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Effect of Total Parenteral Nutrition on Amino Acid and Glucose Transport by the Human Small Intestine

Yoshifumi Inoue, M.D.,* N. Joseph Espat, M.D.,* David J. Frohnapple, Pharm.D.,‡
Howard Epstein, M.D.,† Edward M. Copeland, M.D., F.A.C.S.,*
and Wiley W. Souba, M.D., Sc.D., F.A.C.S.*

From the Department of Surgery, Division of Surgical Research and Urology,† and the Laboratories for Surgical Metabolism and Nutrition, University of Florida College of Medicine, Gainesville, and the Department of Pharmacy,‡ Veterans Administration Hospital, Gainesville, Florida*

Objective
The effect of total parenteral nutrition (TPN) on small intestinal amino acid transport activity was studied in humans.

Summary Background Data
Studies in humans receiving TPN indicate that a decrease in the activities of the dissacharidase enzymes occurs, but morphologic changes are minimal with only a slight decrease in villous height.

Methods
Surgical patients were randomized to receive TPN (n = 6) or a regular oral diet (controls, n = 7) for 1 week before abdominal surgery. Ileum (5 controls, 5 TPN) or jejunum (2 controls, 1 TPN) were obtained intraoperatively and brush-border membrane vesicles (BBMV) were prepared by magnesium aggregation/differential centrifugation. Transport of L-MeAIB (a selective system A substrate), L-glutamine, L-alanine, L-arginine, L-leucine, and D-glucose was assayed by a rapid mixing/filtration technique in the presence and absence of sodium.

Results
Vesicles demonstrated approximately 18-fold enrichments of enzyme markers, classic overshoots, transport into an osmotically active space, and similar 1-hour equilibrium values. TPN resulted in a 26–44% decrease in the carrier-mediated transport velocity of all substrates except glutamine across ileal BBMVs. In the one patient receiving TPN from whom jejunum was obtained, there was also a generalized decrease in nutrient transport, although glutamine was least affected. Kinetic studies of the system A transporter demonstrated that the decrease in uptake was secondary to a reduction in carrier V_{max}, consistent with a decrease in the number of functional carriers in the brush-border membrane.

Conclusions
TPN results in a decrease in brush-border amino acid and glucose transport activity. The observation that glutamine transport is not downregulated by 1 week of bowel rest may further emphasize the important metabolic role that glutamine plays as a gut fuel and in the body's response to catabolic stresses.

The transport of amino acids across the brush-border membrane of the small intestinal epithelial cell is accomplished by functionally distinct amino-acid transport systems. A number of these carriers have been described on the basis of their amino-acid selectivities and kinetic properties.[1] The majority of these carriers are classified as sodium symport systems because they require sodium as a cotransporter ion. Examples of sodium-dependent brush-border carriers include system B, which transports glutamine, alanine, and other short-chained dipolar amino acids and system A, which transports small neutral amino acids and for which the non-metabolizable analog 2-methylaminoisobutyric acid (MeAIB) is a highly selective non-metabolizable substrate. Other amino acids are transported by Na^+-independent carriers, which include system L (leucine and other branched-chain amino acids) and system y^+ (arginine and other cationic amino acids). Like glutamine and alanine, glucose transport across the intestinal apical membrane is Na^+-dependent.[2]

The activity of these brush-border nutrient transporters is regulated by their respective substrates.[3,4] Fluctuations in transport activity may be based on factors such as cost of transporter synthesis and nutrient availability. The presence of ingested substrate in the lumen may be an important initial signal that results in a stimulation of transporter activity to a level above that present when nutrients are not provided. A decrease in transporter activity in the bowel that is not exposed to luminal nutrients may be adaptive if it diminishes the costs of synthesizing and maintaining the membrane transport protein. Consistent with these physiologic responses is the observation that food intake and the presence of nutrients within the gut lumen are important stimuli for the growth and turnover of the gut mucosa. In animals, starvation results in the development of mucosal atrophy and a decrease in the activities of several brush-border enzymes. Similarly, rats nourished with total parenteral nutrition (TPN) develop mucosal hypoplasia and a decrease in brush-border enzymes.[5] In humans receiving TPN for as long as 3 weeks, a decrease in the activities of the dissacharidase enzymes occurs, but morphologic changes are minimal with only a slight decrease in villous height.[6] These changes may be associated with and may even lead to a breakdown of the gut mucosal barrier under certain circumstances.[7,8]

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Address reprint requests to Wiley W. Souba, M.D., Sc.D., Division of Surgical Oncology, Department of Surgery, Massachusetts General Hospital, Boston, MA 02114.

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The effects of TPN on the activities of the major amino-acid transport systems in the brush border of the small intestine of humans has not been studied. Therefore, we examined the effects of TPN on amino acid and glucose transport by the human ileum using brush-border membrane vesicles.

MATERIALS AND METHODS

Reagents and Chemicals

All chemicals and reagents used were of analytical quality and were purchased from Sigma Chemical Co. (St Louis, MO). Radiolabeled L-[G-^{3}H]-glutamine, L-[2,3-^{3}H]-alanine, L-[4,5-^{3}H]-leucine, L-[2,3,4,5-^{3}H]-arginine, and D-[6-^{3}H]-glucose were purchased from Amersham (Arlington Heights, IL). [^{3}H]-MeAIB(2-methylamino isobutyric acid) was obtained from American Radiolabeled Chemicals (St. Louis, MO).

