2	18	18	Died
	9 Pneumonia, sepsis	M	55	72	183	21.5	21	4	Survived
	10 Thoracic and head trauma	M	32	72	176	23.2	19	8	Died
	11 Gangrenous cholecystitis	M	62	72	180	22.2	26	45	Died
Mean		2 F/9 M	59	75	179	23.4	17	13	5 Survived/ 6 Died
SD			15	17	6	4.3	6	12	
GH	1 Crohn's disease, colectomy (surg.)	M	54	75	179	23.4	13	8	Survived
	2 Duodenal ulcer (surg.)	M	66	73	180	22.5	12	18	Died
	3 Colon cancer (surg.)	M	74	46	160	18.0	17	51	Survived
	4 COPD, exacerbation	M	37	53	171	18.1	7	23	Survived
	5 Perforated appendicitis (surg.)	M	72	88	170	30.4	10	8	Survived
	6 Pneumonia, sepsis	F	46	51	170	17.6	14	6	Survived
	7 Alcoholism, pancreatitis	M	52	108	175	35.3	18	7	Died
	8 Ruptured AAA (surg.)	M	55	75	167	26.9	17	14	Survived
	9 Ruptured AAA (surg.)	M	68	85	183	25.4	26	15	Died
	10 Esophageal varices (alcohol)	M	56	80	182	24.2	19	3	Died
	11 Knife wound diaphragm (surg.)	M	26	84	185	24.5	19	4	Survived
Mean		1 F/10 M	55	74	175	24.2	16	14	7 Survived/ 4 Died
SD			15	18	8	5.4	5	14	



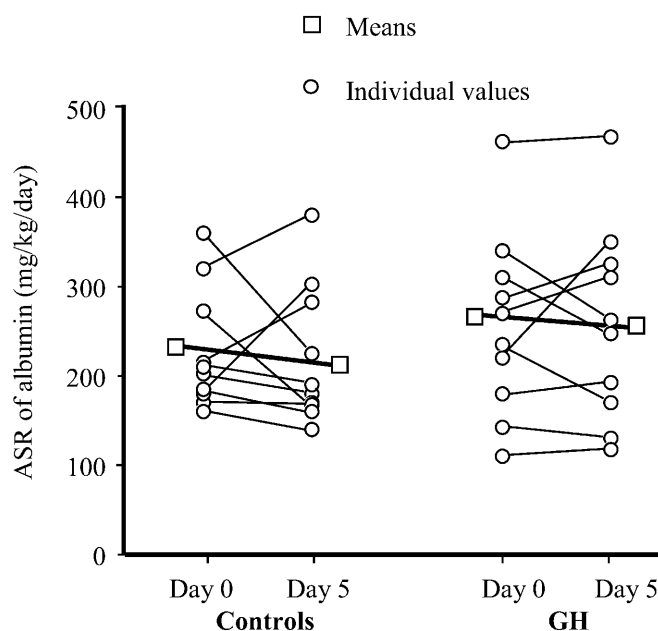
**Fig. 1** Fractional synthesis rates (FSR) of albumin in two groups of critically ill patients, investigated twice, with a 5-day interval. Between measurements the patients in the growth hormone (GH) group ( $n = 11$ ) received daily injections of GH, whereas controls received no treatment

acid-precipitated protein fraction by differential solubility in absolute ethanol [24]. The purity of the albumin fraction was checked with matrix-assisted laser desorption time of flight mass spectrometry (Finnigan Laser mat 2000, Finnigan, Hemel Hempstead, England) showing that a single peak with molecular weight 67,450 kDa had been isolated. Also, in a previous study of albumin synthesis in critically ill patients, it was shown by fast protein liquid chromatography (FPLC) that the separation of albumin by the ethanol technique used here provided pure and reproducible preparations [11], suitable for gas chromatography mass spectrometry (GC-MS). Thereafter, the protein precipitate was suspended in 0.3 M NaOH and reprecipitated in 5% PCA. Following washing with 2% PCA, albumin was hydrolyzed in 6 M HCl for 24 h at 110 °C. The HCl was removed by evaporation in vacuo, and the hydrolysate used for the measurement of isotopic enrichment.

