A Nitrogen-Free Hypocaloric Diet and Recombinant Human Growth Hormone Stimulate Postoperative Protein Synthesis: Fasted and Fed Leucine Kinetics in the Surgical Patient

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Twelve otherwise healthy patients undergoing elective surgery for resection of rectosigmoid adenocarcinoma were randomly allocated to two groups: one group receiving intravenous dextrose 5% 600 to 800 kcal · d^{-1} (DX, n = 6) and the other group receiving the same amount of dextrose intravenously plus recombinant human growth hormone (DX + rGH, n = 6). Supplementation with rGH started on the day of surgery and continued postoperatively for 5 days. No nitrogen was provided in the diet. This regimen was started 3 days before surgery and continued for 5 days after surgery. Protein kinetics were studied over a period of 8 hours in all patients. Following an overnight fast, a primed constant infusion of L-[1-^{13}C]leucine was maintained for 4 hours (fasted state) and continued for a further 4 hours (fed state) during which 5% beet dextrose (low ^{13}C content) with or without rGH was administered. The isotope studies were performed on the day before surgery and 6 days after surgery. Other measurements included urinary nitrogen excretion, gaseous exchange, and plasma concentrations of insulin, GH, and insulin-like growth factor-I (IGF-I). Addition of rGH to the dextrose diet had a significant positive effect on protein synthesis ($P = .02$). Surgery was responsible for a significant increase in postoperative whole-body protein breakdown and synthesis and leucine oxidation ($P < .01$), although lesser changes were observed in the DX group. An interaction between rGH and surgery was associated with a significant increase in protein synthesis ($P = .009$), but not with changes in either protein breakdown or leucine oxidation. Carbohydrate provision in the form of beet dextrose during the fed state of the isotopic study did not attenuate the significant decrease in protein synthesis ($P = .01$) or breakdown ($P = .003$) either before or after surgery, probably reflecting the absence of nitrogen in the diet. No significant interaction was found between rGH and feeding. These results of leucine kinetics indicate that addition of rGH to a low-dextrose intake in the absence of dietary nitrogen can actually promote protein synthesis. The low levels of leucine oxidation could be explained by the fact that amino acids resulting from protein degradation were directed preferentially toward resynthesis of new proteins rather than to oxidative pathways. There was a significant increase in plasma insulin and GH in the group receiving rGH ($P < .05$). The postoperative plasma concentration of IGF-I did not change in the latter group compared with the DX group, in which IGF-I concentration decreased significantly ($P < .05$) as part of the response to combined surgery and dietary restriction. Although both IGF-I and insulin are independently capable of stimulating protein synthesis, elevated levels of either hormone or GH itself may primarily modulate protein synthesis, even with a low intake of carbohydrates.

THE EFFECT OF NUTRIENT WITHDRAWAL in normal healthy subjects has been investigated, and the opinion is unanimous that fasting causes a reduction in protein synthesis.[1] However, from a number of studies conducted in the surgical patient, a postinjury reduction in protein synthesis can no longer be accepted in support of this hypothesis without significant reservations. The evidence is that unless there is absolute comparability of nutritional intake between paired studies, the effect of surgery may be masked by greater changes resulting from nutritional factors including fasting.[2-4]

Patients undergoing major abdominal surgery with gut resection inevitably undergo a period when they are unable to feed orally, and it is routine practice in many surgical units to provide dextrose solutions only after surgery until patients are able to commence eating. The total amount of calories patients receive after surgery normally varies between 600 and 800 kcal · d^{-1}.

Although the immediate nutritional regimen of injured patients should include sufficient calories to spare body proteins, there is a limit to the ability of the body to derive energy from infused glucose. Hypocaloric glucose intake in injured patients cannot suppress endogenous glucose production, although it does in normal subjects.[5] The suppressibility of gluconeogenesis by an exogenous supply of glucose is dose-dependent in normal and in injured surgical patients. Provision of glucose 4 mg · kg^{-1} · min^{-1} to stressed patients has been shown to suppress hepatic glucose production by 55% without adverse effect.[6]

To explain the mechanism by which whole-body protein synthesis increases following administration of recombinant human growth hormone (rGH) in surgical patients receiving a moderate intake of nitrogen and calories, we have previously assessed whole-body protein metabolism in the fasted and the fed state.[7] The findings indicated that the observed postoperative increase in whole-body protein synthesis was achieved through an inhibition of amino acid oxidation.

