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Original Communications

The Effect of Hyperglycemic Hyperinsulinemia on Small-Intestinal Mucosal Protein Synthesis in Patients After Surgical Stress

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ABSTRACT. Hyperglycemic hyperinsulinemia cannot stimulate intestinal protein synthesis in healthy individuals but does so in conditions characterized by an altered somatotropic axis such as diabetes. Only in a state of growth hormone resistance (high growth hormone but low insulin like growth factor [IGF-1] concentrations), extra insulin may acutely reverse the impaired, growth-hormone-induced IGF-1 release, thereby exerting anabolic actions at the intestinal tract. Growth hormone resistance can be also found in patients after surgical stress. Therefore, we wanted to test the hypothesis whether hyperglycemic hyperinsulinemia would stimulate ileal protein synthesis in the latter condition. Mass spectrometry techniques (capillary gas chromatography/combustion isotope ratio mass spectrometry) were used to directly determine the incorporation rate of 1-[^{13}C]-leucine into ileal mucosal protein. All subjects had an ileostomy, which allowed easy access to the ileal mucosa, and consecutive sampling from the same tissue was performed during continuous isotope infusion (0.16 μmol/kg min). Isotopic enrichments and fractional protein synthesis were determined at baseline (period I) and after a 4-hour glucose infusion (170 mg/kg/h) or after infusion of saline (control group) (period II). In controls, ileal protein synthesis declined significantly during prolonged isotope infusion (period I: 1.11 ± 0.14%/h, period II: 0.39 ± 0.13%/h, $p < .01$). In contrast, ileal protein synthesis remained constant during glucose infusion (period I: 1.32 ± 0.35%/h, period II: 1.33 ± 0.21%/h, n.s. *vs* period I, but $p < .005$ *vs* the corresponding value at the end of period II in the control group). Using the continuous tracer infusion technique, ileal protein synthesis seemingly declines over a short time in control subjects. We found evidence that this artificial decline was due to mass effects of a rapidly turning over mucosa protein pool in which an isotopic plateau was reached during the experiment and of which the size amounted to approximately 4% of the total mixed protein pool. Maintenance of ileal protein synthesis during glucose infusion therefore indicates a rise of ileal protein synthesis in a slowly turning over protein pool. This effect in postsurgical patients would be compatible with the concept of intestinal insulin action to depend on the specific clinical state (eg, growth hormone resistance). (*Journal of Parenteral and Enteral Nutrition* **30:**97–107, 2006)

Various substrates participate in regulating small-intestinal protein mass and function. Luminal carbohydrates may be of particular importance because of their trophic action, thereby stimulating mucosal growth, increasing mucosal mass, and maintaining mucosal function.[1,2] However, there is little evidence that such effects can also be obtained by parenteral glucose supply. Thus, in healthy subjects glucose infusion did not alter duodenal protein synthesis[3] or splanchnic amino acid balances.[4] Correspondingly, various degrees of hyperinsulinemia remained without anabolic effects in the splanchnic bed.[5–7] Stimulatory effects on splanchnic protein synthesis were also absent when the insulin-induced hypoaminoacidemia was prevented by simultaneous amino acid infusion.[7] Experiments in adult dogs or pigs in which intestinal protein metabolism could be evaluated selectively confirmed the absent anabolic actions of hyperglycemic[8] or euglycemic hyperinsulinemia[9–12] at the gut.

These findings in healthy individuals are in contrast to a recent study in patients with insulin-dependent diabetes mellitus (IDDM) where even small amounts of insulin increased duodenal protein synthesis *in situ* significantly.[13] Furthermore, carbohydrate-based parenteral nutrition in adult colitis patients could largely maintain intestinal morphology and enterocyte proliferation.[14–17] The potentially different intestinal insulin action in healthy and diseased subjects may be explained by a varying release of secondary insulin-induced mediators important for trophic effects at the intestinal tract. One of those potent mediators is IGF-1, which is known to stimulate mucosal growth and enterocyte proliferation.[18,19] Hyperinsulinemia/hyperglycemia cannot raise concentrations of free dissociable IGF-1 in healthy individuals[20–23] but does so in all conditions known to be associated with a high baseline concentration of growth hormone and low level of IGF-1 (growth hormone resistance). Thus, insulin-mediated IGF-1 release has been observed in hypo-

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glycemia,[22] diabetes,[24] or malnutrition.[25] Growth hormone resistance is also present after surgical injury,[26] and, correspondingly, also in such conditions insulin has been found to accelerate IGF-1 release significantly.[20,27] Therefore, we wanted to test the hypothesis whether a hyperglycemic hyperinsulinemia would stimulate intestinal protein synthesis in patients during postsurgical stress. To create a scenario that is comparable to clinical routine, we used a carbohydrate infusion to establish the desired degree of hyperinsulinemia. The carbohydrate infusion rate was set in a way to produce insulin concentrations low enough not to interfere with intestinal amino acid supply.[7,9]

A further aspect of the present study was to provide an explanation for the rapid, unphysiologic decline of mucosal tracer incorporation and protein synthesis found recently in nonmalignant[28] and malignant[29] intestinal mucosa *in situ* when patients were studied in a metabolic steady state by continuous tracer infusion.

MATERIALS AND METHODS

Subjects

Two groups of postoperative patients (control, n = 7; carbohydrate infusion, n = 6) with cured rectal carcinoma and ileostomies were carefully screened through medical history, physical examinations, and routine blood tests. The groups were comparable with respect to age (control: 60.3 ± 3.1 year; carbohydrate infusion: 61.5 ± 4.8) and body mass index (control: 23.4 ± 0.7 kg/cm^2; carbohydrate infusion: 24.4 ± 0.9). Data of the control group have been presented previously.[28] Patients had limited colorectal cancer and had undergone curative, elective abdominal surgery, which had also included construction of an ileostomy. All patients had a preoperative bowel preparation (orthograde flushing by oral colonoscopy fluids) and received prophylactic IV antibiotics at the time of surgery. Anesthesia consisted of epidural application of analgesic and anesthetic drugs, and was continued no longer than the fifth postoperative day. The patients were studied between days 8 and 10 after surgery, when body protein loss peaks,[29] had an uneventful postoperative course, and were free from signs of organ malfunction and local or systemic infection. Prior to surgery, no patient had a history of previous weight loss or clinical signs of malnutrition or metabolic diseases. Informed consent was obtained after the experimental protocol had been explained in detail. The study was approved by the local institutional review board (protocol # 134/97).

