Growth hormone enhances fat-free mass and glutamine availability in patients with short-bowel syndrome: an ancillary double-blind, randomized crossover study

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ABSTRACT

Background: Benefits of recombinant human growth hormone (rhGH) alone or combined with glutamine in patients with intestinal failure because of short-bowel syndrome remain controversial.

Objective: We explored effects of rhGH on whole-body protein metabolism in patients with short-bowel syndrome with intestinal failure (SBS-IF) to gain insight into its mechanism of action.

Design: Eight stable hyperphagic patients with severe SBS-IF received, in a double-blind, randomized crossover study, low-dose rhGH (0.05 mg · kg⁻¹ · d⁻¹) and a placebo for two 3-wk periods. Leucine and glutamine kinetics under fasting and fed conditions, fat-free mass (FFM), and serum insulin were determined on the final day of each treatment.

Results: rhGH increased FFM and nonoxidative leucine disposal (NOLD; an index of protein synthesis) (P < 0.02), whereas FFM and NOLD were correlated in the fed state (r = 0.81, P = 0.015). With rhGH administration, leucine release from protein breakdown (an index of proteolysis) decreased in the fed compared with fasting states (P = 0.012), which was not observed with the placebo. However, the fast-to-fed difference in leucine release from protein breakdown was not significantly different between rhGH and placebo (P = 0.093). With rhGH, the intestinal absorption of leucine and glutamine increased (P = 0.036) and correlated with serum insulin (r = 0.91, P = 0.002). rhGH increased glutamine de novo synthesis (P < 0.02) and plasma concentrations (P < 0.03) in both fasting and fed states.

Conclusions: In SBS-IF patients, feeding fails to decrease proteolysis in contrast to what is physiologically observed in healthy subjects. rhGH enhances FFM through the stimulation of protein synthesis and might decrease proteolysis in response to feeding. Improvements in de novo synthesis and intestinal absorption increase glutamine availability over the physiologic range, suggesting that beneficial effects of rhGH in hyperphagic patients might be achieved without glutamine supplementation.  


INTRODUCTION

The administration of recombinant human growth hormone (rhGH) may be beneficial in short-bowel syndrome (SBS) patients with chronic intestinal failure (IF) requiring long-term home parenteral nutrition (HPN) (1). Byrne et al (2, 3), in uncontrolled trials performed in 10 HPN-dependent SBS with IF (SBS-IF) patients, were the first to suggest a positive effect of rhGH combined with oral glutamine and a modified diet. Since that time, 5 randomized, double-blind, placebo-controlled studies have examined the effect of rhGH alone or combined with glutamine in SBS-IF adult patients (4–8). The 4 crossover studies reported improvement in both body weight (5–8) and lean body mass (5, 7), rhGH also increased intestinal absorption assessed at the end of treatment (5–7) but not when measured 5 d after rhGH discontinuation (8). However, contrary to the more recent randomized trial of Byrne et al (4), those 4 crossover studies did not seek to reduce parenteral nutrition (PN) dependence. Whatever the benefits, they were transient and disappeared within weeks after the final injection of rhGH, presumably because of the short half-life of rhGH and rapid turnover of human enterocytes (∼5 d) (9). In light of these data and the known side effects of rhGH, the latter is not routinely used in SBS-IF patients in Europe (10), and a recent Cochrane database review showed insufficient evidence for recommending rhGH in SBS-IF patients.  

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2 Granted by the Délégation à la Recherche Clinique, Assistance Publique-Hôpitaux de Paris, Paris, France. A fellowship of DS was granted by a prize from the Société Francophone Nutrition Clinique et Métabolisme and the Fondation pour la Recherche Médicale, France. Pharmacia & Upjohn AB, Sweden, generated the randomization sequence and provided blinded growth hormone and placebo treatments.

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4 Abbreviations used: FFM, fat-free mass; GHBP, growth hormone binding protein; GLP-2, glucagon-like peptide 2; HPN, home parenteral nutrition; IF, intestinal failure; IGF-1, insulin-like growth factor 1; IGFBP-3, insulin-like growth factor binding protein 3; NOLD, nonoxidative leucine disposal; PN, parenteral nutrition; rhGH, recombinant human growth hormone; SBS, short-bowel syndrome; SBS-IF, short-bowel syndrome with intestinal failure.

Received January 7, 2014. Accepted for publication June 26, 2014.

First published online July 30, 2014; doi: 10.3945/ajcn.113.071845.
rhGH despite its positive effect on weight gain and energy absorption (1). Likewise, the randomized trials produced conflicting data on the rationale (7) and benefits of glutamine supplementation in a group of 12 patients with SBS-IF (7).

rhGH and glutamine are thought to enhance intestinal adaptation, thereby enabling the remnant bowel to compensate for impairment of its absorptive capacities (9). Very few studies have addressed mechanisms that explain the clinical efficacy of such treatments in SBS-IF patients.

Thus, the aim of this ancillary study was to gain insight into the mechanism of the beneficial effects of rhGH at the end of a 3-wk course of a low-dose treatment compared with a placebo on whole-body protein metabolism in patients with SBS with permanent IF.