Ward et al,[8] using a 24-hour ^{15}N-glycine infusion, reported a significant increase in postoperative whole-body protein breakdown and synthesis in a group of surgical patients receiving a nonnitrogenous hypocaloric diet and rGH supplementation. They used urinary nitrogen excretion as the measure of amino acid oxidation in the calculation of protein synthesis. Some concern has been expressed with reference to isotope studies conducted over a 24-hour period, when caloric supplementation has not been necessarily constant, and to the possible specific effects of rGH on glycine metabolism.

Although the insulin-like growth factor-I (IGF-I) response to rGH has been shown to depend on adequate dietary carbohydrate,[9] it is far from clear what is the minimal amount of

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carbohydrate required for the injured patient to modulate the action of rGH.

The present study was undertaken to investigate the effect of rGH supplementation together with a nonnitrogen minimal-calorie diet on postoperative protein metabolism using a similar experimental design in which the direct effect of nutrients on aspects of leucine kinetics were assessed in both fasted and fed states.[7] In addition, changes in plasma hormone profiles and gaseous exchange were determined.

SUBJECTS AND METHODS

Twelve patients scheduled for elective sigmoid and anterior resection of localized adenocarcinoma of the rectum were entered onto the study. None of the patients had metastatic diseases or suffered from malnutrition or weight loss, and they were otherwise healthy. The study was approved by the Ethics Committee of Northwick Park Hospital, and all patients supplied written informed consent.

Nutrition

All patients entering the study were seen 10 days before surgery and began a dietary regimen consisting of nitrogen 0.1 g · kg^{-1} · d^{-1} and 20 kcal · kg^{-1} · d^{-1}. This diet was changed to a nitrogen-free hypocaloric regimen of 10 kcal · kg^{-1} · d^{-1} (600 to 800 kcal) provided by 5% dextrose 3 days before surgery and continued for 5 days after surgery according to current surgical practice in our hospital.

The patients were randomly allocated to two groups of six patients each: one group receiving only dextrose solution ([DX] control group; mean ± SD age, body weight, and body mass index [BMI], 65 ± 5 years, 64 ± 8 kg, and 23 ± 4 kg/m^2, respectively) and the other group receiving dextrose and rGH ([DX + rGH] treatment group; mean ± SD age, body weight, and BMI, 71 ± 9 years, 67 ± 6 kg, and 24 ± 2 kg/m^2). In the latter group, rGH (Genotrophin; Kabi-Pharmacia, Milton Keynes, UK) was administered subcutaneously (0.15 μU · kg^{-1} · d^{-1}) at 12:00 noon on the day of surgery and for 5 days postoperatively. In the DX group, an equivalent volume of 0.9% NaCl was administered subcutaneously.

Anesthesia and Surgical Care

General anesthesia consisted of thiopentone, vecuronium, enflurane, and nitrous oxide in oxygen. Postoperative pain relief was achieved and maintained for 3 to 4 days with a subcutaneous infusion of papaveretum at a rate of 3 to 5 mg · h^{-1}. The duration (mean ± SD) of surgery was similar in both groups; 156 ± 32 minutes for the DX group and 174 ± 44 minutes for the DX + rGH group. Blood loss was 678 ± 343 and 803 ± 295 mL for the two groups, respectively. Hartmann's solution (4 to 6 mL · kg^{-1} · h^{-1}) was infused intravenously during surgery.

Protein Kinetics

Leucine kinetic studies during the fasted and fed states were conducted on the day before surgery and on the sixth postoperative day. The protocol for the leucine study is shown in Fig 1. All patients fasted for 8 hours before the isotope studies, which were started at 8:00 AM. A superficial vein in the dorsum of the hand was cannulated to provide access for infusion of L-[1-^{13}C]leucine. L-[1-^{13}C]leucine (99% ^{13}C) and 99% ^{13}C-sodium bicarbonate ($NaH^{13}CO_3$) were purchased from Cambridge Isotope Laboratories (Cambridge, MA).