Samples of plasma for the determination of free phenylalanine enrichment were treated with 8% sulfosalicylic acid to precipitate protein. The amino acid-containing supernatants were purified in cation exchange columns (Biorad AG, 50W-X8, (H<sup>+</sup>) form, 100–200 mesh) and eluted from columns with 4 M NH<sub>4</sub>OH. The samples were then dried in vacuo.

#### Mass spectrometry

The determination of enrichment of L-[<sup>2</sup>H<sub>5</sub>]phenylalanine in plasma albumin and plasma has been described previously [23]. In short, the enrichment of L-[<sup>2</sup>H<sub>5</sub>]phenylalanine from albumin hydrolysates was determined by measuring the mass-to-charge ratio ( $m/z$ ) at 106 and 109 of the *n*-heptafluorobutyl derivative of phenylethylamine on a Fisons MD 800 mass spectrometer under electron ionization (Fisons, Beverly, Mass., USA). The L-[<sup>2</sup>H<sub>5</sub>] enrichment of free phenylalanine in plasma was measured by monitoring the ions at  $m/z$  336 and 341 of the *t*-butyldimethylsilyl deriv-



**Fig. 2** Absolute synthesis rates (ASR) of albumin in two groups of critically ill patients: “controls” (no growth hormone (GH) treatment) and “GH” (GH daily injections), measured twice, with a 5-day interval

ative on an HP 5972 mass spectrometer (Hewlett Packard, Palo Alto, Calif., USA) under electron ionization.

#### Other analytical procedures

Plasma albumin concentrations and liver function tests were analyzed by routine laboratory methods. Radioimmunoassays were used to determine the serum concentrations of insulin, IGF-I, cortisol and glucagon.

#### Calculation and statistics

The fractional synthesis rate (FSR) of albumin, i.e. the fraction of the intravascular albumin pool that is synthesized per day (%/day), was calculated by the formula in a previously described study [25]:

$$\text{FSR} = (P_{(2)} - P_{(1)}) \times \frac{100}{\text{AUC}}$$

where  $P_{(2)}$  and  $P_{(1)}$  represent enrichment of phenylalanine in albumin at two time points after the curve of albumin enrichment becomes linear (between 50 min and 90 min samples). AUC is the area under the curve for enrichment of plasma free phenylalanine between the same time points, adjusted for the secretion time, i.e. the temporal lag period due to processing in the liver before the appearance of labeled albumin in plasma. The secretion time for each patient was assessed by plotting the individual regression line for the linear part of the albumin enrichment curve and extrapolating to the intercept of the time axis [25]. Calculation of absolute synthesis rates for albumin (ASR) was calculated by multiplying the FSR of albumin by the intravascular albumin mass, cal-

**Table 2** Plasma albumin concentration, plasma volume, intravascular albumin mass (*IAM*) and secretion time in critically ill patients, during the period that the effect of growth hormone (*GH*) on albumin synthesis was determined. Results are presented as

Parameter	Controls		GH	
	Day 0	Day 5	Day 0	Day 5
Plasma albumin concentration (g/l)	28.2 ± 6.6	27.1 ± 3.8	30.2 ± 4.6	29.6 ± 5.9
Plasma volume (l)	3.9 ± 1.2	3.9 ± 1.1	3.9 ± 1.0	4.1 ± 1.1
IAM (g)	105 ± 22	104 ± 28	106 ± 45	117 ± 36
Secretion time (min)	27.6 ± 2.2	26.4 ± 2.8	27.5 ± 1.8	27.3 ± 2.3

means and standard deviation. Secretion time is the temporal lag period from injection of isotope until appearance of labeled albumin in plasma *GH* growth hormone

**Table 3** Concentrations of glucose in plasma and of insulin, insulin-like growth factor I (*IGF-I*), cortisol and glucagon in serum in critically ill patients during the period that the effect of growth hormone (*GH*) on albumin synthesis was determined. Normal values