Patient Selection

Adult surgical patients admitted to the Shands Hospital at the University of Florida or the Gainesville Veterans Administration Hospital were eligible to participate in the study. Patients were randomized to receive a regular hospital diet (28.2 nonprotein kcal/kg/day [34% fat], and 1.25 g protein/kg/day [0.20 g N/kg/day]) or TPN (30 nonprotein kcal/kg/day [34% fat as Intralipid], and 1.27 g protein as Aminosyn/kg/day [0.20 gm N/kg/day]). Patients were assigned to the particular feeding regimen on an every other patient basis, alternating between TPN and the regular diet. All patients participating in the study were judged to be healthy without evidence of diabetes, metastatic malignant disease, infection, or significant weight loss. The studies were designed to evaluate the effects of TPN and bowel rest on amino acid transport by the human small intestine in relatively healthy patients without malnutrition. The protocol was approved by the Institutional Review Board at the University of Florida College of Medicine and by the Subcommittee for Clinical Investigation at the Gainesville Veterans Administration Medical Center.

Segments of distal ileum were obtained intraoperatively from ten healthy patients. Patients underwent (a) right hemicolectomy for tumors of the cecum or ascending colon (3 controls, 3 TPN); (b) total abdominal colectomy (1 control); or (c) cystectomy and ileal loop for carcinoma of the bladder (1 control, 2 TPN). Transport by jejunal brush-border vesicles from two previously studied control patients[9] was compared with transport by jejunal vesicles from one patient receiving 1 week of TPN before cystpancreatico-jejunostomy. After opening

the abdomen, a 10- to 15-cm section of ileum or jejunum was obtained, placed on ice, and immediately transported to the laboratory. The specimen was not devascularized until immediately before passing it off the operating table. In the laboratory, the mucosa was rinsed with 0.9% ice-cold saline, scraped with a glass slide, and stored in liquid nitrogen. Brush-border membrane vesicles (BBMV) from human small intestinal mucosa were prepared as described.

Membrane Vesicle Preparation

Brush-border membrane vesicles were prepared by a Mg^{++} aggregation/differential centrifugation technique according to Stevens et al.[10] with minor modifications that we have already described in detail.[9] The previously frozen mucosa was first thawed. All steps of the preparation were conducted at 0–5 C. Briefly, each gram of thawed mucosal scrapings was homogenized in 8 ml of buffer containing 300 mmol/l mannitol in 1 mmol/l N-2 hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES)-TRIS (pH 7.5) buffer with a Polytron homogenizer (Brinkman, Westbury, NY) for 15 seconds at setting number 6. Homogenates from each group were then treated with 100 mmol/l $MgCl_2$ in 1 mmol/l HEPES/TRIS (pH 7.5) to yield a final concentration of 10 mmol/l $MgCl_2$. After stirring for 20 minutes, the homogenate was centrifuged for 5 minutes at 1500*g*. The supernatant containing brush-border material was collected and this step was repeated once. The supernatant was then centrifuged at 45,000*g* for 30 minutes. The brush-border membrane pellet was resuspended in 350 mmol/l mannitol/50 mmol/l HEPES/TRIS (pH 7.5) and centrifuged again at 45,000*g* for 30 minutes. The final pellet was resuspended in the same buffer to yield a final protein concentration of 10 to 15 mg/ml. The brush-border enzymes alkaline phosphatase and gamma glutamyl transpeptidase were routinely measured to assay for brush-border vesicle purity.[11]

Transport Measurements

The transport of radiolabeled glutamine (Gln), alanine (Ala), MeAIB, leucine (Leu), arginine (Arg), and D-glucose was measured using a rapid mixing/filtration technique.[9,10] For each uptake measurement, 10 μl of BBMV and 40 μl of the radioactive uptake buffer were placed separately at the bottom of a 12 × 75 polystyrene tube (Fisher Scientific Inc, Pittsburgh, PA). The uptake buffer components were adjusted so that the final concentration mixture contained initial gradients of 125 mmol/l NaCl or KCl and labeled substrates at 100 μmol/l concentrations. An electronically controlled device was used to initiate the reaction by rapidly vibrating the tube.[9] After the prescribed reaction period (10 sec–1 hr), 1 ml of ice-cold stop buffer (150 mmol/l NaCl/10 mmol/l Hepes/TRIS, pH 7.5) was added to quench the reaction. The quenched reaction mixture was then filtered through a prewet and chilled 0.45 μm nitrocellulose filter (product no. 63068 Gn-6, Gelman Scientific, Ann Arbor, MI) by low pressure vacuum to separate intravesicular from extravesicular substrate. The filters were rapidly washed twice with a total of 8 ml of ice-cold stop buffer and then dissolved in Aquasol Scintillation cocktail (New England Nuclear, Boston, MA). The radioactivity trapped by the vesicles was measured by liquid scintillation counting (Beckman LS 7800, Beckman Scientific Instruments, Irvine, CA). All transport assays were carried out at 22 C. With the exception of uptake time courses, reaction was terminated at the 10-second timepoint to ensure measurement of initial rates under these assay conditions.