SUBJECTS AND METHODS

Patients

Adult patients with SBS-IF (a remnant small bowel <150 cm) were enrolled from a cohort followed at the accredited center for HPN at Lariboisière Saint-Lazare Hospital, Paris, France. The length of postduodenal remaining gut structures was determined as described previously (7). Patients with chronic IF requiring HPN ≥1 y and ad libitum oral feeding ≥1.0-fold their estimated basal metabolic rate (calculated by using the Harris-Benedict equation) were considered for inclusion (11). Ongoing medication was continued. Severe malnutrition according to Detsky et al (12), active disease or malignancy, recent sepsis or surgery, an unstable condition, severe extra intestinal organ failure, and diabetes were exclusion criteria as described previously (7). The protocol was approved by the Ethics Committee for Medical Research, Paris, France, in accordance with the principles of the Helsinki Declaration of 1975 as revised in 1983. All included patients provided written signed consent.

Study setting

This was a double-blind, placebo-controlled, randomized, 7-wk crossover study (Figure 1). Three-week placebo- and active-treatment periods were randomly assigned at the first visit. Treatment were administered by a subcutaneous injection daily at 0800. The two 3-wk periods (days 1–21 and 29–49) were separated by a 1-wk washout period. During the third week of each period, patients were hospitalized (days 17–21 and 45–49) for the return of used and unused vials to verify compliance and the performance of the intestinal absorption study. Leucine and glutamine kinetics were performed the last day of each hospital stay (days 21 and 49). Consequently, ≥23 d elapsed between the assessment of placebo and rhGH treatments (Figure 1). During the study, the HPN composition (energy and nitrogen) was unchanged, patients were encouraged to maintain their usual ad libitum hyperphagic diets; their food habits and preferences were respected.

Random assignment and masking

The randomization sequence of the 2 crossover periods was generated independently of investigators by the Department of Biostatistics and Data Management (Pharmacia & UpJohn AB) by using a computer procedure that was based on a block size of 4. Placebo and active treatments were prepared and numbered...
Whole-body leucine and glutamine kinetics

Infusion protocol

Whole-body leucine and glutamine kinetics were determined in postabsorptive and fed states after overnight fasting as described elsewhere (Figure 1) (13, 14). The night before the final day of each treatment period, patients ate dinner at 1900 and fasted until 1200 the next day. During the night, patients received neither energy (glucose or lipid emulsion) nor nitrogen (amino acid solution) via the parenteral route but only water and electrolytes as needed. From 0900 to 1600, primed continuous infusions of sterile pyrogen-free saline solutions of L-[1-13C]leucine (99% 13C) and L-[2-15N]glutamine (99% 15N) (Mass Trace) provided 4 and 6 μmol · kg\(^{-1}\) · h\(^{-1}\), respectively, and were administered intravenously by using the same calibrated syringe pump (Perfusor IV; Braun). Labeled amino acids were infused in the fasting state for 3 h (from 0900 to 1200) and in the fed state while patients ingested 8 identical snacks that were based on an isonitrogenous mixed meal (Mixés NP; Novartis Nutrition SA) that were given at 30-min intervals until 1600. These snacks corresponded to one-third of each patient’s daily overall energy intake on the basis of a baseline dietary history obtained by the dietitian. To limit the natural enrichment in 13C of the exogenous glucose more commonly extracted from cornstarch, beet glucose was used in PN bags from the day of patients’ inclusion in the study. Throughout the isotope-infusion study, patients remained supine and resting with their heads placed under the ventilated canopy of an indirect calorimeter (Deltatrac II; Datex Instrumentation), which enabled the continuous measurement of the carbon dioxide (CO\(_2\)) rate and oxygen consumption (O\(_2\)) rate. Blood and breath samples were obtained just before the start of the tracer infusion and at 12- and 15-min intervals, respectively, during the final hour of fasting (1100–1200) and fed states (1500–1600). During sampling periods, the patient’s hand was warmed in a heated box (air temperature: 60–65°C) to produce arterialized venous blood (15). Samples were centrifuged immediately at 4°C, and 0.5-mL aliquots of plasma were stored at −80°C until analysis. Breath samples were collected in 0.5-L breath bags and immediately transferred to 10-mL evacuated tubes (Becton Dickinson) to determine exhaled 13CO\(_2\).

Analytic methods

Plasma [\(^{13}\)C]leucine and [\(^{15}\)N]glutamine enrichments were determined by electron-impact gas chromatography–mass spectrometry (GCMS MSD 5972; Hewlett-Packard) by using N-trifluoroacetyl-n-butyl ester and N-acetyl-n-propyl ester derivatives, respectively, as previously described (16–18). Appropriate standard curves were run simultaneously for the determination of enrichments. The enrichment of expired 13CO\(_2\) was determined by using gas chromatography–isotope ratio mass spectrometry (IRMS Tracer Mass; Europa Scientific). Plasma and breath CO\(_2\) isotopic enrichments were calculated from preinfusion-corrected isotope ratios (19, 20).