Experimental Protocol

All subjects were inpatients of the general surgical service. After surgery, patients received a mixed diet (approximately 25 kcal/kg/d of which 5 kcal/kg/d were administered as protein and free amino acids); one-third of the calories was administered enterally (liquids) and the other two-thirds parenterally. After 10 PM, all subjects remained postabsorptive, except for consumption of mineral water, and parenteral nutrition was discontinued. A primed-constant infusion of 1-[^{13}C]-leucine (Tracer Technologies, Sommerville, MA; 99.3 atom % enrichment) was started at 7 AM the next day. The isotope infusion rate was 0.16 μmol/kg min (prime 9.6 μmol/kg). A blood sample was collected before isotope infusion to determine the background enrichment of protein-bound and free plasma leucine. Plasma background enrichments were used as an indicator of intracellular protein-bound and free leucine background enrichments.[30]

The first mucosal biopsy was performed after 180 minutes of isotope infusion, the second after 360 minutes, and the third after 600 minutes. The baseline period (period I) ranged from 180 to 360 minutes. Between minutes 360 and 600 (after the second biopsy, period II), patients in the carbohydrate group received a continuous glucose infusion (170 mg/kg h). Patients in the control group remained in a fasting state. Assignment to the control or carbohydrate group was done by random order. Arterialized blood samples for measurement of glucose and insulin concentrations were taken at the same time as mucosal biopsies. The minimum distance between biopsy sites was 2 cm. The average biopsy size was 10 mg wet weight.

Procedures

Study methods and data analysis were discussed in detail previously.[30] The free and protein-bound amino acids in tissue biopsies were separated by protein precipitation. After protein hydrolysis, amino acids were separated from the accompanying impurities by cation-exchange chromatography. For capillary gas chromatography and combustion isotope ratio mass spectrometry analysis, amino acids from proteins (on average 7–8 ng) were then converted to the *N*-acetyl *n*-propyl ester. For gas chromatography and quadrupole mass spectrometry analysis, we prepared the tert-butyldimethylsilyl derivative from free intracellular amino acids. *N*-acetyl *n*-propyl-amino acid derivatives were analyzed in a capillary gas chromatography and combustion isotope ratio mass spectrometry system that consisted of a Hewlett-Packard 5890 Series II gas chromatograph interfaced to a mass spectrometer Delta S (Finnigan MAT, Bremen, Germany). Tert-butyldimethylsilyl derivatives were analyzed by a gas chromatography and quadrupole mass spectrometry system (MSD 5971D, Hewlett-Packard, Houston, TX). Isotopomere ratios of the sample were obtained by electron impact ionization and selected ion monitoring at mass-to-charge ratios 303 and 302. Data were expressed as tracer/tracee ratios.

Plasma glucose concentration was determined enzymatically, and insulin concentrations were measured *via* immunoassay.

Calculations

The tissue fractional synthetic rate in the metabolic steady state was calculated by dividing the increment in protein-bound 1-[^{13}C]-leucine tracer/tracee ratio by enrichment of the precursor pool.[30] As a substitute for the true precursor pool enrichment (tRNA-bound amino acid enrichment), we used the free homogenate

TABLE I
*Tracer/tracee ratios of 1-[^{13}C]-leucine in free intracellular leucine of ileal mucosa during continuous tracer infusion**

	Sample 1 (180 min)	Sample 2 (360 min)	Sample 3 (600 min)
Control	5.24 ± 0.46	5.37 ± 0.47	5.46 ± 0.35
Glucose infusion	5.01 ± 0.89	6.15 ± 1.14	6.48 ± 1.28†

*Values are given as % (mean ± SEM). Sample 1 was taken after 180 minutes, sample 2 after 360 minutes, and sample 3 after 600 minutes of isotope infusion. Patients in the glucose infusion group received 170 mg/kg/h glucose between 360 and 600 minutes. Control patients continued to fast throughout the study.
†$p < .05$ *vs* sample 1.

1-[^{13}C]-leucine tracer/tracee ratio.[28] Delta increments of protein-bound 1-[^{13}C]-leucine enrichment between biopsy samples were calculated from isotope ratios [^{13}C]/[^{12}C] using a correction factor that takes into account dilution of the label at the carboxyl position by the other carbon atoms in the derivatized leucine.[30] The fractional synthetic rate (FSR) was then calculated as follows:

$$FSR = \frac{R_L(t_{i+1}) - R_L(t_i)}{\{R_L'(t_i) + R_L'(t_{I+1})\}/2} * \frac{1}{\Delta t} * 60 * 100 \quad (\%/h). \qquad (1)$$

$R_L(t_i)$ and $R_L(t_{i+1})$ correspond to the tracer/tracee ratio of ileal protein-bound leucine in 2 subsequent samples (i and i + 1), separated by the time interval Δt (min). $R_L'(t_i)$ and $R_L'(t_{i+1})$ indicate the tracer/tracee ratios of ileum-free leucine in 2 subsequent samples. Average values between $R_L'(t_i)$ and $R_L'(t_{i+1})$ were used as precursor enrichments for ileal protein synthesis. The factors 60 (min) and 100 are needed to express the fractional synthetic rate in percentage per hour.