Substance	Controls		GH	
	Day 0	Day 5	Day 0	Day 5
Glucose (3.5–6.4 mmol/l)	8.6 ± 2.7	8.4 ± 2.1	9.2 ± 2.3	10.4 ± 4.2
Insulin (3–35 mU/l)	26 ± 29	19 ± 7	36 ± 27	120 ± 134 <sup>b,c</sup>
IGF-I (µg/l) (Age dependent)	78 ± 36	89 ± 44	82 ± 64	200 ± 127 <sup>a,c</sup>
Cortisol (140–700 nmol/l)	686 ± 180	699 ± 257	502 ± 196	503 ± 240
Glucagon (10–40 pmol/l)	65 ± 57	65 ± 58	63 ± 38	67 ± 42

<sup>a</sup>*P* < 0.01 vs. Day 0

<sup>b</sup>*P* < 0.05 vs. Day 0

<sup>c</sup>*P* < 0.05 vs. Day 5, controls

are in parentheses. In healthy individuals, serum cortisol concentration has a diurnal variation, being highest in the morning. Results are presented as means and standard deviation

culated as the product the concentration of plasma albumin concentration and the measured plasma volume.

Data are presented as means and standard deviation (SD). The differences of the FSR and ASR of albumin, respectively, as well as blood chemistry parameters, were assessed by ANOVA for repeated measures and, if significant (*P* < 0.05), Student's *t*-test for comparison within and between groups. Correlation was assessed using Pearson's coefficient of correlation.

## Results

The FSR of albumin was similar in the two groups for the first measurement, 16.3 ± 4.1%/day in the control group (*n* = 11) and 17.0 ± 4.7%/day in the GH group (*n* = 11). When compared with the first measurement, no changes were observed after 5 days, neither in the control group, 15.7 ± 4.2%/day, nor in the GH group, 16.7 ± 5.5%/day (Fig. 1). Furthermore, no differences were observed between the groups when the results from the second measurement were compared (after 5 days).

The ASR of albumin was also similar in the two groups in the first measurement, 233 ± 67 mg/kg and day in the control group (*n* = 10) and 261 ± 100 mg/kg and day in the GH group (*n* = 10). After 5 days, the rates were 218 ± 78 mg/kg and day in the control group and 256 ± 110 mg/kg and day in the GH group. Accord-

ingly, no differences were observed in the second measurement, neither within the groups when compared with the first measurement, nor between groups (Fig. 2).

There were no differences within or between the two groups in any of the two measurements regarding plasma albumin concentration, plasma volume or intravascular albumin mass (Table 2). The secretion time, i.e. the time elapsed from the injection of phenylalanine to the appearance of isotope in albumin in peripheral blood, was also similar in the two groups (Table 2).

The serum concentrations of IGF-I and insulin increased in response to GH treatment (*P* < 0.01 and *P* < 0.05, respectively, vs the first measurement). Consequently, the serum concentrations of IGF-I and insulin were higher after the 5-day GH treatment than in the control group (*P* < 0.05 in both cases) (Table 3). However, although increased compared with healthy individuals, no differences were observed within or between the groups regarding the concentrations of glucose in plasma or of cortisol and glucagon in serum (Table 3). Also, the mean values for the liver function tests were affected in both groups compared with healthy individuals, but there were no significant differences within or between the groups (Table 4).

During the 5-day study period, the patients in the control group received a total of 119 g (range 0–496 g) albumin as plasma or albumin solution. This was not dif-

**Table 4** Liver function tests (serum concentrations). Normal values are in parentheses. Results are presented as means and standard deviation. (*ALP* alkaline phosphatase, *ALT* alanine amino

transferase, *CRP* C-reactive protein, *GH* growth hormone, *GT* glutamyl transferase)

	Controls		GH	
	Day 0	Day 5	Day 0	Day 5
CRP (< 10 mg/l)	135 ± 79	148 ± 101	116 ± 63	105 ± 75
ALT (< 0.70 µkat/l)	0.98 ± 0.42	1.40 ± 0.94	1.51 ± 1.44	1.17 ± 0.71
ALP (< 4.6 µkat/l)	5.7 ± 2.2	7.5 ± 3.7	5.9 ± 3.7	5.4 ± 3.0
γ GT (< 1.0 µkat/l)	3.4 ± 1.4	4.6 ± 3.2	2.8 ± 2.5	2.8 ± 1.8
Bilirubin (3.5–21 µmol/l)	53 ± 61	73 ± 111	75 ± 75	81 ± 105
Prothrombin complex (80–120%)	73 ± 26	71 ± 23	61 ± 18	67 ± 15

ferent from the supplementation in the GH group, which was 173 g (range 0–487 g). No plasma or albumin solution was provided during sampling periods. Furthermore, there was no correlation between the supplementation of albumin and ASR of albumin in the second measurement, either in any of the groups separately, or in both.