Calculations

The isotopic steady state in both fasting and fed states was defined by a plateau in plasma and breath CO\(_2\) enrichments with a CV, ie,

\[
CV = 100 \times SD/\text{mean} \quad (1)
\]

defined by a plateau in plasma and breath CO\(_2\) enrichments with a CV, ie,

\[
CV = 100 \times SD/\text{mean} \quad (1)
\]
of 3 consecutive measurements <10%. Leucine and glutamine appearance rates (Ra\(_{\text{Leu}}\) and Ra\(_{\text{Gln}}\)) and leucine oxidation (Ox\(_{\text{Leu}}\)) were calculated by using equations for steady state conditions as previously described and were expressed in micromoles per kilogram per hour (20).

In the fasting state, because leucine is an essential amino acid, Ra\(_{\text{Leu}}\) is entirely derived from the protein breakdown, whereby

\[
B_{\text{Leu}} = R_{\text{aLeu}} \quad (2)
\]

and represents an index of whole-body proteolysis. The rate of leucine incorporation into protein synthesis (S\(_{\text{Leu}}\)), which corresponds to nonoxidative leucine disposal (NOLD) and represents an index of whole-body protein synthesis, was estimated as follows (19):

\[
\text{NOLD} = S_{\text{Leu}} = B_{\text{Leu}} - O_{\text{XLeu}} \quad (3)
\]

In contrast, because glutamine is a nonessential amino acid, both the release of glutamine from protein breakdown (B\(_{\text{Gln}}\)) and de novo glutamine synthesis (D\(_{\text{Gln}}\)) contribute to Ra\(_{\text{Gln}}\) (20, 21) as follows:

\[
R_{\text{aGln}} = B_{\text{Gln}} + D_{\text{Gln}} \quad (4)
\]

With consideration that the release of glutamine and leucine from protein breakdown is directly proportional to their relative abundance in whole body protein

\[
B_{\text{Gln}} = k \times B_{\text{Leu}} \quad (5)
\]

where \(k = 0.423\) and corresponds to the ratio of glutamine to leucine abundance in body protein; consequently (22)

\[
D_{\text{Gln}} = R_{\text{aGln}} - (0.423 \times B_{\text{Leu}}) \quad (6)
\]

In the fed state, Ra\(_{\text{Leu}}\) results from the release of leucine from both body protein breakdown (B\(_{\text{Leu}}\)) and oral protein intake (I\(_{\text{Leu}}\)), whereby

\[
B_{\text{Leu}} = R_{\text{aLeu}} - I_{\text{Leu}} \quad (7)
\]

Similarly, in the fed state, D\(_{\text{Gln}}\) can be calculated as follows:

\[
D_{\text{Gln}} = R_{\text{aGln}} - (0.423 \times B_{\text{Leu}}) - (0.423 \times I_{\text{Leu}}) \quad (8)
\]

where I\(_{\text{Leu}}\) (in μmol · kg\(^{-1}\) · h\(^{-1}\)) is calculated from the amount of net nitrogen absorbed by the intestine after oral
intake (amount of protein intake multiplied by the percentage of net intestinal absorption for nitrogen), with consideration of a content of 0.08 of leucine per gram of protein, and $k = 0.423$ is the ratio of bound glutamine to leucine in protein (20).

**Variables that reflected effects of rhGH**

Because the current study was arduous, it was presented to the first 8 consecutive patients in the 12 patients enrolled in the 2003 published trial as an optional extension (7). Because this extension was carried out simultaneously, the following selected data from these 8 patients were obtained from the main study: body weight, FFM assessed by using bioelectric impedance analysis, 3-d net intestinal nitrogen absorption expressed as the percentage of oral nitrogen intake (one of the macronutrients that represented the primary criteria of judgment in the main study) by using the duplicated diet method, and PN dependence (expressed as the percentage of total daily energy given via glutamine-free PN divided by the basal metabolic rate assessed by the Harris-Benedict equation multiplied by 1.5, which is a coefficient that corresponded to the estimate of the total energy expenditure) (23). Results of ensuing blood tests were also obtained for the 8 enrolled patients were as follows: serum growth hormone binding protein (GHBP), insulin-like growth factor 1 (IGF-1), insulin-like growth factor binding protein 3 (IGFBP-3), insulin, and glucose concentrations.

On day 21 of each treatment period, plasma amino acid concentrations were measured both in fasting and fed states by using ion-exchange liquid chromatography (Beckman 6300 chemistry analyzer; Beckman Coulter) (24). Insulin resistance was evaluated by using the HOMA-IR index (25), whereby

$$\text{HOMA-IR} = \frac{\text{fasting glucose (in mmol/L)}}{\text{fasting insulin (in μU/mL)}} \times 22.5$$

**Statistics**

In this crossover design, a sample size of 8 patients corresponded to the number required to obtain statistically significant data (significant level of 0.05 with power at 90%), with consideration of the primary endpoint of the main study, which was a 30% increase in macronutrient absorption when rhGH was given, with an SD of 20% and a correlation of 0.2 between repeated measures (7). Consequently, the trial was not designed to calculate the power of any variables studied in the ancillary study.