Patients in the carbohydrate group demonstrated a significant increase in the precursor pool enrichment during the 10-hour isotope infusion (Table I), possibly indicating a non–steady-state situation. In this case, an equation different from equation 1 would have to be used to calculate FSR.[31] This equation requires knowledge of tracer and tracee concentrations in the free amino acid and protein pool, which are, however, difficult to obtain from biopsies of ileal mucosa *in situ*. As an alternative, equation 1 can still be used, if piecewise constant functions between pairs of consecutive sampling points can be identified.[31] Because the free tissue homogenate leucine enrichment did not differ significantly between samples taken at 180 and 360 minutes and 360 and 600 minutes, respectively, one may assume a steady-state-like condition between sampling points 1 and 2 and 2 and 3, justifying use of equation 1 to calculate FSR separately for those 2 study intervals.

To analyze the speed of tracer incorporation separately in periods I and II, we calculated the absolute increase of the protein-bound tracer/tracee ratio per hour during the 10-hour isotope infusion by the formula:

$$\Delta R_L/h = [\{R_L(t_{i+1}) - R_L(t_i)\}/\Delta t] * 60 \qquad (2)$$

Tracer incorporation during the study is measured in a mixed protein sample but is known to occur in different protein pools with different turnover. Isotopic enrichments in these protein pools may reach an isotopic plateau before the first sample is taken (very rapidly turning over protein pool), between the first and the last sample (rapidly turning over protein pool), or after the last sample (slowly turning over protein pool). For such protein pools with different turnover, several boundary conditions may be derived from the data measured in the control group (with stable experimental conditions). The theory behind this multiple pool hypothesis and deduction of corresponding boundary conditions are presented in the Appendix. These boundary conditions must be compatible with each other if the pool concept is correct. If so, individual pool sizes may eventually be estimated.

Statistics

Data are expressed as mean ± SEM. Protein synthetic rates from control patients and patients in the carbohydrate group (6-hour isotope infusion) were compared by the unpaired *t* test. The paired *t* test was used to compare protein synthetic rates and the speed of tracer incorporation within each group between periods I and II. ANOVA (analysis of variance) was used to compare the free homogenate leucine tracer/tracee ratios between the 2 groups and between the 3 different sampling points. A *p* value of .05 or less was taken as indicating a significant difference.

RESULTS

Plasma glucose and insulin concentrations rose significantly during carbohydrate infusion (glucose, end of period II: 165.0 ± 5.9 mg/dL, $p < .05$ *vs* 111.5 ± 4.5 at the end of period I; insulin, end of period II 25.1 ± 4.3 μUl/mL, $p < .05$ *vs* 9.4 ± 1.4 μU/mL at the end of period I), whereas glucose and insulin concentrations remained unchanged in control patients (glucose, end of period II: 96.3 ± 2.0 mg/dL, n.s. *vs* 103.5 ± 3.8 at the end of period I; insulin, end of period II 7.2 ± 1.1 μUl/mL, n.s. *vs* 6.3 ± 0.6 μU/mL at the end of period I), resulting in a significant difference of insulin and glucose concentrations between controls and subjects receiving glucose at the end of period II ($p < .05$).

Analysis of tracer data showed that only in the control group did the tracer/tracee ratio in the mucosa-free homogenate leucine pool (= precursor pool for protein synthesis) not change significantly over time (Table I). In patients receiving carbohydrates, the precursor pool enrichment rose significantly over time. Thus, the enrichment in sample 3 (600 minutes of isotope infusion) was significantly higher than the corresponding enrichment in sample 1 (180 minutes of isotope infusion).

Table II and Figure 1 show the values of protein-bound leucine enrichment and the time curve of tracer incorporation into ileal mucosa over time. In both groups, tracer incorporation was not linear. On average, in patients receiving carbohydrates the tracer/tracee ratio rose at 0.0056 ± 0.004% per hour in period I (hours 3–6) and at 0.083 ± 0.004% per h in period II (hours 6–10, period of glucose infusion, $p < .05$). In contrast, incorporation slowed down significantly over time in control patients (0.057 ± 0.005%

TABLE II
*Tracer/tracee ratios of 1-[^{13}C]-leucine in ileal mucosa protein**

	Sample 1 (180 min)	Sample 2 (360 min)	Sample 3 (600 min)
Glucose infusion			
1	0.20	0.33	0.63
2	0.15	0.35	0.54
3	0.33	0.45	0.71
4	0.28	0.42	0.67
5	0.14	0.39	0.60
6	0.23	0.37	0.82
Control			
1	0.15	0.36	0.54
2	0.04	0.21	0.27
3	0.16	0.33	0.30
4	0.15	0.34	0.42
5	0.06	0.25	0.28
6	0.17	0.34	0.44
7	0.23	0.30	0.35

*Values are given as %. Sample 1 was taken after 180 minutes, sample 2 after 360 minutes, and sample 3 after 600 minutes of isotope infusion. Patients in the glucose infusion group received 170 mg/kg/h glucose between 360 and 600 minutes. Control patients continued to fast throughout the study.

per h in period I, 0.019 ± 0.005% per h in period II, $p < .001$). The speed of tracer incorporation in period II differed also significantly between groups ($p < .05$).

During carbohydrate infusion fractional protein synthesis remained stable. Thus, ileal protein synthesis amounted to 1.32 ± 0.35%/h in period I and to 1.33 ± 0.21 in period II (n.s.). In contrast, ileal fractional protein synthesis in control patients declined significantly over time (period I: 1.11 ± 0.14%/h, period II: 0.39 ± 0.13%/h, $p < .05$ *vs* period I and *vs* the corresponding value at the end of period II in the carbohydrate group; Figure 2).