## Discussion

Despite the relatively extensive disparities within (but not between) the two groups of patients in this study, in terms of diagnosis, age, and APACHE II score, as well as in the time after admission to the ICU when the patients were investigated, a relatively small scatter in albumin synthesis rates was observed, both within and between the two groups (Table 1, Figs. 1 and 2). Furthermore, the mean value for albumin FSR of approximately 16%/day found in both groups studied was consistently higher than in other investigations of albumin synthesis in healthy individuals, where mean values of approximately 6–9%/day have been found (Fig. 1) [23, 25]. The present results are in accord with previous findings in critically ill patients [3, 11], where increased albumin synthesis rates were found. It is notable that the albumin synthesis rates in these critically ill patients approach the rates found in nephrotic patients (mean value approximately 18%/day), who also have very low plasma albumin concentrations [26], suggesting that the elevation is an attempt to compensate for the low concentration. However, even though GH is capable of stimulating albumin synthesis and increasing the level of albumin mRNA in healthy individuals [17, 18], provision of GH in pharmacological doses to these intensive care unit patients did not increase albumin FSR above the level observed in the control group (Fig. 1). It is therefore a reasonable hypothesis that albumin synthesis in the critically ill patients is not stimulated by GH because it is already stimulated maximally, presumably by the low albumin concentration. However, the result from this study may also be looked upon from the other

point of view, that GH provision does not entail negative effects on albumin synthesis in these patients, as a result of hepatic substrate depletion secondary to the retention of nitrogen in the periphery, found previously in the critically ill [14, 15]. Therefore, it seems less plausible that such a mechanism may have contributed to the increased mortality observed in critically ill patients in association with GH treatment [20], at least where albumin synthesis is concerned.

Despite the findings that GH reduces net protein loss in the critically ill [14, 15], there are other studies in which GH treatment has not been shown to reduce protein catabolism, and might even be associated with increased mortality [20, 27, 28]. This discrepancy may be attributed to the difficulties encountered in standardizing these patients. For example, it is crucial to characterize the patients who are investigated, i.e. the degree of acute illness, for which the APACHE II score is a valuable tool, even though it may not be used as a prognostic marker for the individual patient. Also, the time factor seems to be of importance, i.e. when in relation to the catabolic insult the study is performed [27]. Furthermore, there have been reports that indicate an even more pronounced GH resistance in hypercatabolic patients in the ICU than after elective surgery [29], indicating that the dose of GH might be important.

Protein synthesis rates are commonly measured employing stable isotope techniques, i.e. analysis of the incorporation of a labeled amino acid into a target protein. The isotopically labeled amino acid is provided either for several hours as a constant infusion or as a short-term injection (the so-called “flooding” technique), and the duration of the periods of measurement differ correspondingly. Even though there has been considerable debate over the years as to which of the two methods is the more appropriate for measuring protein synthesis [30, 31], it may be said that the specific conditions of a given experiment should decide which method should be employed [32]. Thus, even though the constant infusion method is considered more appropriate for measurements of whole body protein turnover and synthesis of proteins with slow turnover rates during