Quantitative data were expressed as medians (IQRs 25–75%). Three effects were tested according to the crossover design of the study as follows: treatment, period, and treatment × period interaction. Wilcoxon’s signed rank test was used to compare differences between rhGH and placebo values, whatever their order of administration. When appropriate, fasting and fed states were also compared with each other by using the same test. The period effect was tested by comparing differences between rhGH and placebo values between the 2 groups (rhGH in first and placebo in first) by using Wilcoxon’s rank-sum test. The treatment × period interaction was tested by comparing the average of rhGH and placebo values between the 2 groups (rhGH in first and placebo in first) by using Wilcoxon’s rank-sum test. Correlations between quantitative data were studied by using Spearman’s test. Statistical analyses were performed with SAS software (version 9.2; SAS Institute).

**RESULTS**

**Patients**

Patients were recruited and completed the study between November 1997 and April 1998. Six patients had a jejunocolonic anastomosis, and 2 patients had an end jejunostomy; none of the patients had a remnant ileum or ileocecal valve (Table 1). Although patients consumed an ad libitum diet that provided a median (IQR 25–75%) of 2.1 times (1.6–2.9 times) the calculated baseline basal metabolic rate defined as hyperphagic (26), all patients had chronic IF and received 1361 kcal/d (803–1677 kcal/d) via PN, which corresponded to an HPN dependence of 61% (36–75%) (Table 1). The 8 patients enrolled received the planned dose of treatment and completed the study. One-half of patients received the placebo first and then rhGH (the other one-half of patients received treatments in the reverse order). The tolerance was satisfactory without serious adverse events observed. No significant period effect and no interaction between treatment and period were detected for any measured variable.

**rhGH effects**

Three-day spontaneous energy and nitrogen oral intakes were not significantly different between placebo and rhGH treatments (2842 kcal/d (2363–4018 kcal/d) compared with 3482 kcal/d (2790–4395 kcal/d) and 17.9 g N/d (15.3–25.4 g N/d) compared with 18.9 g N/d (18.8–23.4 g N/d), respectively). Body weight and FFM (in kg and percentage of body weight) increased with the rhGH treatment. rhGH was associated with higher concentrations of IGF-1 and IGFBP-3 compared with placebo values, whereas concentrations of GHBP decreased. The median for insulin was 6.2 μU/mL, and the high end of the normal range was 15 μU/mL. Insulin concentrations tended to be higher than during the placebo period ($P = 0.069$), whereas fasting serum glucose concentrations were unaltered. When rhGH was given, HOMA-IR tended to increase ($P = 0.069$), its median was 1.35, and the high end of the normal range was 2.5 (Table 2).

Fasting baseline plasma concentrations (in μmol/L) of leucine (126; 88–144), glutamine (690; 478–795), arginine (17; 11–23), arginine (54; 32–74), and total amino acids (3092; 2950–3403) were not different from placebo period concentrations. The leucine plasma concentration decreased with the placebo during the fed compared with fasting states ($P = 0.05$). Compared with the placebo treatment, the rhGH treatment increased the leucine appearance rate ($\text{Ra}_{\text{Leu}}$) and NOLD in both fasting ($P = 0.012$ and $P = 0.012$, respectively) and fed states ($P = 0.05$ and 0.012, respectively). $\text{Ra}_{\text{Leu}}$ and NOLD decreased during the fed state compared with the fasting state during rhGH ($P = 0.012$) and placebo ($P = 0.017$) treatments. Feeding failed to affect the leucine release from protein breakdown ($\text{B}_{\text{Leu}}$) during placebo treatment. The estimated release of leucine from oral intake ($I_{\text{Leu}}$) (taking into account the net intestinal absorption of
Initial characteristics of SBS-IF patients enrolled in a crossover study design (n = 8)

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>Age (y)</th>
<th>BMI (kg/m²)</th>
<th>Albumin (g/dL)</th>
<th>Oral energy intake (kcal)</th>
<th>Oral nitrogen intake (g)</th>
<th>Duration of HPN (d)</th>
<th>HPN infusions dependence (%)</th>
<th>Delay since last bowel surgery (d)</th>
<th>Remnant small bowel resection (cm)</th>
<th>Remnant colon resection (cm)</th>
<th>Postduodenal remnant small-bowel length (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>SBV</td>
<td>26</td>
<td>18.2</td>
<td>4.3</td>
<td>20%</td>
<td>100%</td>
<td>32 (26–40)</td>
<td>56 (48–64)</td>
<td>20 (16–24)</td>
<td>56 (48–64)</td>
<td>20 (16–24)</td>
<td>56 (48–64)</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>SBV</td>
<td>38</td>
<td>17.1</td>
<td>3.8</td>
<td>27%</td>
<td>100%</td>
<td>32 (26–40)</td>
<td>56 (48–64)</td>
<td>20 (16–24)</td>
<td>56 (48–64)</td>
<td>20 (16–24)</td>
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<tr>
<td>3</td>
<td>F</td>
<td>SBV</td>
<td>34</td>
<td>17.9</td>
<td>4.0</td>
<td>27%</td>
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<td>32 (26–40)</td>
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<td>M</td>
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<td>MI</td>
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Median (IQR 25–75%)

DISCUSSION

To our knowledge, our findings provide new insight into whole-body anabolic and intestinotrophic effects of low-dose rhGH in hyperphagic patients with severe SBS-IF. In 2010, a Cochrane review on trials on rhGH in SBS-IF patients suggested a positive but transient effect of rhGH on weight gain and energy absorption but questioned the usefulness of combining rhGH with glutamine and did not report any information about protein turnover (1). This review prompted us to analyze isotopic data that were collected during our clinical study (7).