The Na^+-dependent component of substrate transport for glutamine, MeAIB, alanine, and glucose was calculated by subtracting uptake in the presence of potassium (Na^+-independent uptake, quadruplicate determinations) from that observed in the presence of sodium (total uptake, quadruplicate determinations). Carrier-mediated, Na^+-independent arginine and leucine transport was determined in the absence of sodium and in the presence and absence of a 10 mmol/l excess of unlabeled arginine. Saturable, carrier-mediated Na^+-independent arginine and leucine transport was calculated by subtracting non-saturable uptake (defined as the component of uptake resistant to inhibition by 10 mmol/l amino acid) from total Na^+-independent uptake (uptake in the absence of 10 mmol/l amino acid). Transport kinetic characteristics were determined by assaying uptake as described earlier and varying the initial concentration of substrate from 50 μmol/l to 5 mmol/l. Kinetic experiments were performed at the 10-second timepoint.

Osmotic adjustments for varying concentrations of test substrates were made with mannitol. In all transport experiments blank values (no vesicle present) were determined and subtracted from the corresponding substrate uptakes. Protein concentration was determined by the Bio-Rad protein assay (Bio-Rad Laboratories, Richmond, CA) with gamma globulin as the protein standard and all uptakes were normalized to protein. Data are expressed as Na^+-dependent or Na^+-independent uptake velocity in pmol/mg protein/unit time.

Statistical Analysis

All data are expressed as mean ± standard error. Data were compared using the unpaired two-tailed Student's *t*-test or, when appropriate, analysis of variance (ANOVA) followed by Fischer LSD if a significant F value was obtained. A p value less than 0.05 was considered statistically significant.

RESULTS

Validation Studies

Brush-Border Purification

The purity of the brush-border membranes was ascertained by determining the activities of the brush-border marker enzymes alkaline phosphatase (Alk Phos) and gamma-glutamyl transpeptidase (GGT). Both enzymes showed a 16–18-fold enrichment in vesicles from control and TPN patients when compared with the crude homogenate (Fig. 1).

Transport Versus Nonspecific Binding

To determine whether substrate uptake by BBMV represents binding of the substrate to the vesicle surface or actual transport into an intravesicular space, the osmotic sensitivity of substrate uptake was examined. We investigated the uptake of glutamine (100 μmol/l) at 20 minutes as a function of increasing the incubation medium osmolarity (osmolarity was changed with mannitol). The relationship between glutamine uptake and 1/osmolarity was linear with a correlation coefficient of 0.960. Extrapolating the line to infinite osmolarity showed minimal uptake (intercept = 20.373 pmol/mg protein). These results indicate that uptake of glutamine by BBMV is principally (92.3%) the result of transport of the substrate into the intravesicular space with negligible binding to the vesicle surface (Fig. 2).

Vesicular Size and Permeability Coefficients

Transport of 100 μmol/l glutamine was examined as a function of time both in the presence and absence of sodium. In the presence of sodium, glutamine transport was found to be rapid and linear for the first 30 seconds and showed a distinct "overshoot" phenomenon that peaked at approximately 1 minute. Equilibrium was reached after 60 minutes of incubation. Equilibrium uptake values for vesicles from either group of patients were highly comparable, indicating uniformity of vesicle size. Intravesicular volumes calculated from equilibrium levels of glutamine were 0.75 ± 0.11 and 0.73 ± 0.17 μl/mg protein for vesicles from control and TPN-fed patients, respectively.

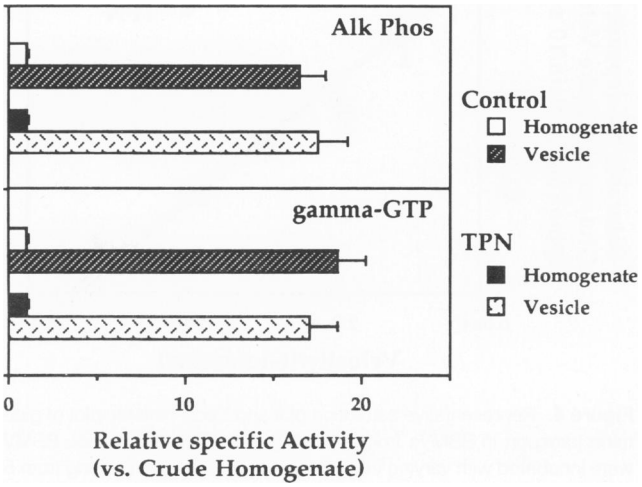

Figure 1. Specific activities of marker enzymes in the brush-border membrane. The specific activities of the marker enzymes alkaline phosphatase (Alk Phos) and γ-glutamyl transpeptidase (gamma-GTP) in BBMVs were compared with those in crude homogenates. The values were expressed as relative ratio to crude homogenates (the value of crude homogenates = 1).