Blood samples were collected from an intravenous cannula placed in the contralateral arm together with an expired-air sample before the infusion to measure basal ^{13}C enrichment, after which priming doses of $NaH^{13}CO_3$ 0.08 mg · kg^{-1} and L-[1-^{13}C]leucine 0.5 mg · kg^{-1} were administered. Continuous infusion of labeled leucine was started immediately and continued for 8 hours. Two hours after the start of isotope infusion, at isotopic steady state, venous blood and expired-air

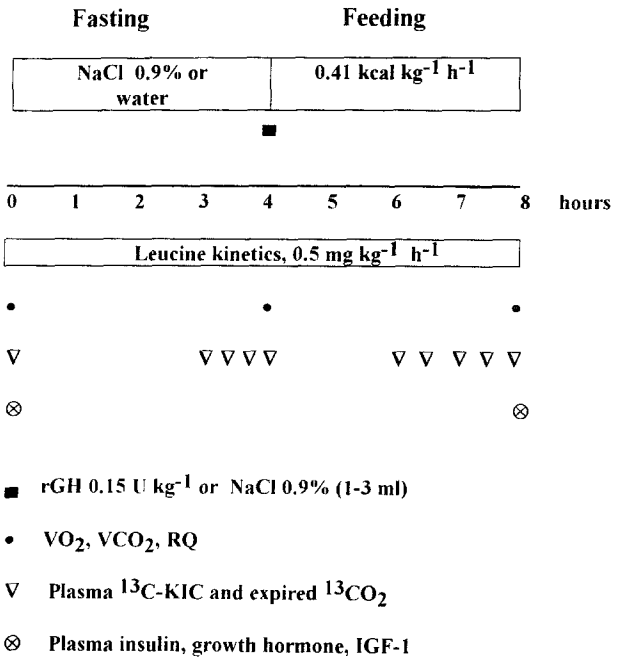

Fig 1. Diagram of the protocol followed in the fasted/fed states before and after surgery.

samples were collected at intervals of 15 minutes for the next 2 hours (a total of 4 hours in the fasted state). Isotope infusion was then continued for a further 4 hours (fed state) (4 to 8 hours of the overall protocol), during which time a 5% beet dextrose solution at a rate of 0.41 kcal · kg^{-1} · h^{-1} was administered. In the DX + rGH group, a subcutaneous injection of rGH was administered at the beginning of the fed state (4 hours from the start), while the DX group received a subcutaneous injection of 0.9% NaCl (the same volume as the rGH injection). The time of injection of rGH during the study corresponded to that of the daily postoperative administration.

During the fed state, crystallized beet sugar (Dextrose anhydrous; Avebe, Foxhol, Holland) was used as a 5% dextrose preparation due to its low natural-abundance ^{13}C content, which would thus not interfere with the leucine oxidation estimations. This solution was prepared in the hospital pharmacy under sterile conditions, and was shown to be pyrogen-free. It had been infused previously in three healthy volunteers, when it was demonstrated that there was no detectable perturbation of $^{13}CO_2$ enrichment in expired air over several hours. The rate of intravenous infusion of dextrose solution was set according to body weight.

Venous blood and expired-air samples were taken at 15-minute intervals for the last 2 hours of the fed state, when the tracer was assumed to have again reached isotopic steady state. All blood samples were centrifuged immediately at 4°C, and the plasma was stored at −70°C until required for analysis.

Plasma α-[^{13}C]ketoisocaproate (α-KIC) and expired-air $^{13}CO_2$ enrichment were measured as described previously.[10] In the fasted state, leucine can only originate from protein breakdown, and therefore it is assumed that leucine flux reflects whole-body protein breakdown. In the calculation of leucine oxidation, factors of 0.76 for the fasting state (1 to 4 hours) and 0.90 for the fed state (4 to 8 hours) were used to account for the fraction of $^{13}CO_2$ released from leucine but retained in the bicarbonate pool of the body.[11]

Urinary Nitrogen

Twenty-four-hour urine collections were made 2 days before surgery and daily for 5 consecutive days after surgery. Nitrogen content of urine

samples was measured by chemiluminescence,[12] and 24-hour excretion of nitrogen was calculated.

Gas Exchange

Indirect calorimetry (Datex; Deltatrac, Helsinki, Finland) was performed before and after surgery over a 30-minute period during plasma ^{13}C-leucine steady state in both the fasted and fed periods. Rates of oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) were measured, and the respiratory quotient (RQ) was calculated.