steady state conditions, the difficulty in obtaining a correct assessment of the true precursor pool for protein synthesis (i.e. aminoacyl-tRNA), using surrogate measures of plasma and tissue fluid enrichments, remains a problem [32, 33]. On the other hand, an important question concerning the flooding technique is whether the large dose of tracee amino acid alters protein synthesis. Recent results implying such an effect [34, 35], are contradicted by the results of other studies where this finding has not been substantiated, including those in which an alternative method for protein synthesis measurement (ribosome analysis) has been employed [36, 37]. An alternative explanation may be that the labeling of the precursor amino acid was altered by the flood through stimulation or inhibition of shared metabolic and transport pathways [30]. Still, the results presented by Smith et al. raise concern over the use of the flooding technique, even though this concern seems less applicable when short periods of measurement are used [32]. Despite this concern, it was decided to employ the flooding technique in the present study for four reasons: (1) this technique does not require a metabolic steady-state and therefore renders studies of rapidly changing conditions possible (e.g. during critical illness), including investigations of proteins with high turnover rates (e.g. liver proteins); (2) the transcapillary escape rate of albumin, which is approximately 5%/h in healthy individuals, and probably even higher in ICU patients [38, 39], becomes less critical (long periods of measurement would imply a significant loss of labeled albumin into the extravascular space, please note below); (3) the problem of labeled amino acids reappearing in the precursor pool as a result of proteolysis (i.e. recycling) is minimized, and (4) since all potential precursor pools are flooded by the amino acid given, the isotopic enrichment of plasma is representative of its intracellular enrichment [16, 40].

Although the concentration of albumin was low in the present study, it did not fall further during the 5-day period of study, implying that both synthesis and degradation of albumin were elevated. This study, there-

fore, confirms the results from previous investigations, that albumin synthesis is increased in critical illness [3, 10, 11], not the opposite, as commonly believed (Fig. 1). The reason for the low plasma albumin concentration in critically ill patients, which develops very early in the illness, is more likely to be due to an increase in vascular permeability. This has the potential to change plasma albumin very rapidly and to a greater extent than changes in synthesis and degradation, occurring at a rate of up to several times higher than synthesis (and presumably degradation) in critical illness [38, 39].

Liver function tests were affected to a varying degree in many of the patients in this study, even though there was no difference between the groups in this respect (Table 4). Thus, GH treatment did not further impair liver function as assessed by biochemical parameters in the blood. In this context it may be of interest that albumin synthesis capacity is markedly depressed only in patients with severe liver failure [41], none of whom were included in this study. Considering the acute-phase response as well as the relatively high rates of albumin synthesis found in this and previous studies, it may be speculated that liver protein synthesis (including albumin synthesis) is one of the hepatic functions that seems to be given priority in critical illness.

In conclusion, this study has shown that albumin synthesis is consistently high in critically ill patients, and that provision of GH for 5 days does not alter this rate. Possibly more importantly, no inhibition of albumin synthesis was observed, which may indicate that GH treatment was not associated with negative effects on liver protein metabolism in critical illness.

**Acknowledgments** This investigation was supported by grants from the Swedish Medical Research Council (project 17X-04210, to J. W.), the County Council of Stockholm (to J. W.) and the Swedish Society of Medicine (to H. B.). Hans Barle is the grateful recipient of the 1993 ESPEN-Clintec Research Fellowship as well as of the 1999 ESPEN Research Fellowship. The skilled technical and nursing assistance of Mr. George Casella, Mrs. Viveka Gustavsson and Mrs. Eva Skoog is gratefully acknowledged.

## References

- Breuille D, Rose F, Arnal M, Melin C, Obled C (1994) Sepsis modifies the contribution of different organs to whole-body protein synthesis in rats. *Clin Sci* 86: 663-669
- Sjölin J, Stjernström H, Friman G, Larsson J, Wahren J (1990) Total and net muscle protein breakdown in infection determined by amino acid effluxes. *Am J Physiol* 258:E856-E863
- Mansoor O, Cayol M, Gachon P, Boirie Y, Scoeffler P, Obled C, Beaufrère B (1997) Albumin and fibrinogen synthesis rates increase while muscle protein synthesis decreases in head-injured patients. *Am J Physiol* 273:E898-E902
- Rosenblatt MD, Clowes GHA, George BC, Hirsch E, Lindberg B (1983) Exchange of amino acids by muscle and liver in sepsis. *Arch Surg* 118: 167-175
- Clowes GHA, Randall HT, Cha CJ (1980) Amino acid and energy metabolism in septic and traumatized patients. *JPEN* 4: 195-205
- Hunter KA, Ballmer PE, Anderson SE, Broom J, Garlick PJ, McNurlan MA (1995) Acute stimulation of albumin synthesis rate with oral meal feeding in healthy subjects measured with [ring-<sup>2</sup>H<sub>5</sub>]phenylalanine. *Clin Sci* 88: 235-242