The current study is the first study to our knowledge to assess protein kinetics in the fed state in patients receiving rhGH. Our results show a strong positive effect of rhGH on protein metabolism thanks to its anabolic effect. They bring new information on the pathophysiology of protein metabolism in SBS-IF patients showing that the feeding state failed to reduce the protein breakdown as it is physiologically observed in healthy subjects. In addition, our results strongly suggest that there is no benefit from the addition of glutamine to rhGH in SBS-IF patients.

The leucine metabolic clearance rate

\[ \text{MCR}_{\text{Leu}} = \frac{\text{Ra}_{\text{Leu}}}{\text{Leu}} \quad (10) \]
that compared each patient to himself or herself allowed for the metabolism in SBS-IF patients. The choice of a crossover design that supported a positive effect of rhGH treatment on protein error of 5%, this isotopic study provided significant information on nonsignificant variables. However, with consideration of a type I error of 5%, the latter showed a strong tendency to lower insulin sensitivity.

Because of the low sample size, it was not possible to conclude on subnormal nutritional status may have potentiated effects of rhGH compared with malnourished subjects (29–31). In these simulations, because effect sizes ranged from 0.45 to 1.10 respectively for each variable by using the same study design. In these simulations, because effect sizes ranged from 0.45 to 1.10 with a mean value of 0.64, the recommended sample size for a future study can be calculated. With a power of 80% and a correlation value of 0.5 between repeated measures, it would be necessary to recruit 31, 15, 32, 31, 39, and 8 subjects respectively for each variable by using the same study design. In these simulations, because effect sizes ranged from 0.45 to 1.10 with a mean value of 0.64, the recommended sample size for a subsequent study would be 20 subjects.

The short residual small-bowel length (31 cm), long HPN duration (5.2 y), and high degree of PN dependence (61%) indicated that subjects enrolled in the current study suffered from severe SBS with permanent IF. At 15 y follow-up, 2 patients died, 4 patients were still on HPN, and 2 patients were weaned from PN after 21 and 22 y. All patients but one received rhGH for only 3 wk during the trial; patient 6 was again treated with low-dose rhGH 11 y later and could be weaned from PN within 2 mo. Note that no clinical or statistical argument suggested a remnant effect of rhGH during the placebo period when rhGH was administered first, considering that the testing of a crossover design was not powerful.

Variations in FFM (although evaluated by bioelectric impedance analysis that presented a limitation), IGF-1, IGFBP-3, GHB, amino acid kinetics, and loss of correlation between fasting plasma citrulline and amounts of daily PN energy intake confirmed that patients were responsive to rhGH. Although some patients had low BMI, their body weights were stable, and they were not considered severely malnourished as assessed by IGF-1, plasma leucine, and glutamine concentrations (24, 27, 28). This subnormal nutritional status may have potentiated effects of rhGH compared with malnourished subjects (29–31). In these SBS-IF patients, hyperphagia, which is a key player for intestinal adaptation, should have enhanced IGF-1 secretion, which is the main effector of rhGH, in contrast with standardized diet used in previous rhGH trials (6, 8). As an illustration, the increase in IGF-1 concentrations was of the same range by using a low dose (current study) and higher dose (0.12–0.14 mg · kg⁻¹ · d⁻¹) of rhGH (6, 8). Moreover, probably because of the low-dose rhGH used, enrolled patients did not develop diabetes, and their serum glucose concentrations and HOMA-IR remained within normal values. However the latter showed a strong tendency to lower insulin sensitivity.

Because the current work constitutes an extension of our previous study, the sample size was only determined for the latter (7). Because of the low sample size, it was not possible to conclude on nonsignificant variables. However, with consideration of a type I error of 5%, this isotopic study provided significant information that supported a positive effect of rhGH treatment on protein metabolism in SBS-IF patients. The choice of a crossover design that compared each patient to himself or herself allowed for the improvement of the precision of estimated differences between placebo and active treatments and fasting and fed states and, therefore, increased the power of the study. With consideration that the differences observed between medians were +15% for RaLeu, +17% for NOLD, +17% for [Gln], +14% for RaGln, and +15% for D_Glu in the fasting state between placebo and rhGH and reached −29% for B_iLeu in the rhGH period between fasting and fed states, a sample size for a future study can be calculated. With a power of 80% and a correlation value of 0.5 between repeated measures, it would be necessary to recruit 31, 15, 32, 31, 39, and 8 subjects respectively for each variable by using the same study design. In these simulations, because effect sizes ranged from 0.45 to 1.10 with a mean value of 0.64, the recommended sample size for a subsequent study would be 20 subjects.