Plasma Hormones

Blood samples for plasma insulin, GH, and IGF-I were taken during studies in the fasted and fed periods before and 6 days after surgery. Plasma insulin and GH and serum IGF-I concentrations were measured as reported previously.[7]

Statistical Analysis

Results are expressed as the mean ± 1 SD. Paired and unpaired Student's *t* tests were used when appropriate for urinary nitrogen, plasma insulin, GH, and IGF-I. Results for leucine kinetics and gas exchange were analyzed using ANOVA (Genstat statistical software package, Oxford Science, Oxford, UK) with three factors and one random factor-subject. The effect of rGH was assessed relative to the variation between subjects, and the effects of feeding (RX) and surgery (OP) and their interactions with rGH were assessed relative to the variation within subjects. Statistical significance was accepted at *P* less than .05.

RESULTS

The two groups were similar with regard to age, body weight, and BMI.

Leucine Kinetics

The data on leucine kinetics for groups DX and DX + rGH during the fasted and fed states before and after surgery are shown in Table 1. *P* values from ANOVA regarding factors rGH, OP, and RX and their interactions are presented in Table 2.

Preoperative whole-body protein breakdown and synthesis and leucine oxidation were comparable in both groups.

Effect of surgery. On the sixth day after surgery, there was a significant increase in whole-body protein synthesis ($P = .002$) and breakdown ($P = .001$) and leucine oxidation ($P = .009$).

Table 1. Leucine Kinetics of the Two Groups Before and After Surgery in the Fasted and Fed (DX) State Receiving rGH (mean, μmol · kg^{-1} · h^{-1})

Leucine Kinetics	Before Surgery		After Surgery		Between-Subject SE (column)	Within-Subject SE (row)
	Fasted	Fed	Fasted	Fed		
Flux/breakdown						
DX	105	98	116	104	9.9	6.0
DX + rGH	110	103	136	121		
Oxidation						
DX	22	22	26	33	3.1	2.5
DX + rGH	20	22	24	25		
Synthesis						
DX	83	76	88	72	5.0	3.5
DX + rGH	90	85	111	98		

NOTE. Statistical significance and *P* values related to this table are presented in Table 2.

Abbreviation: SE, standard error.

Table 2. *P* Values for Leucine Kinetics (fasted and fed states) From Table 1

Factor	*df*	*P*		
		Synthesis	Flux/Breakdown	Oxidation
Between subjects	11	.02*	.15	.3
rGH	1			
Within subjects	36	.001*	.003*	.8
RX	1			
OP	1	.002*	.001*	.009*
rGH · RX	1	.8	.8	.7
rGH · OP	1	.009*	.05*	.3
RX · OP	1	.17	.3	.9
rGH · RX · OP	1	.9	.9	.5

Abbreviations: RX, feeding; OP, surgery.

*Statistically significant.

Close analysis of the interaction between rGH and OP indicated that there was a greater increase in postoperative protein synthesis in the DX + rGH group ($P = .009$) compared with the DX group. Conversely, leucine oxidation did not change ($P = .30$) and only changed protein breakdown moderately ($P = .05$).

Effect of rGH and feeding. Supplementation of rGH to the dextrose diet had no effect on leucine oxidation or whole-body protein breakdown, but protein synthesis was increased ($P = .02$). This corresponded to mean increase of 34% seen particularly in the postoperative period following 5-day supplementation with rGH. The transition from the fasted to the fed state was accompanied by a significant decrease in protein synthesis ($P = .001$) and breakdown ($P = .003$), whereas there were no changes in leucine oxidation ($P = .80$). The decrease occurred both before and after surgery, probably reflecting the absence of nitrogen in the diet. There was no significant interaction between rGH and RX.

Urinary Nitrogen

Urinary nitrogen excretion was comparable in both groups before surgery (0.23 ± 0.07 g · kg^{-1} · d^{-1} for DX and 0.21 ± 0.03 g · kg^{-1} · d^{-1} for DX + rGH). Cumulative postoperative nitrogen excretion was lower in the latter group (0.97 ± 0.30 g · kg^{-1}) than in the DX group (1.24 ± 0.30 g · kg^{-1}), although this difference was not statistically significant.

Gas Exchange

Gas exchange values in the two groups studied before and after surgery and in the fasted and fed states are shown in Table 3. A significant increase in $\dot{V}O_2$ ($P = .03$) was associated with surgery. Feeding with 5% beet dextrose and supplementation with rGH caused a significant increase in $\dot{V}O_2$ ($P < .05$). Similarly, with the interactions between rGH and RX and rGH and OP, $\dot{V}O_2$ increased significantly. No changes were observed in $\dot{V}CO_2$. The RQ decreased significantly after surgery in the DX + rGH group ($P = .03$).