As outlined in the Appendix, the rapid decline of tracer incorporation and mucosa protein synthesis over time in controls does not represent a true change of tissue protein synthesis but results from mass effects of a rapidly turning over protein pool in which an isotopic plateau is reached during the experiment. For such a rapidly turning over mucosa protein pool, several different boundary conditions can be derived from the data measured in the control group (with stable experimental conditions). As can be seen in the Appendix section, none of the boundary conditions conflicts with any other, and the size of the rapidly turning over protein pool can be estimated, approximately amounting to 4% of the total protein pool in the sample.

DISCUSSION

This study shows that after major abdominal surgery, a mild hyperglycemia/hyperinsulinemia stimulates protein synthesis in ileal mucosa significantly. Thus, after a 4-hour infusion of 2.8 mg/kg/min glucose, ileal fractional protein synthetic rate was >300% higher than the corresponding rate in control patients (Figure 2).

To interpret the carbohydrate effect correctly, it is essential to analyze the changes of ileal protein synthesis over time in the control group. Thus, during a 10-hour tracer infusion in postabsorptive patients, we observed a rapid, marked decline of protein synthesis, falling significantly from about 1.11%/h–0.39%/h. As can be seen in Figure 1, tracer incorporation into mucosal protein in fasting postoperative patients (control group) was not linear over time but slowed down between the sixth and 10th hour of isotope infusion, resulting in a rapid decrease of small-bowel protein synthesis during this time. Similar observations have been made in rat liver[32–34] when tracers were given continuously over prolonged periods of time. It can be concluded that this rapid marked decrease resulted from the effect of fasting because, by use of published sources,[35,36] one can calculate that human small-bowel protein synthesis declines by only 1.3%/h during a 36-hour fasting period.

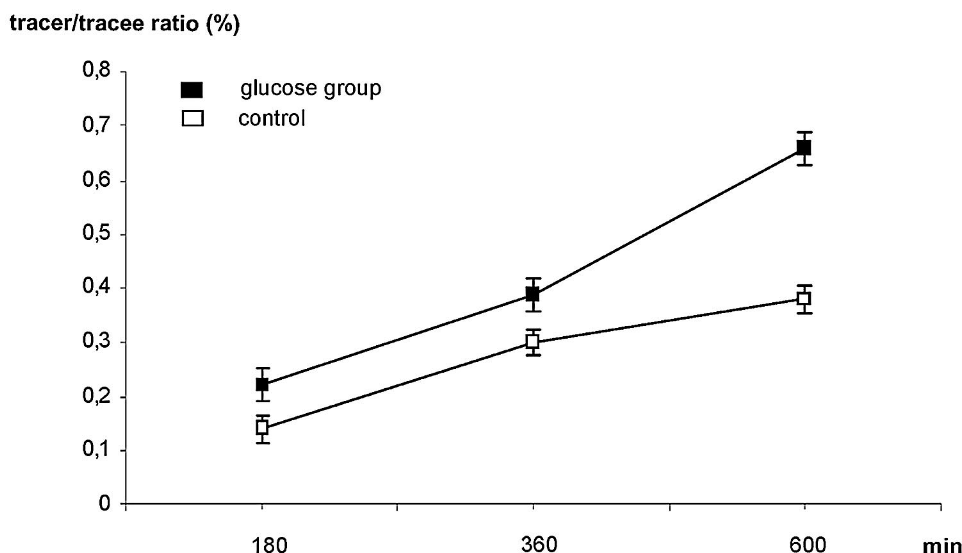

FIGURE 1. Time curves of tracer incorporation into ileal mucosa in control patients and patients receiving carbohydrates during a 10-hour isotope infusion. Patients in the glucose infusion group received 170 mg/kg h glucose between 360 and 600 minutes. Control patients continued to fast throughout the study.

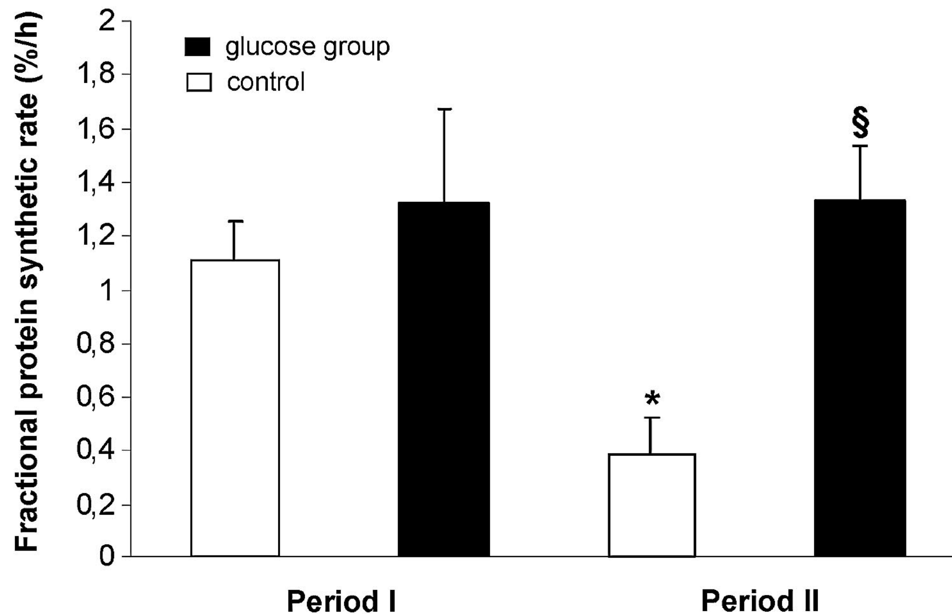

FIGURE 2. Effect of carbohydrate infusion on ileal protein fractional synthetic rate (%/h, mean ± SEM). Period I ranged from hour 3 to hour 6; period II, from hour 6 to hour 10. Patients in the carbohydrate group received a glucose infusion (170 mg/kg h) during period II. $^{*}p < .05$ *vs* period I. §$p < .05$ *vs* control.