7. Moshage HJ, Janssen JAM, Franssen JH, Hafkenscheid JCM, Yap SH (1987) Study of the molecular mechanism of decreased liver synthesis of albumin in inflammation. *J Clin Invest* 79: 1635–1641
8. McNurlan MA, Sandgren A, Hunter K, Essén P, Garlick PJ, Wernerman J (1996) Protein synthesis rates of skeletal muscle, lymphocytes, and albumin with stress hormone infusion in healthy man. *Metabolism* 45: 1388–1394
9. van Acker B, Hulsewé KWE, Wagenmakers AJM, Deutz NEP, von Meyenfeldt MF, Soeters PB (1998) Effect of surgery on albumin synthesis rate in humans (abstract). *Clin Nutr* 17 [Suppl 1]: 14
10. Dahn M, Mitchell RA, Lange MP, Smith S, Jacobs LA. (1995) Hepatic metabolic response to injury and sepsis. *Surgery* 117: 520–530
11. Essén P, McNurlan MA, Gamrin L, Hunter K, Calder G, Garlick PJ, Wernerman J. (1998) Tissue protein synthesis rates in the critically ill. *Crit Care Med* 26: 92–100
12. Liljedahl S-O, Gemzell, C.-A., Plantin, L.-O. and Birke G. (1961) Effect of growth hormone in patients with severe burns. *Acta Chir Scand* 122: 1–14
13. Herndon D, Barrow RE, Kunkel KR, Broemeling L, Rutan RL (1990) Effects of recombinant human growth hormone on donor-site healing in severely burned children. *Ann Surg* 212: 424–429
14. Voerman HJ, Strack van Schijndel RJM, Groenewald ABJ, de Boer H, Nauta JP, van der Veen EA, Thijs LG (1992) Effects of recombinant human growth hormone in patients with severe sepsis. *Ann Surg* 216: 648–655
15. Douglas RG, Humberstone DA, Haystead A, Shaw JHF (1990) Metabolic effects of recombinant human growth hormone: isotopic studies in the postabsorptive state and during parenteral nutrition. *Br J Surg* 77: 785–790
16. Barle H, Essén P, Nyberg B, Olivecrona, Tally M, McNurlan MA, Wernerman J, Garlick PJ (1999) Depression of liver protein synthesis during surgery is prevented by growth hormone. *Am J Physiol* 276:E620–E627
17. McNurlan M, Garlick PJ, Frost RA, deCristofaro K, Lang CH, Steigbigel R, Fuhrer J, Gelato MC (1998) Albumin synthesis and bone collagen formation in human immunodeficiency virus-positive subjects: differential effects of growth hormone administration. *J Clin Endocrinol Metab* 83: 3050–3055
18. Barle H, Råhlén L, Essén P, McNurlan MA, Garlick PJ, Holgersson J, Wernerman J (2001) Stimulation of human albumin synthesis and gene expression by growth hormone treatment. *Clin Nutr* 20: 59–67
19. Peterson S, Jeevanandam M, Shahbazian LM, Holaday NJ (1997) Reprioritization of liver protein synthesis resulting from recombinant human growth hormone supplementation in parenterally fed trauma patients: the effect of growth hormone on the acute-phase response. *J Trauma* 42: 987–996
20. Takala J, Ruokonen E, Webster N, Nielsen M, Zandstra D, Vundelinckx G, Hinds C (1999) Increased mortality associated with growth hormone treatment in critically ill adults. *N Engl J Med* 341: 785–792
21. Knaus W, Draper EA, Wagner DP, Zimmerman JE (1985) APACHE II: A severity of disease classification system. *Crit Care Med* 13: 818–829
22. McNurlan MA, Essén P, Thorell A, Calder AG, Anderson SE, Ljungqvist O, Sandgren A, Grant I, Tjäder I, Ballmer PE, Wernerman J, Garlick PJ (1994) Response of protein synthesis in human skeletal muscle to insulin: an investigation with L-[<sup>2</sup>H<sub>5</sub>]-phenylalanine. *Am J Physiol* 267:E102–E108