Plasma Hormones

Insulin. Preoperative fasting plasma insulin was elevated in the DX + rGH group (6.0 ± 5.6 μU · mL^{-1}), although it was

Table 3. Gas Exchange of the Two Groups Before and After Surgery and in the Fasted and Fed States

Parameter	Before Surgery		After Surgery		Between-Subject SE (column)	Within-Subject SE (row)
	Fasted	Fed	Fasted	Fed		
$\dot{V}O_2$ (mL)						
DX	273	262	272	282	37	26
DX + rGH	274	297	308	318		
$\dot{V}CO_2$ (mL)						
DX	208	200	206	211	24	19
DX + rGH	231	227	231	237		
RQ						
DX	0.76	0.77	0.76	0.75	1.5	3.2
DX + rGH	0.84	0.77	0.75	0.74		

NOTE. Statistical values are in the text.

not significantly different from the level in the DX group ($3.9 \pm 3.3\ \mu U \cdot mL^{-1}$). No significant changes in plasma insulin concentration were observed after surgery in the DX group. Conversely, a significant increase occurred in the DX + rGH group ($P < .01$). The transition from the fasted to the fed state before surgery did not show any significant changes in either group, whereas after surgery the mean insulin concentration increased significantly in the DX + rGH group ($29.7 \pm 2.2\ \mu U \cdot mL^{-1}$, $P < .05$).

GH. Preoperative fasting plasma GH concentrations in the DX and DX + rGH groups were 8.4 ± 11.8 and $5.7 \pm 10.9\ \mu U \cdot mL^{-1}$, respectively. There were no changes in the circulating concentration of GH during the preoperative period in the DX group. Supplementation with rGH in the treatment group increased plasma GH significantly ($P < .01$). During the postoperative period, plasma GH increased significantly in the fed state in the DX + rGH group ($63.8 \pm 17.6\ \mu U \cdot mL^{-1}$, $P < .05$) but not in the DX group ($20.7 \pm 24.0\ \mu U \cdot mL^{-1}$).

IGF-I. Preoperative fasting plasma IGF-I concentrations in the DX and DX + rGH groups were 97 ± 20 and $135 \pm 26\ \mu U \cdot mL^{-1}$, respectively ($P < .01$). Dextrose feeding with or without rGH supplementation did not alter the plasma concentration of IGF-I before or after surgery. Surgery was associated with a significant decrease in plasma IGF-I in the DX group ($64 \pm 15\ \mu U \cdot mL^{-1}$, $P < .05$), although no changes were observed in the DX + rGH group ($118 \pm 56\ \mu U \cdot mL^{-1}$).

DISCUSSION

In the present study, preoperative fasting levels of whole-body protein turnover and synthesis were lower than those previously reported in the literature for subjects in the same age group and in a recent investigation by our group.[7] This could well be explained by the minimal amount of calories and the absence of nitrogen in the diet administered to these patients during the period of study. Short-term withdrawal of nitrogen, whether in volunteers[1] or in injured patients,[13] is associated with a negative nitrogen balance and a reduction in the rate of protein synthesis.

In the present study, there was a minimal increase in postabsorptive, postoperative whole-body protein breakdown and synthesis when dextrose alone was infused after surgery. In contrast, addition of rGH to dextrose infusion resulted in a significant increase in the postabsorptive rate of whole-body protein breakdown and synthesis, with the greatest change occurring in the latter. The increase in protein breakdown and synthesis was to some extent similar to that observed in our previous investigation in which a nitrogenous and normocaloric diet was administered.[7] Leucine oxidation, on the contrary, was not affected by administration of rGH.

The effect of a low-calorie diet on protein metabolism in surgical patients has been examined by Harrison et al,[14] who administered dextrose perioperatively in a protocol similar to the present one and failed to show a change in whole-body protein synthesis and breakdown on the third postoperative day. However, at day 7 after surgery, the rates of leucine oxidation and protein synthesis were significantly elevated compared with the preoperative values, and were greater than those found in this study. Although there is no clear explanation for this discrepancy with the present results, it has to be emphasized that those patients, in comparison to our group, received a normal diet preoperatively as evidenced by the greater rate of leucine oxidation.