The rhGH treatment increased protein synthesis. During fasting, as already documented in other diseases and the fed state, rhGH increased the NOLD (an index of whole-body protein synthesis) (32–38). Gains in body weight and FFM may have been the consequence of salt and fluid retention secondary to treatment as reported with higher doses of rhGH (4, 6, 8). However, the significant correlation between fed NOLD and FFM during placebo and rhGH periods provided support that the anabolic effect observed during isotope infusion translated into an increase in lean body mass rather than an increase in extracellular fluid retention. This increase could be sustained by the increase in leucine turnover with rhGH compared with placebo in the fasting state (where RaLeu is equivalent to B_iLeu, which is an index of whole body protein synthesis), because higher lean body mass involved a potentially higher level of proteolysis during fasting. These results were consistent with the increase in glutamate de novo synthesis (D_Glu) in both fasting and fed states with rhGH.

During placebo administration, the leucine release from protein breakdown failed to decrease with feeding as observed in healthy subjects (20, 39, 40), which suggested that the load of nutrients absorbed by the remnant intestine may be insufficient to inhibit proteolysis in severe SBS-IF patients. During rhGH, the leucine release from protein breakdown decreased by −29%, which suggested that rhGH decreases proteolysis in response to feeding, whereas the fast-to-fed decrease in leucine release tended to be higher with rhGH than placebo, probably because of an insufficient statistical power. This feeding decline in

### TABLE 2

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Normal range</th>
<th>Baseline</th>
<th>Placebo</th>
<th>rhGH</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>—</td>
<td>58.9 (49.5–63.8)</td>
<td>59.4 (50.7–64.8)</td>
<td>61.7 (51.5–67.6)</td>
<td>0.012</td>
</tr>
<tr>
<td>FFM (kg)</td>
<td>—</td>
<td>49.2 (40.2–52.9)</td>
<td>49.1 (40.7–53.7)</td>
<td>52.4 (43.6–55.0)</td>
<td>0.017</td>
</tr>
<tr>
<td>FFM (% body weight)</td>
<td>—</td>
<td>82 (77–85)</td>
<td>82 (77–86)</td>
<td>84 (79–87)</td>
<td>0.05</td>
</tr>
<tr>
<td>GHB (% radioactivity)</td>
<td>19.4–48.7</td>
<td>26.5 (24.1–29.0)</td>
<td>26.9 (20.5–36.3)</td>
<td>19.6 (18.1–30.4)</td>
<td>0.012</td>
</tr>
<tr>
<td>IGF-1 (ng/mL)</td>
<td>100–226</td>
<td>130 (123–160)</td>
<td>133 (100–137)</td>
<td>421 (259–563)</td>
<td>0.012</td>
</tr>
<tr>
<td>IGFBP-3 (µg/mL)</td>
<td>1.8–4</td>
<td>2.3 (1.5–3.0)</td>
<td>2.2 (1.4–3.0)</td>
<td>3.0 (2.6–4.4)</td>
<td>0.012</td>
</tr>
<tr>
<td>Insulin (µg/mL)</td>
<td>3.0–15</td>
<td>4.2 (3.5–5.8)</td>
<td>4.7 (3.4–6.8)</td>
<td>6.2 (4.4–9.8)</td>
<td>0.069</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>4.0–5.5</td>
<td>4.4 (4.1–4.6)</td>
<td>4.3 (4.0–4.5)</td>
<td>4.3 (4.1–4.6)</td>
<td>NS</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>&lt;2.50</td>
<td>0.86 (0.66–1.12)</td>
<td>0.87 (0.61–1.40)</td>
<td>1.35 (0.83–1.76)</td>
<td>0.069</td>
</tr>
</tbody>
</table>

1. \(P\) values were derived from Wilcoxon’s signed rank test for 0.05 mg rhGH during 3 wk compared with a placebo. FFM, fat-free mass; GHB, growth hormone binding protein; IGF-1, insulin-like growth factor 1; IGFBP-3, insulin-like growth factor binding protein 3; rhGH, recombinant human growth hormone; SBS-IF, short-bowel syndrome with intestinal failure.

2. Median; IQR 25–75% in parentheses (all such values).
TABLE 3
Amino acid metabolism after placebo and rhGH treatments of SBS-IF patients enrolled in a crossover study design (n = 8) 1