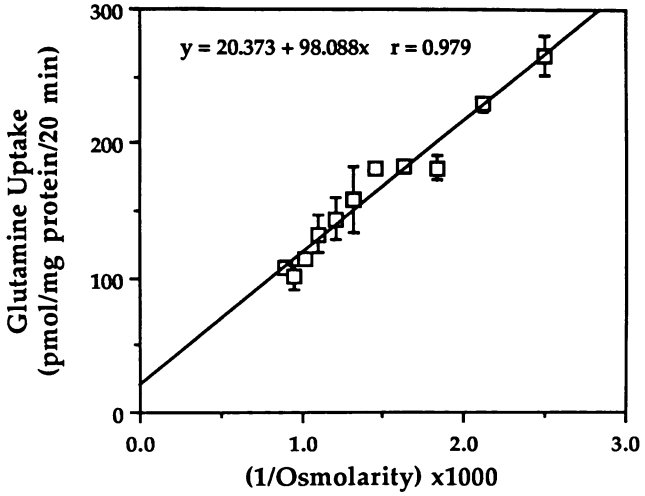

Figure 2. Effect of osmolarity on glutamine transport in BBMVs. BBMVs were preloaded with 350 mmol/l mannitol and 50 mmol/l HEPES/TRIS. Incubation was performed for 20 minutes in an uptake buffer containing 125 mmol/l NaCl, 100 μmol/l glutamine, and an amount of mannitol required to give the indicated osmolarity. Each point represents mean ± SEM.

Effect of TPN on Amino Acid and Glucose Transport

A representative time course of Na^+-dependent Gln and MeAIB transport by BBMV from control and TPN-fed patients are shown in Figure 3. The time courses of glutamine transport in both patients were identical with similar uptake rates at all examined incubation timepoints. On the other hand, the initial Na^+-dependent MeAIB uptake rate was 37.5% lower and peak accumulation at 1 minute was 32.3% lower than that observed in control vesicles.

To investigate the change in ileal amino-acid transport activity observed in vesicles from TPN-fed patients, ki-

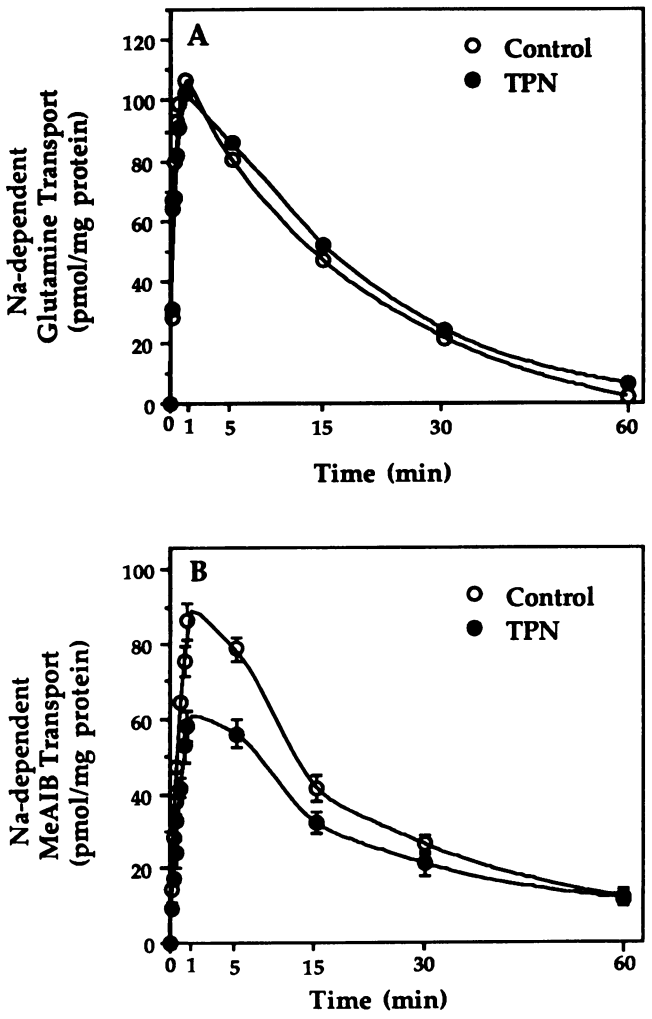

Figure 3. Time course of glutamine and MeAIB uptake by BBMVs from control and TPN-fed patients. BBMVs were incubated with 100 μmol/l glutamine (A) or 100 μmol/l MeAIB (B) uptake buffer as described in Methods. The figure is a representative time course of three assays performed separately from three different BBMV preparations. Each data point represents mean ± SEM of quadruplicate measurements. When not shown, the error bars are contained within the symbol.

netic transport experiments were undertaken as a function of concentration (50 μmol/l −5 mmol/l) in the presence of 125 mmol/l NaCl or KCl. Incubations were performed for 10 seconds. The initial rate of Na^+-dependent MeAIB and glutamine uptake is shown in Figures 4 and 5, respectively. Na^+-dependent glutamine transport was almost identical at all extravesicular glutamine concentrations. Eadie-Hofstee linear transformation of the data showed similar transport V_{max} (maximal velocity of transport; 321.63 ± 38.41 pmol/mg protein/10 sec for control and 322.86 ± 25.24 for TPN) and Km (transporter affinity; 0.6 mmol/l for control and TPN). In the case of MeAIB transport, vesicles from TPN-fed patients showed significantly decreased uptake at all extravesicular MeAIB concentrations (Fig. 5A). Regression analysis of Eadie-Hofstee linear transformation of the data (Fig. 5B) indicated that the decrease was due to a 24% decrease in the transport Vmax (137.76 ± 11.4 pmol/mg protein/10 sec for control vs. 105.25 ± 8.4 for TPN, $p < 0.01$) without alteration of Km (0.26 mmol/l for both control and TPN).