For rat liver, this phenomenon has been explained by different protein pools with different protein turnover rates and corresponding different speeds of isotope incorporation.[32] The existence of such differing pools has been shown in rodent and dog liver[33,37] and in rodent small intestine[38] and intestinal brush border.[39] To explain the decline in hepatic tracer enrichment over time mathematically, one has to describe mixed protein enrichments (as obtained from mucosal biopsies) as the sum of all enrichments measured at a certain point of time in all the various protein pools in a tissue.

It is obvious that turnover of individual proteins varies between a few minutes and many days.[37,40,41] Therefore, the time will also vary after which all proteins in a specific pool will be labeled during a continuous tracer infusion and at which point the isotopic plateau is being reached. Beyond that point, only those individual proteins or protein pools will add to the rising enrichment of the total pool that have not yet reached their isotopic plateau. Because of mass effects of the different pools, the speed of tracer incorporation will be lower at this later period of tracer infusion, resulting in seemingly reduced rates of protein synthesis over time (see Appendix for details).

Assumption of a comparable plateau effect in intestinal mucosa of our study would require gut proteins in which the enrichment reaches its plateau not later than 6 hours after the beginning of the isotope infusion. Proteins of this type would belong to a rapidly turning over protein pool. Rapidly turning over proteins should have a fractional synthetic rate of at least 100%/6 hours, or about 17%/h. With protein mass and turnover constant, this synthetic rate would correspond to a protein half-life of approximately 3 hours. A variety of enzymes or epithelial glycoproteins such as lactosaminoglycans or proteoglycans were found to have turnover rates in that range,[42–45] and a significant portion of cellular protein shows a half-life of 3.5 hours.[46] Although half-lives of certain glycoproteins, such as sucrase, lactase or disaccharidase, were found to be somewhat higher in *in vitro* experiments (around 12 hours), these proteins are susceptible to turnover acceleration by a various physiologic and pathophysiologic stimuli, most likely resulting in a significantly shorter *in vivo* half-life.[42,46–51]

For such a rapidly turning over protein pool, several boundary conditions can be defined from the data measured in the control group (with stable experimental conditions). These boundary conditions must be compatible with each other if the concept of a rapidly turning over protein pool is correct. As can be seen in the Appendix section, none of the 5 boundary conditions conflicts with any other, thereby supporting the hypothesis of a rapidly turning over pool effect. Furthermore, we could calculate from our data an approximate size of the rapidly turning over protein pool of about 4% (of the total mixed pool).

This pool size estimation for those rapidly turning over proteins in our study cannot be compared with any human data. In rats, it was found that sucrase-isomaltase and maltase account for approximately 1% of mucosa proteins.[52] Total glycoprotein synthesis was found to be responsible for 6%–10% of total tracer incorporation in the mucosa,[53] whereas in rat liver about 10% of all proteins show a rapid turnover in the above range.[37,41] Our calculation of a size of 4% for such a rapidly turning over protein pool in human postoperative ileal mucosa would be in the range of these estimates and would, therefore, also support the validity of the rapidly turning over pool concept for explaining the enrichment changes that were seen during continuous isotope infusion over prolonged periods of time in ileal mucosa from controls.

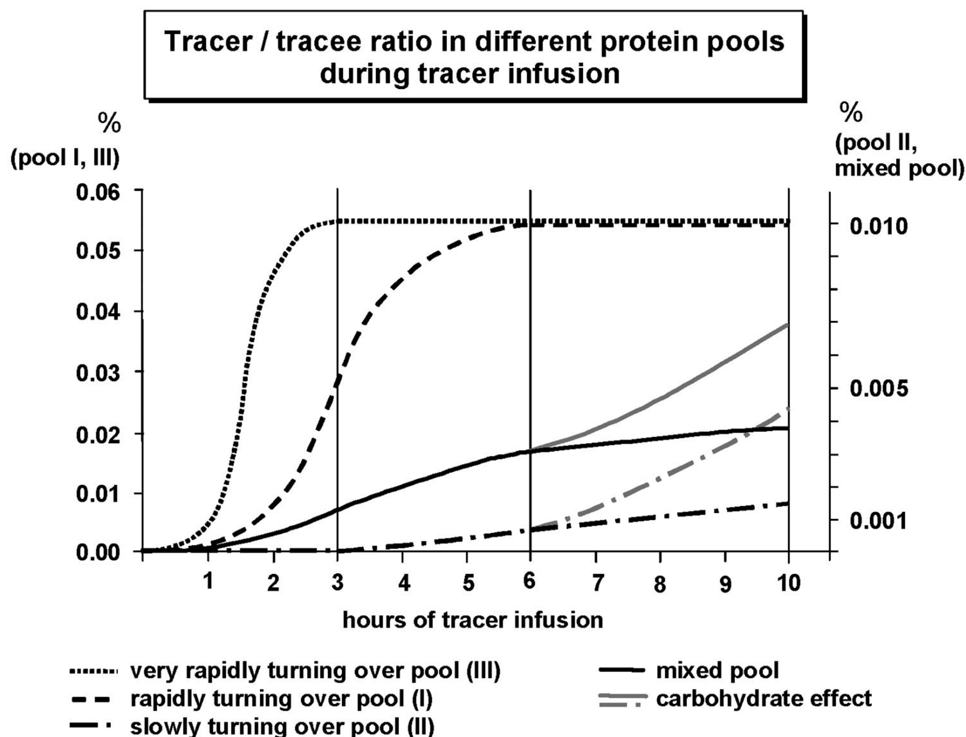

FIGURE 3. Hypothetical tracer/tracee ratios in different mucosa protein pools during a 10-hour tracer infusion. Numbers for individual pools were estimated as indicated in the Appendix section. An approximate size of the very rapidly turning over protein pool of 0.5% and of the rapidly turning over protein pool of 3.8% was assumed.