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Placebo</th>
<th>rhGH</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leu</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting state</td>
<td>[Leu] (μmol/L)</td>
<td>127 (107–143)</td>
<td>117 (106–133)</td>
<td>NS</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>RaLeu = BLeu (μmol · kg⁻¹ · h⁻¹)</td>
<td>106 (96–128)</td>
<td>122 (101–148)</td>
<td>0.012</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>OxLeu (μmol · kg⁻¹ · h⁻¹)</td>
<td>18 (15–25)</td>
<td>18 (16–22)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>NOLD (μmol · kg⁻¹ · h⁻¹)</td>
<td>87 (79–100)</td>
<td>102 (89–122)</td>
<td>0.012</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>MCRLeu (mL · kg⁻¹ · h⁻¹)</td>
<td>895 (822–1105)</td>
<td>1103 (936–1188)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Fed state</td>
<td>[Leu] (μmol/L)</td>
<td>117 (93–138)</td>
<td>110 (90–129)</td>
<td>NS</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>RaLeu (μmol · kg⁻¹ · h⁻¹)</td>
<td>97 (86–112)</td>
<td>104 (93–134)</td>
<td>0.05</td>
<td>0.017</td>
</tr>
<tr>
<td></td>
<td>OxLeu (μmol · kg⁻¹ · h⁻¹)</td>
<td>22 (18–25)</td>
<td>19 (15–23)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>NOLD (μmol · kg⁻¹ · h⁻¹)</td>
<td>74 (63–91)</td>
<td>83 (76–113)</td>
<td>0.012</td>
<td>0.017</td>
</tr>
<tr>
<td></td>
<td>Bo (μmol · kg⁻¹ · h⁻¹)</td>
<td>100 (70–109)</td>
<td>87 (74–115)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Ile (μmol · kg⁻¹ · h⁻¹)</td>
<td>6 (4–14)</td>
<td>17 (6–28)</td>
<td>0.036</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>MCRLeu (mL · kg⁻¹ · h⁻¹)</td>
<td>864 (728–1116)</td>
<td>934 (809–1202)</td>
<td>0.036</td>
<td>NS</td>
</tr>
<tr>
<td>Gln</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting state</td>
<td>[Gln] (μmol/L)</td>
<td>624 (541–774)</td>
<td>728 (551–876)</td>
<td>0.025</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>RaGln = BGln (μmol · kg⁻¹ · h⁻¹)</td>
<td>333 (293–405)</td>
<td>381 (324–471)</td>
<td>0.012</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>DGln (μmol · kg⁻¹ · h⁻¹)</td>
<td>284 (253–356)</td>
<td>326 (272–421)</td>
<td>0.012</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>MCRGln (mL · kg⁻¹ · h⁻¹)</td>
<td>542 (517–594)</td>
<td>570 (479–644)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Fed state</td>
<td>[Gln] (μmol/L)</td>
<td>621 (541–737)</td>
<td>725 (569–847)</td>
<td>0.021</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>RaGln (μmol · kg⁻¹ · h⁻¹)</td>
<td>301 (276–351)</td>
<td>363 (300–382)</td>
<td>0.017</td>
<td>0.012</td>
</tr>
<tr>
<td></td>
<td>DGln (μmol · kg⁻¹ · h⁻¹)</td>
<td>251 (232–307)</td>
<td>318 (258–331)</td>
<td>0.017</td>
<td>0.036</td>
</tr>
<tr>
<td></td>
<td>Ile (μmol · kg⁻¹ · h⁻¹)</td>
<td>15 (12–35)</td>
<td>42 (15–71)</td>
<td>0.036</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>MCRGln (mL · kg⁻¹ · h⁻¹)</td>
<td>490 (452–524)</td>
<td>508 (447–576)</td>
<td>NS</td>
<td>0.036</td>
</tr>
<tr>
<td>Cit</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting state</td>
<td>[Cit] (μmol/L)</td>
<td>17 (8–24)</td>
<td>19 (14–23)</td>
<td>NS</td>
<td>0.017</td>
</tr>
<tr>
<td>Fed state</td>
<td>[Cit] (μmol/L)</td>
<td>14 (7–20)</td>
<td>16 (13–20)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Arg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting state</td>
<td>[Arg] (μmol/L)</td>
<td>58 (33–74)</td>
<td>56 (22–72)</td>
<td>NS</td>
<td>—</td>
</tr>
<tr>
<td>Fed state</td>
<td>[Arg] (μmol/L)</td>
<td>60 (28–68)</td>
<td>64 (35–77)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Overall AAs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting state</td>
<td>[AA] (μmol/L)</td>
<td>3043 (2619–3302)</td>
<td>2929 (2829–3669)</td>
<td>NS</td>
<td>—</td>
</tr>
<tr>
<td>Fed state</td>
<td>[AA] (μmol/L)</td>
<td>2808 (2543–3032)</td>
<td>2890 (2752–3562)</td>
<td>NS</td>
<td>0.012</td>
</tr>
</tbody>
</table>

1All values are medians; IQRs 25–75% in parentheses. P values were derived from Wilcoxon’s signed rank test. AA, amino acid; [AA], amino acid plasma concentration; B, release from protein breakdown; D, release from de novo synthesis; I, release from net intestinal absorption of nitrogen after oral intake; MCRAA = RaAA − [AA], amino acid metabolic clearance rate; NOLD, nonoxidative leucine disposal; Ox, oxidation; Ra, rate of appearance; rhGH, recombinant human growth hormone; SBS-IF, short-bowel syndrome with intestinal failure.

2Comparison of 0.05 mg rhGH · kg⁻¹ · d⁻¹ 3 wk compared with a placebo.

3Comparison of fasting compared with fed states during the placebo period.

4Comparison of fasting compared with fed states during the rhGH-treatment period.

proteolysis with rhGH may arise from 1) a true inhibition of protein breakdown, 2) improved release of leucine from oral intake (I_{Leu}), or 3) a combination of both, as suggested by a good correlation between I_{Leu} and insulin secretion with rhGH (r = 0.91, P = 0.002). Inhibition of whole body proteolysis is a main effect of insulin in vivo (41). Therefore, the decrease in proteolysis observed in our study during rhGH in the fed state might have been mediated by increased insulin secretion. However, because we did not assess insulin or IGF-1 concentrations in the fed state, we could not determine whether the drop in fed proteolysis was due to insulin, IGF-1, or their combined effect. In healthy subjects, by using [1-13C]leucine infusion technique, IGF-1 was shown to decrease fasting proteolysis (42, 43). In our patients, the correlation between IGF-1 and insulin shown during rhGH treatment suggested that the 2 hormones act together to slow down the average daily rate of proteolysis.