To determine the specificity of the effects of TPN on other brush-border transporters, the ileal transport of alanine, leucine, arginine, and glucose by BBMV was also measured. The uptake of all substrates was significantly decreased in the BBMVs from TPN patients (Fig. 6). Figure 7 shows the summary of these changes for all sub-

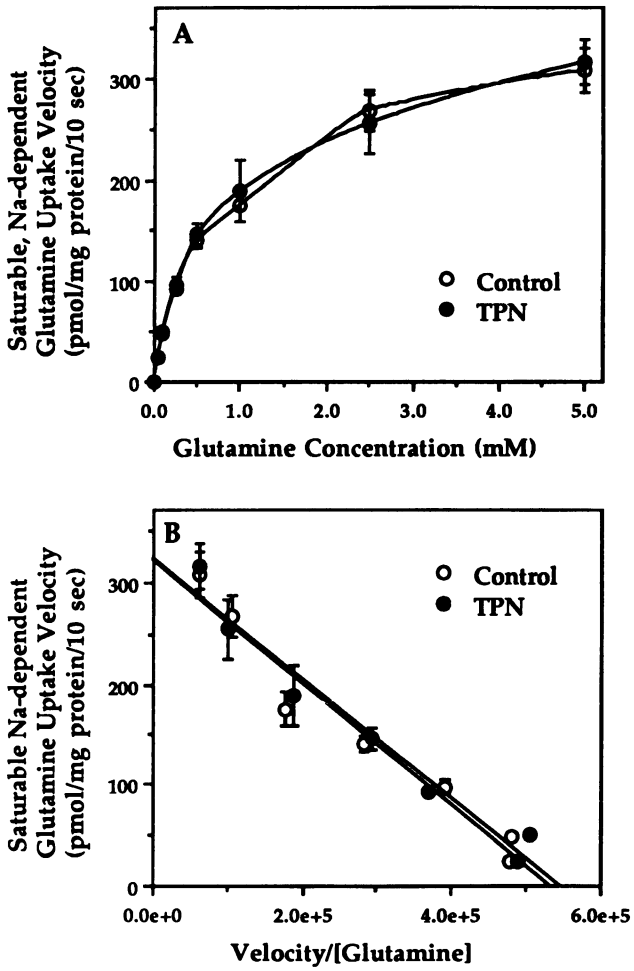

Figure 4. Representative saturation plot and Eadie-Hofstee plot of glutamine transport in BBMVs from a control and a TPN-fed patient. BBMVs were incubated with varying concentrations of glutamine ranging from 50 μmol/l to 5 mmol/l for a period of 10 seconds. Na^+-dependent glutamine uptake was determined as described in Methods and uptake velocity was plotted as a function of glutamine concentration (A) or as a function of velocity/[glutamine] (B). In Figure B, the y-axis intercept represents maximal transport velocity (V_{max}) and negative slope of the line corresponds to the apparent K_m (transporter affinity). Data from three separate runs indicated no change in V_{max} or Km.