Assumption of such a pool concept would also affect the interpretation of experiments performed to alter mucosal protein synthesis. In our studies, manipulations started after the second sample (at 6 hours of isotope infusion, after a baseline period). If a manipulation would then stimulate only protein synthesis in the rapidly turning over protein pool, it would be missed because the plateau enrichment cannot surmount the precursor pool enrichment in any case. On the other hand, effects on protein synthetic rates in the slowly turning over protein pool (which reaches its isotopic plateau only after the 10th hour of tracer infusion) will be detected because corresponding enrichments in this pool will rise, whereas the plateau enrichment in the rapidly turning over protein pools will remain constant. Therefore, it is safe to conclude that carbohydrate infusion must at least have stimulated ileal protein synthesis in this slowly turning over pool (Figure 3).

Principally, 2 mechanisms could have been responsible for the stimulation of ileal protein synthesis during glucose infusion. Thus, carbohydrate infusion did not only lead to hyperinsulinemia but also increased plasma glucose concentrations. However, changes in glucose concentration *per se* are commonly believed not to affect protein metabolism significantly, because hyperglycemia in combination with constant insulin concentrations was without effect on plasma leucine concentration or appearance rate,[54,55] or on intestinal glucose uptake.[56] Therefore, variations in insulin concentration are usually considered the main effectors of changes in total body or muscle protein metabolism observed during hyperinsulinemic hyperglycemia.[57]

However, in healthy postabsorptive individuals these anabolic effects are apparently absent at the intestinal tract. Thus, in dogs and humans, various degrees of hyperinsulinemia did not change splanchnic or gut amino acid balances significantly.[5,9,10] Furthermore, when regional catheter techniques were combined with amino acid tracer infusion, studies in healthy subjects revealed that euglycemic hyperinsulinemia was associated even with a fall in splanchnic protein synthesis (calculated without direct measurement of tracer incorporation).[6] In dogs in which gut protein metabolism could be examined selectively, euglycemic hyperinsulinemia was found to reduce intestinal leucine use and production simultaneously, thereby explaining the unchanged gut amino acid balances.[10] The inhibitory effects of insulin on gut protein synthesis and amino acid use are usually explained by the simultaneous, insulin-mediated intestinal deficit of amino acids.[57] However, even with simultaneous amino acid replacement, euglycemic hyperinsulinemia did not stimulate splanchnic protein synthesis in healthy subjects[7] or gut protein synthesis in pigs.[11,12] Therefore, it is surprising that a recent study in insulin-dependent diabetic subjects[13] found that only small amounts of insulin increased duodenal mucosal protein synthesis significantly (measured directly *via* tracer incorporation) and that carbohydrate-based parenteral nutrition prevented mucosal atrophy and dysfunction in catabolic colitis patients.[14–17] These observations and our findings are in clear contrast to the results of O'Keefe et al[3] in healthy volunteers in whom hyperglycemic hyperinsulinemia did not alter duodenal protein synthesis *in situ*.

A possible explanation for those discrepant results may be found in the accompanying circumstances and mechanisms determining insulin action in health and disease. Catabolic illnesses such as surgical stress, malnutrition, but also NIDDM are all characterized by a disturbed somatotropic axis.[25,26,58] Associated pathologic changes are characterized by growth hormone resistance due to reduced hepatic growth hormone receptor expression. The latter results from portal insulin deficiency in NIDDM, protein deficiency in malnutrition, or increased cytokine release in inflammatory or posttraumatic disorders.[25,26,58] As a common consequence shared by all these diseases, plasma concentrations of free and total IGF-1 decline causing a secondary, compensatory rise in pituitary GH release. When hepatic insulin availability rises acutely in such a situation (either by stimulated endogenous release or exogenous infusion), a strong permissive effect on hepatic GH action can be observed. Due to its stimulatory action on hepatic GH receptor expression,[59] insulin will acutely reverse the impaired hepatic GH effects, thereby acutely increasing IGF-1 synthesis and plasma concentration significantly. Corresponding insulin effects on IGF-1 have been demonstrated in all of the above diseases.[20,24,25,27] This endocrine scenario also explains why insulin-induced IGF-1 release does not occur in healthy subjects where pituitary growth hormone output and IGF-1 levels are normal, thereby abolishing the permissive effect of insulin on hepatic IGF-1 production.[20–23] Considering the strong anabolic effects of IGF-1 at the gut,[18,19] the same scenario may explain why hyperinsulinemia stimulates intestinal protein synthesis in diseased but not in healthy subjects.

CONCLUSIONS

In conclusion, our results clearly suggest that hyperglycemic hyperinsulinemia does have a profound effect on human ileal protein metabolism after surgical trauma. They may also explain how IV carbohydrates can contribute to the maintenance of villous height and enterocyte proliferation, and to the conservation of mucosal protein, DNA and RNA content observed clinically during parenteral nutrition in catabolic diseases, despite the absence of a luminal substrate supply.[14–17] Furthermore, our results demonstrate that at least at the gastrointestinal tract, findings from healthy subjects cannot necessarily be applied to diseased individuals.

APPENDIX

Rationale of the 3-Pool Model

Turnover of individual proteins varies between a few minutes and many days. When tracer is given continuously, after a certain amount of time all existing protein in an individual pool will have incorporated the tracer. The amount of time required for this state to be reached depends on the individual turnover rate and can be used to calculate fractional protein synthesis and half-life if turnover and mass remain constant. Thus, after a linear phase with a continuous increase of tracer enrichment, the enrichment in a particular protein will reach a plateau of which the magnitude corresponds to the magnitude of tracer enrichment in the precursor pool. The enrichment measured at a certain time in a mixed protein pool will, therefore, combine enrichments of proteins in which the incorporation is still rising and of those in which the enrichment is already at plateau. The relative contribution of these individual enrichments to the total enrichment will depend on the time passed since the beginning of the tracer infusion (Figure 3).