The current results on glutamine are consistent with our earlier reports in clinically stable, nonmalnourished enterectomized...
patients (24, 44, 45). Indeed, we showed that, compared with healthy subjects, SBS patients presented a significant increase in fasting plasma glutamine concentrations, whereas the fasting glutamine turnover was toward lower normal rates. These changes likely reflected the enterocyte mass reduction that induces a decline in glutamine use (44). rhGH treatment was associated with enhanced glutamine availability by 15% (ie, over the physiologic range in both fasting and fed states) (24, 44). Compared with the placebo, rhGH, by its systemic anabolic effect, increased the plasma glutamine concentration, glutamine turnover (RaGln), and de novo synthesis. These results may also have been related to a better functioning of the enterocyte mass because of the rhGH intestinotrophic effect that induced increased dietary glutamine absorption and generated more demand for a glutamine supply, which consequently increased glutamine turnover. However, plasma citrulline concentrations, which are recognized to be related to functional enterocyte mass (24), did not change significantly with rhGH, contrary to an earlier report in SBS-IF children (46). This result reflected the fact that, with rhGH, the rise in the production of glutamine is proportionally higher than the production of citrulline. This outcome could result from the combination of both a systemic anabolic effect (on de novo synthesis) and an intestinotrophic effect (on intestinal absorption) of rhGH on glutamine, whereas a rise of citrulline only results from the rhGH intestinotrophic effect (on enterocyte mass).

These data do not support the use of glutamine supplementation in SBS-IF patients; indeed, the combined administration of rhGH and oral glutamine warrants discussion (4, 6, 8). Whether such a supplementation is indicated when only an intestinotrophic analog such as glucagon-like peptide 2 (GLP-2) is used is still a matter of debate (47). The effectiveness of glutamine alone in the treatment of SBS-IF patients has not been shown (48, 49). One randomized controlled study with a 3-arm design compared the respective effects of oral glutamine compared with rhGH compared with rhGH plus oral glutamine in SBS-IF patients (4). The combined treatment was not more effective than rhGH alone, and the study design did not include a fourth placebo group, which precluded the assessment of the oral glutamine effect per se. Therefore, the putative benefit of a combined administration of oral glutamine and rhGH remains unproven, particularly in hyperphagic, well-nourished SBS-IF patients in whom fasting glutamine concentrations are already increased, as in the current study (24, 49).

The current study adds mechanistic information to our previous article on clinical outcomes by showing that, in hyperphagic SBS-IF patients, proteolysis was not inhibited in the feeding state, and compared with normal ranges, glutamine concentrations were higher, whereas glutamine kinetics remained lower. In the light of previous studies, the beneficial effects of rhGH seem to result from a combination of whole-body anabolic effects on lean body mass and improvement in the net intestinal absorption of oral food intake, with the knowledge that the latter wears off within the month after discontinuation of rhGH (4–8). Systemic and intestinotrophic effects of low-dose rhGH in SBS-IF patients may generate a third insulin-induced effect that allows rhGH, IGF-1, and insulin to act synergistically, possibly potentiating one another, as reported by Wheeler et al (50) in vitro. Taken together, these effects sustain our hypothesis that rhGH treatment enhances the decrease of proteolysis in response to feeding, thereby suggesting that decreased intestinal absorption in SBS-IF patients might cause insufficient insulin secretion that is unable to impede proteolysis.

In conclusion, our results provide support that interventional studies should consider the benefit of rhGH in SBS-IF patients in additional circumstances without additional glutamine but with respect for their hyperphagic habits. First, because of its whole-body effects, rhGH could be used as a booster of intestinal adaptation (1). Second, in addition to the recent encouraging results obtained with GLP-2 (51), the current study suggests to support the use of rhGH in combination with the GLP-2 analog for additional intestinotrophic and systemic anabolic effects of rhGH. This method could be of benefit when GLP-2 alone is insufficient to wean patients off PN, especially those with remnant ileum or ileocolon in whom basal GLP-2 concentrations are high (52).

We thank MC Morin for carrying out dietary inquiries, SK Lim (Service de Biochimie, Hôpital St Antoine, Assistance Publique-Hôpitaux de Paris) for the assay of plasma amino acid concentrations, and Pharmacia & Upjohn AB, Sweden, for the generous supply of rhGH and placebo treatments.

The authors’ responsibilities were as follows—DS, DD, AD, FT, LC, and BM: designed the research; DS, FT, and BM: conducted the research; LC: provided essential reagents and materials; DS and AD: analyzed data; DS: wrote the draft of the manuscript; DS, DD, AD, LC, AC, FG, and BM: critically revised the manuscript; DS, DD, and BM: had primary responsibility for the final content of the manuscript; and all authors: read and approved the final version of the manuscript for submission. None of the authors had a personal or financial conflict of interest.

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