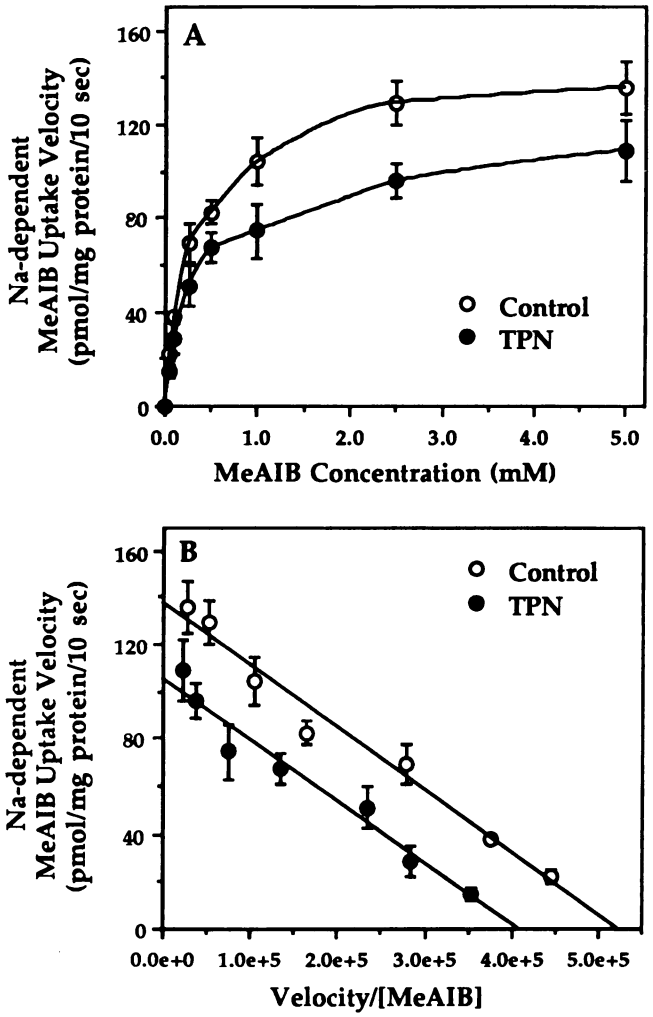

Figure 5. Representative saturation plot and Eadie-Hofstee plot of MeAIB transport in BBMVs from a control and a TPN-fed patient. BBMVs were incubated with varying concentrations of glutamine ranging from 50 μmol/l to 5 mmol/l for a period of 10 seconds. Na^+-dependent MeAIB uptake was determined as described in Methods and uptake velocity was plotted as a function of MeAIB concentration (A) or as a function of velocity/[MeAIB] (B). In Figure B, the y-axis intercept represents maximal transport velocity (V_{max}) and negative slope corresponds to the apparent K_m (transporter affinity). Data from three separate runs demonstrated that TPN feeding resulted in a 44% decrease in V_{max} with no change in transporter Km.

strates we measured as percent change relative to control uptakes. Except for glutamine, the uptake of all substrates we measured decreased from 26%–44%.

In the one patient studied who received TPN for 1 week and had jejunum harvested, a generalized decrease in amino acid and glucose transport in BBMV occurred compared with jejunum from two control patients. MeAIB uptake decreased by 12%, alanine transport was reduced by 24%, leucine transport was diminished by 26%, glucose uptake decreased by 15%, arginine uptake

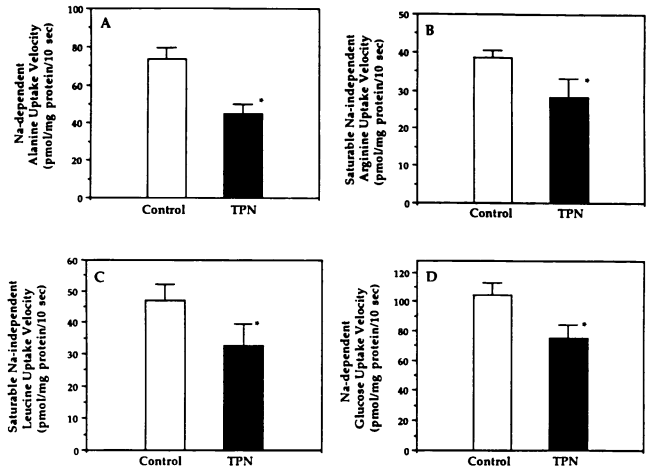

Figure 6. Na-dependent nutrient transport in BBMVs from control and TPN-fed patients. BBMVs from control and TPN-fed patients were incubated in 100 μmol/l alanine (A), 100 μmol/l arginine (B), 100 μmol/l leucine (C), and 100 μmol/l D-glucose (D) for a period of 10 sec as described in Methods. The data represent the mean ± SEM of three assays performed separately using BBMVs from at least three control and three TPN-fed patients. Each data point represents the mean ± SEM. *$p < 0.01$ vs. control.

was decreased by 11%, and glutamine transport was diminished by 10%.

DISCUSSION

The effects of total parenteral nutrition on small intestinal amino-acid and glucose transport were studied in

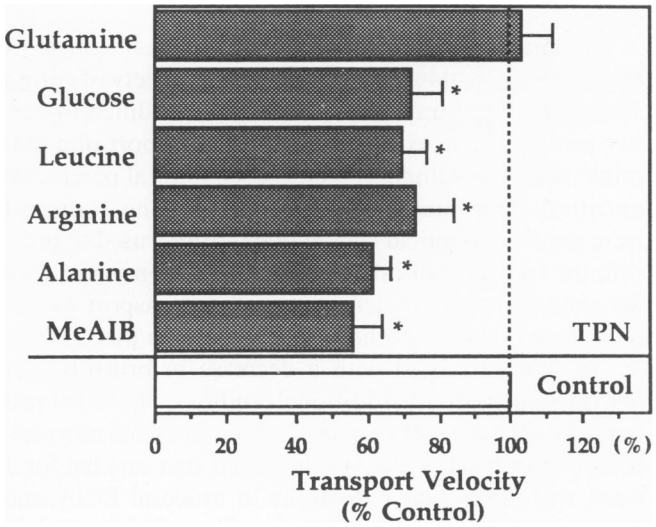

Figure 7. Effects of TPN on amino-acid and glucose transport in ileal BBMVs. Ileal BBMVs from control and TPN-fed patients were incubated in 100 μmol/l concentrations of glutamine, alanine, leucine, arginine, MeAIB or glucose for 10 sec as described in Methods. The TPN data (3–5 patients) are expressed as mean ± SEM relative to control values (5 patients) normalized to 100%. *$p < 0.05$ vs. control.