However, during a limited experiment (as ours with a 10-hour duration) with several sampling points (3 in our experiment), not all protein pools with different turnover will affect the enrichment measured in a mixed sample to the same degree. To account for this phenomenon, a 3-pool model (Figure 3) has been introduced by Koch.[34] In the first pool, some proteins will have a turnover slow enough that a plateau is not being reached during an experiment (in our case, a 10-hour experiment). In these proteins, tracer incorporation will be linear and these proteins will add adequately to the continuous rise of tracer enrichment in the samples, although individual synthetic rates will vary. Then, there will be other proteins (second pool), of which turnover is so rapid that a plateau is already reached before the first sample is taken (after 3 hours of tracer infusion in our case). Irrespective of their individual synthetic rate, these proteins will not add to the rise of tracer enrichment observed in the remaining proteins in all subsequent samples. Because protein synthesis is calculated from the absolute difference between enrichments in samples obtained at different times, synthesis of proteins in this very rapidly turning over protein pool cannot be measured by an experimental design as ours.

Finally, there will be other proteins (third pool) of which the enrichment reaches a plateau during the experiment (between the third and the 10th hour of isotope infusion in our study). Samples taken before that plateau is reached will still show a linear rise in enrichment because tracer incorporation is still ongoing in all proteins. Samples taken after some proteins have reached a plateau will show disproportionately lower enrichments in mixed protein samples, because those proteins will no longer contribute to the enrichment of the mixed sample. Thereby, a deceleration of tracer incorporation will be observed in the mixed protein pool if 3 subsequent samples are compared with each other and if the size if this third pool is of sufficient magnitude. Calculation of fractional protein synthesis will then yield a seeming fall of protein synthesis over time.

This effect will be maximal if the percentage of these plateauing proteins in the mixed sample is high and if they reach their plateau between the first and the second of 3 consecutive samples. Deceleration of tracer incorporation will be lower if the plateau is reached between the second and third sample. In that case, enrichments in the proteins plateauing after the second sample, and, therefore, in the proteins of the mixed protein pool in the second sample, will be lower than corresponding enrichments in that sample, which

would have been measured if the enrichment in the respective proteins had plateaued before the second sample was taken. With a later plateau, the absolute enrichment difference between the second and third sample would rise, reducing the decelerating effect of the plateau mechanism on tracer incorporation between the second and third sample.

Boundary Conditions for the Mucosal 3-Pool Model and Estimation of Pool Size in Control Subjects

A 3-pool model of protein turnover in intestinal mucosa is being proposed. One pool (I) consists of rapidly synthesized proteins in which tracer incorporation would reach a plateau during the tracer infusion (with a maximum effect when the plateau is reached after 6 hours); this rapidly turning over protein pool should have an estimated fractional synthetic rate of 15%–20%/h and a half-life of 2–3 hours. Another pool with slower turnover rates (II) would show a continuous linear tracer incorporation during the whole experiment (third to 10th hour). Proteins with an extremely rapid turnover (reaching their isotopic plateau before the third hour of tracer infusion) are part of still another pool (III) with a fractional synthetic rate of >33%/h and a half-life of 1–1.5 hours or less.

Using the enrichments observed in the mixed mucosa protein pool (Table II) and the constant precursor pool enrichment (Table I) of controls, and using the above assumption on the specific qualities of the different pools, it is possible to define boundary conditions for the 3-pool model. These conditions must all be compatible with each other if the multiple pool model is correct. If so, they could also be used to estimate the relative size (k) of the rapidly turning over protein pool (I) in postoperative ileal mucosa.

In the following calculations, the variables for relative tracer and tracee amounts in different pools and samples are defined as indicated in Table III.

Tracer/tracee values in the mixed protein pool represent mean values from actual measurements in postoperative controls in samples obtained after 3, 6, and 10 hours of isotope infusion (as presented in Table II). k and k′ indicate the amount of tracee in the rapidly and very rapidly turning over protein pool (pool size) as referred to an arbitrary amount of tracee of 1000 in the mixed pool, which combines the 3 other pools. The amount of tracee in the slowly turning over protein pool is obtained by subtracting k and k′ from 1000. R_i, r_i, s_i, and m_i indicate the amount of tracer in the very rapid, rapid, slow, and mixed turnover pools after 3, 6, and 10 hours of isotope infusion.

For a 3-pool model, the following boundary conditions can be defined and possibly be used to estimate k:

1. If the rapidly turning over protein pool reaches its plateau between the third and sixth hour of tracer infusion, then

$$r_6/k = r_{10}/k \text{ or } r_6 = r_{10} \quad (1)$$

2. Between hours 0 and 6 of the tracer infusion, enrichments in the rapidly turning over protein pool should rise linearly up to a turning point from which the plateau is being approached. During this temporary linear rise, the enrichment (r_t/k) at a certain time t that has passed since the beginning of the isotope infusion will follow the equation:

$$r_t/k = a * t + b \quad (2)$$

At time $t = 0$, the enrichment (tracer/tracee ratio) should be also 0. Therefore, b equals 0, leaving

$$r_t/k = a * t \quad (3)$$

The magnitude of "a" equaling the slope of the linear curve will depend on the exact time at which the turning point and the plateau enrichment, respectively, are being reached. If the plateau is to be reached before or at the sixth hour of tracer infusion, "a" will have to be larger than the slope "a_h" of a hypothetical, permanently linear curve, which would rise uniformly and without a turning point from time zero until the value of the plateau enrichment is crossed exactly after 6 hours of tracer infusion. This hypothetical curve can be described by the equation:

$$y_t = a_h * t \quad (4)$$

t equals the time passed since the beginning of the tracer infusion. Because at $t = 6$ (after 6 hours of tracer infusion), y_6 should equal 0.055 (enrichment in the pool of the free intracellular amino acids = plateau enrichment of the rapidly turning over pool, Table I), a_h will then be $0.055/6 = 0.0092$. From $a > a_h$ and from $a = r_t/(kt)$, according to equation 3, follows $r_t/k > 0.0092 * t$. Thus, after 3 hours of isotope infusion, r_3/k will have to be larger than 0.0275 or

$$r_3 > 0.0275 * k \quad (5)$$

3. Because the plateau enrichment in the rapidly and very rapidly turning over protein pool corresponds to the precursor pool enrichment (enrichment in the pool of the free intracellular amino acids, Table I), it amounts to approximately 5.5%, or 0.055 (average value for all measurements in postsurgical control patients). Therefore,

$$r_6/k = r_{10}/k = 0.055, \text{ or } r_6 = r_{10} = 0.055k \quad (6a)$$

$$R_3/k' = R_6/k' = R_{10}/k' = 0.055, \text{ or } R_3 = R_6 = R_{10} = 0.055k' \quad (6b)$$