23. Barle H, Nyberg B, Essén P, Andersson K, McNurlan MA, Wernerman J, Garlick PJ (1997) The synthesis rates of total liver protein and plasma albumin determined simultaneously in vivo in man. *Hepatology* 25: 154–158
24. Korner A, Debro JA (1956) Solubility of albumin in alcohol after precipitation by trichloroacetic acid: a simplified procedure for separation of albumin. *Nature* 178: 1067
25. Ballmer PE, McNurlan M, Milne E, Heys SD, Buchan V, Calder AG, Garlick PJ (1990) Measurement of albumin synthesis in humans: a new approach employing stable isotopes. *Am J Physiol* 259:E797–E803
26. Ballmer PE, Weber BK, Roy-Chaudhury P, McNurlan MA, Watson H, Power DA, Garlick PJ (1992) Elevation of albumin synthesis rates in nephrotic patients measured with [<sup>13</sup>C]leucine. *Kidney Int* 41: 132–138
27. Roth E, Valentini L, Semsroth M, Hölzenbein T, Winkler S, Blum WF (1995) Resistance of nitrogen metabolism to growth hormone treatment in the early phase after injury of patients with multiple injuries. *J Trauma* 38: 136–141
28. Dahn MS, Lange P, Jacobs LA (1988) Insulin-like growth factor I production is inhibited in human sepsis. *Arch Surg* 123: 1409–1414
29. Ross R, Chew SL (1995) Acquired growth hormone resistance. *Eur J Endocrinol* 132: 655–660
30. Garlick PJ, McNurlan, MA, Essén P, Wernerman J (1994) Measurement of tissue protein synthesis rates in vivo: a critical analysis of contrasting methods. *Am J Physiol* 266:E287–297
31. Rennie MJ, Smith K, Watt PW (1994) Measurement of human tissue protein synthesis: an optimal approach. *Am J Physiol* 266:E298–E307
32. Davis TA, Reeds PJ (2001) Of flux and flooding: the advantages and problems of different isotopic methods for quantifying protein turnover in vivo: II. Methods based on the incorporation of tracer. *Curr Opin Clin Nutr Metab Care* 4: 51–456
33. Ljunqvist OH, Persson M, Ford GC, Nair KS (1997) Functional heterogeneity of leucine pools in human skeletal muscle. *Am J Physiol* 273: E564–E570
34. Smith K, Downie S, Barua JM, Watt PW, Scrimgeour CM, Rennie MJ (1994) Effect of a flooding dose of leucine in stimulating incorporation of constantly infused valine into albumin. *Am J Physiol* 266:E640–E644
35. Smith K, Reynolds N, Downie S, Patel A, Rennie MJ (1998) Effects of flooding amino acids on incorporation of labeled amino acids into human muscle protein. *Am J Physiol* 275:E73–E78
36. McNurlan MA, Essén P, Heys SD, Buchan V, Garlick PJ, Wernerman J (1991) Measurement in human skeletal muscle: further investigation of the flooding technique. *Clin Sci* 81: 557–564
37. Jahoor F, Zhang X-J, Baba H, Sakurai Y, Wolfe RR (1992) Comparison of constant infusion and flooding dose techniques to measure muscle protein synthesis rate in dogs. *J Nutr* 122: 878–887
38. Fleck A, Colley CM, Myers MA (1985) Liver export proteins and trauma. *Br Med Bull* 41: 265–273
39. Fleck A, Hawker F, Wallace PI, Raines G, Trotters J, Ledingham I McA, Calman KC (1985) Increased vascular permeability: a major cause of hypoalbuminemia in disease and injury. *Lancet* 1: 781–784
40. Davis TA, Fiorotto ML, Nguyen HV, Burrin DG (1999) Aminoacyl-tRNA and tissue free amino acid pools are equilibrated after a flooding dose of phenylalanine. *Am J Physiol* 277:E103–E109
41. Ballmer PE, Walshe D, McNurlan MA, Watson H, Brunt PW, Garlick PJ (1993) Albumin synthesis rates in cirrhosis: correlation with Child-Turcotte classification. *Hepatology* 18: 292–297