surgical patients using BBMVs. Vesicles have been used successfully in the past by us[9] and by others[12] to study small bowel luminal amino-acid transport. The use of plasma membrane vesicles to assess brush-border substrate transport activity offers several advantages over other models such as cultured enterocytes. Transport activity is reflective of that occurring *in vivo* and can be evaluated apart from other confounding influences such as metabolism and trans-stimulation/inhibition. Alterations in membrane transport activity are preserved during the preparation and storage of vesicles.[10] In the present study, BBMVs from control and TPN-fed patients demonstrated enrichments of enzyme markers and overshoots, indicating vesicle purity and functionality.

Our results demonstrate, for the first time, that 1 week of TPN and bowel rest leads to a decrease in the activities of several amino-acid transporters in the brush-border of the small intestine of humans. Calorie and nitrogen intake was almost identical in control and TPN-fed patients indicating that the changes we observed are secondary to the route of nutrient delivery rather than the quantity of nutrients delivered to the patients. The exact mechanism by which the route of feeding influences transport in the small intestine is unclear. The presence of specific nutrients in the lumen may influence transporter activity. However, the bulk of nutrient transport occurs in the jejunum and we observed a decrease in ileal amino-acid and glucose transport in the TPN group. These results suggest that other factors such as alterations in gut hormone production, changes in regional blood flow, and changes in bowel microflora may also modulate brush-border nutrient transport in the patient receiving total parenteral nutrition.

The magnitude of the decrease in ileal transport ranged from 26–44% as assessed using a variety of amino acids, which are transported by distinctly different carrier proteins. The exception was the transport of glutamine, which was unaltered by 1 week of total parenteral nutrition. Our measurements of glutamine transport were made in identical vesicle preparations used to measure the transport of other amino acids. Consistent with the changes in amino-acid and glucose transport we observed are previous studies that have shown that TPN in humans is associated with a decrease in brush-border hydrolase activities.[6] Additional studies in TPN-fed rats have shown the development of small intestinal morphometric atrophy. Levine demonstrated that rats fed for 1 week with TPN had reductions in mucosal DNA and protein as well as reductions in villous height and in crypt depth.[5] These structural alterations are accompanied by decreases in mucosal dissacharidases[5] and in an increased incidence in the translocation of luminal bacteria to the mesenteric lymph nodes.[13]

In contrast to the histologic changes that occur in the rat receiving TPN, such changes do not appear to occur in the human small intestine. Although we did not measure mucosal morphometrics in the patients we studied, others have shown that longer periods of TPN does not lead to villous atrophy.[6] In addition, we did not culture luminal contents or regional lymph nodes; in animals, such parameters may be altered by the route of feeding.[13] Thus, the consequences of the biochemical changes that occur in patients who are receiving total parenteral nutrition remain to be fully defined.

Luminal amino acids are transported across the brush-border membrane of the small intestine by several well-described transport systems.[1,14] Each system relates to a homogeneous population of transporter proteins which reside in the cell membrane and function to bind structurally related amino acids and transport them into the enterocyte. Each transporter is selective for a group of amino acids (i.e., neutrals, cationics, bipolars, etc.) rather than one specific molecule. Translocation of luminal substrates into the cytoplasm of the enterocyte occurs via three separate pathways: sodium-dependent routes, sodium-independent pathways, and by diffusion, which reflects the permeability of the membrane to the substrate. The sodium-dependent route is usually the dominant pathway. In the case of glutamine and alanine and other dipolar amino acids, their transport is predominantly Na^+-dependent and is mediated by a carrier recently named system B.[14] Alanine can also be transported by the lower affinity Na^+-dependent system A, whereas leucine is mainly transported by the sodium-independent system L and carrier-mediated arginine uptake is primarily mediated by the Na^+-independent system y^{+1}. Glucose uptake from the lumen is accomplished by a sodium dependent co-transporter.[2] Under normal circumstances the rate of transporter synthesis is balanced by the rate of carrier degradation such that a steady state exists and the number of copies of transporter molecules on the brush-border remains fairly constant.

Kinetic analysis of the system A carrier was studied using the non-metabolizable amino acid analog MeAIB. This compound is highly selective for system A in that it is not transported by other carriers and therefore transport exclusively by system A can be evaluated. The Eadie-Hofstee plot shown in Figure 5 indicates that the reduction in system A activity is due to a decrease in V_{max} rather than a change in Km. Most likely, this decrease in V_{max} is due to a decrease in the number of functional transporters in the brush-border membrane as a consequence of nutrient absence. Our observations are consistent with the observations of Diamond and Karasov[4] who studied the influence of diet on the adaptive regulation of intestinal nutrient transporters. Their work indicated that the basal rate of luminal transport can be

upregulated by dietary substrate. This observation may be important in catabolic patients because it suggests that enteral nutrition may help offset the decrease in luminal transport activity that occurs during starvation and during critical illness. Maintenance of transport function may also be important in such individuals since dietary substrate can profoundly influence protein synthesis in the gut mucosa. Furthermore, enteral feedings improve outcome in critically ill patients,[15] an event that may be related to reprioritization of hepatic protein synthesis[16] and/or preservation of mucosal integrity.[7]