4. The amount of tracer in the mixed pool must be the sum of tracer in all other pools. Therefore,

$$R_3 + r_3 + s_3 = 1.4\ (= m_3) \quad (7)$$

$$R_6 + r_6 + s_6 = 3.0\ (= m_6) \quad (8)$$

$$R_{10} + r_{10} + s_{10} = 3.8\ (= m_{10}) \quad (9)$$

Since $r_6 = r_{10}$ = constant,

$$\text{the difference } s_{10} - s_6 \text{ must equal } m_{10} - m_6 = 0.8 \quad (10)$$

(for the 4-hour tracer infusion between hours 6 and 10 of the experiment).

5. In contrast to the rapidly turning over pool, enrichment in the slowly turning over pool should rise linearly during tracer infusion. Therefore,

Table III

Definitions of tracer and tracee relative masses in postoperative ileal mucosa for the total mucosa protein pool (mixed turnover pool) and for a hypothetical pool model with different rates of protein synthesis (rapid, very rapid, and slow turnover pool)

	3-h Sample	6-h Sample	10-h Sample
Tracer/tracee (rapid turnover pool I)	r_3/k	r_6/k	r_{10}/k
Tracer/tracee (slow turnover pool II)	$s_3/(1000 - k - k')$	$s_6/(1000 - k - k')$	$s_{10}/(1000 - k - k')$
Tracer/tracee (very rapid turnover pool III)	R_3/k`	R_6/k`	R_{10}/k'
Tracer/tracee (mixed turnover pool)	m_3 (= 1.4)/1000	m_6 (= 3.0)/1000	m_{10} (= 3.8)/1000

$$s_6 - s_3 = (s_{10} - s_6) \times 3/4 = (m_{10} - m_6) \times 3/4 = 0.6 \quad (11)$$

The correction ¾ is necessary because only 3 hours elapse between the third and sixth sample, in contrast to the 4 hours separating the 6-hour and 10-hour samples.

Eqs. 8–11 can be transformed to yield expressions for the amounts of tracer in r and s, depending on k and k′:

$$s_{10} = 3.8 - r_{10} - R_{10} = 3.8 - 0.055\,(k + k') \quad (12)$$

$$s_6 = 3.0 - r_6 - R_6 = 3.0 - 0.055\,(k + k') \quad (13)$$

$$s_3 = s_6 - 0.6 = 2.4 - 0.055\,(k + k') \quad (14)$$

$$r_3 = 1.4 - s_3 = 0.055\,(k + k') - 1.0 \quad (15)$$

Combination of eqs. 5 and 15 yields boundary condition #1 not depending on k′:

$$k > 36.4 \quad (16)$$

Since all r and s must be >0, further boundary conditions depending on k′ can be derived from eqs. 12–15. Thus, $k < 69.1 - k'$ (#2, from eq. 12), $k < 54.6 - k'$ (#3, from eq. 13), $k < 43.6 - k'$ (#4, from eq. 14), and $k > 18.2 - k'$ (#5, from eq. 15).

Combining conditions #1 with #4 yields an upper estimate for k′:

$$36.4 < 43.6 - k', \text{ or } k' < 7.2 \quad (17)$$

Therefore, referred to a hypothetical pool size of tracee in the mixed pool (1000), the size of the very rapidly turning over protein pool (III) should not be >0.7% of the total, mixed pool. This size would be compatible with experimental findings. Thus, the fraction of this labile protein pool with a very rapid turnover has been estimated in the past in growing prokaryotic cells. k′ was found to be about 10–20 (1%–2% of the total protein pool) but is significantly less in eukaryotic cells.[60,61]

Assuming a maximum of k′ of 7 (corresponding to 0.7%), the following boundary conditions for $k_{k'7}$ can be deduced from eqs. 12–15. Thus, $k_{k'7} < 52.1$ (from eq. 12), $k_{k'7} < 47.6$ (from eq. 13), $k_{k'7} < 36.6$ (from eq. 14), and $k_{k'7} > 11.2$ (from eq. 15). Combining all these condition with condition #1 yields:

$$36.4 < k_{k'7} < 36.6.$$

Assuming a hypothetical value of $k' = 0$ and using again the above conditions, one obtains:

$$36.4 < k_{k'0} < 43.6.$$

It is evident that all corresponding boundary conditions are compatible with each other, indicating that the measured data in the control group would be compatible with the proposed pool model. Furthermore, it can be deduced that, depending on the precise size of k′, k can lie between 36.4 and 43.6. Referred to a hypothetical pool size of tracee in the mixed pool (1000), the size of the rapidly turning over protein pool may be about 4% of the total, mixed pool.

Assuming an approximate size of the very rapidly turning over protein pool of 0.5% and of the rapidly turning over protein pool of 3.8%, one may, for example, calculate tracer/tracee ratios at all sampling points for the slowly turning over protein pool and for the 3-hour sample of the rapidly turning over protein pool (see Table III above). Considering the different plateau effect of the rapidly and very rapidly turning over protein pool, hypothetical graphs can be designed that reflect the principal changes over time of tracer/tracee ratios in the different pools (Figure 3).

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