Ward et al[8] studied a group of patients undergoing major abdominal surgery who received an amount of dextrose similar to that used in the present study and also supplemented with rGH during the postoperative period. Using ^{15}N-glycine as the labeled tracer, they showed a greater postoperative increase in both protein breakdown and synthesis compared with the present study. The greater changes observed may well be explained by the larger doses ($0.2\ U \cdot kg^{-1} \cdot h^{-1}$) of rGH used and/or the greater amount of dextrose administered during part of the isotope study. Such an increase in protein synthesis in the presence of minimal caloric support may be explained by the fact that amino acids derived from muscle catabolism become incorporated into new proteins while oxidative losses remain minimal. This is in net contrast to our previous findings, which demonstrated that when rGH is added to a nitrogenous normocaloric diet with rGH, the increase in protein synthesis is achieved by modulation of amino acid oxidation.[7]

During the fed state with dextrose, there was an overall decrease in protein breakdown and synthesis, with this change being greater after surgery and independent of whether rGH was supplemented. Leucine oxidation did not change in the fed state, and there was no difference between groups, implying a minimal effect of rGH in modulating oxidative and catabolic losses. The minimal changes in leucine oxidation agree, to some extent, with the data on urinary nitrogen excretion, although this investigation was not intended to make a comparison between the rate of leucine oxidation and 24-hour urinary nitrogen excretion. Until a more accurate index of body nitrogen is available, urinary nitrogen excretion is still widely accepted as an index of body protein catabolism.

The lack of changes in amino acid oxidation during infusion of dextrose (fed state) and addition of rGH could be explained by the prolonged fast, the catabolic state, or both influencing GH secretion. Ho et al[15] observed that 5 days of fasting was accompanied by decreasing levels of IGF-I. However, the response to GH-releasing hormone (GHRH) was normal, suggesting that the changes in GH secretion were not due to changes in pituitary sensitivity to GHRH. The low IGF-I levels after protein restriction may be a postreceptor defect in the GH-tissue axis, since GH binding to the liver is normal.[16]

Similarly depressed circulating levels of IGF-I have been reported in critically ill patients.[17]

In this context, the significant decrease in circulating IGF-I in the DX group after surgery was to some extent expected, and could be explained by the prolonged absence of nitrogen intake causing GH resistance and therefore obliterating the ability of GH to increase plasma IGF-I.[18] In contrast, supplementation of dextrose with rGH caused a significant increase in the postoperative concentration of insulin and a steady stimulation of IGF-I. It is believed that an intake of 12 to 18 kcal/kg with a significant proportion of the calories (>70%) as carbohydrate is required for an optimal IGF-I and nitrogen-sparing response to rGH.[9] However, these findings have not been validated in surgical patients.

Although both IGF-I and insulin are independently capable of increasing the rate of protein synthesis, it is not possible to determine in this study if the elevated levels of either hormone or GH itself primarily modulated protein metabolism. It is possible that amino acids derived from the breakdown of proteins were then made preferentially available for the synthesis of new proteins rather than being oxidized. When exogenous nitrogen is available, amino acid oxidation increases, although the increase can be contained by supplementation with rGH.[7] The energy needed for this process may have been provided by lipid oxidation, as shown by the significant increase in oxygen consumption in the DX + rGH group.

There is no consensus as to the optimal dose of rGH required to effectively modulate protein synthesis. Studies on the effect of rGH on nitrogen balance in injured patients have reported a beneficial effect with doses ranging from 12 to 30 $U \cdot d^{-1}$. One study showed that 0.2 and 0.4 $U \cdot kg^{-1} \cdot d^{-1}$ were equally potent in terms of nitrogen retention.[19] However, in all these studies where nitrogen and calories were supplied, there is a considerable range in the amounts administered.

In contrast, Jauch et al[20] showed a dose-response effect, with nitrogen retention increasing with increasing doses of rGH 0.075, 0.15, and 0.3 $\mu U \cdot kg^{-1} \cdot d^{-1}$, although nitrogen and energy were also given in these studies.

This study demonstrated a modulatory effect of rGH on protein metabolism. More specifically, a small amount of carbohydrate and rGH enhanced protein synthesis directly even in the absence of dietary nitrogen. We do not know whether there are possible advantages of such a therapeutic combination in the nutritional management of surgical patients who are unable to receive oral nutrition for a short period. It remains to be seen whether, by providing a greater amount of dextrose in the diet with addition of rGH, the nitrogen-sparing effect of dextrose can be enhanced.

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