It is unclear why the transport of glutamine across the brush border was not attenuated by bowel rest and TPN, especially in view of the diminished transport of alanine, another system B substrate. Possibly, other carriers in the apical membrane also mediate the transport of glutamine, although kinetic studies indicated a homogeneous population of carriers. Alternatively, alanine may also be transported by the system A carrier or the system ASC carrier.[1] One might speculate that glutamine present in bile and glutamine released by sloughed dying enterocytes might maintain luminal concentrations high enough to prevent a decrease in transport activity. If this were the case, one might expect the maintenance of system B activity to also be reflected in preservation of alanine transport activity via system B. Conceivably, the previously reported alanine inhibition of luminal glutamine transport could be non-competitive in nature but studies in the human Caco-2 cell line show this to not be the case.[17] Thus, additional studies should explain these discrepancies and provide further insight into the nutrient-induced divergent regulation of amino-acid transport observed in TPN-fed patients. Longer periods of TPN and bowel rest may result in a downregulation of luminal glutamine transport.

Regardless of the reasons for the selective preservation of glutamine transport activity in TPN-nourished patients, these data may emphasize the importance of this amino acid for bowel metabolism and function. Unlike glutamine, the other naturally occurring amino acids (leucine, alanine, arginine) we studied as well as glucose are present in total parenteral nutrition solutions. Infusion of these compounds provides a circulating source of amino acids that can be taken up by the gut epithelial cell across the basolateral membrane. Consequently, uptake from the lumen may not be as high a priority as it is for glutamine, which is absent from TPN solutions. Because glutamine uptake by the gut mucosa is a balance between luminal and circulating consumption,[18] preservation of brush-border glutamine transport activity in TPN-fed patients may ensure adequate amounts of this key fuel for the gut mucosal cells.

Although we did not measure circulating glutamine levels in these healthy patients participating in this study, others have shown that blood levels of this amino acid do not decrease in unstressed patients receiving TPN. Endogenous production of glutamine may be adequate in such individuals and significant glutamine depletion may not develop unless the patient becomes stressed. Therefore, our results are consistent with a growing body of knowledge that indicates that glutamine is a key mucosal substrate which is essential for intestinal structure and function.[19–21] The role of this amino acid in supporting the gut during critical illness should be further investigated.

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Discussion

DR. JOHN P. GRANT (Durham, North Carolina): It is a pleasure to discuss this paper and to have the opportunity to review the manuscript before this discussion. There has certainly been a lot of research published lately in the experimental animal model concerning the alterations in gut histology and function during intravenous nutrition. This paper reporting for the first time a generalized decrease in amino-acid transport function in the human is of particular interest in that it does deal with the human model. I have several questions I would like the authors to address. First, do the authors feel that this altered amino-acid transport function is due to bowel atrophy or is it due to some altered metabolism with the intravenous nutrition amino-acid profile? To this end, what would happen, or have they evaluated, the administration of a nonabsorbable bulk agent to these patients who are on intravenous nutrition? Is it simply a matter of bulk stimulation of the mucosa or is it due to a nutrient abnormality? Second question, the authors report on altered amino-acid transport from the terminal ileum in this experiment. This is perhaps the least active area of the intestine with respect to amino-acid absorption and may be sensitive to alterations in dietary intake or intravenous feeding. I wonder if they have looked more proximally in the bowel, say in the proximal two-thirds or, in particular, in the midgut to see if it is as sensitive. Perhaps this is just simply a matter of location of study in the small bowel. Third, this was a short-term study. Most of the intravenous feeding done in the hospitalized patient occurs for 2 or 3 weeks on the average and perhaps this 1-week study is a transient phenomenon. Have they evaluated any other patients who have gone to surgery who have perhaps been on long-term TPN for similar findings? One of the authors in this study, Dr. Inoue, spent 2 years in my laboratory evaluating intravenous nutrition and its impact in an experimental animal model. He, like others, identified significant mucosal atrophy occurring during the intravenous feeding; however, this atrophy was completely reversed when 1 to 2% of the amino-acid content of the intravenous solution was substituted with glutamine. He subsequently did an experiment whereby he injected animals with intraperitoneal *E. coli* and found that those animals given intravenous feeding without glutamine had about a 40% survival, whereas those with supplemented glutamine had about a 95% survival. The question therefore becomes, is glutamine capable of completely reversing their findings and is this simply a matter of its absence in the intravenous nutrition solution? Have the authors studied any patients supplementing either with oral or intravenous glutamine for similar findings of the transport proteins?

DR. JOSEF E. FISCHER (Cincinnati, Ohio): This is another in a series of really excellently done studies by Dr. Souba and Dr. Copeland, the quality of which we have become accustomed. What it shows is that the number of carriers in small bowel of man for the first time is decreased, although the confirmation of the carriers as suggested by KM remains the same. Now the questions is, the data is fairly complex and I suppose I am having a little difficulty in understanding some of the consistencies in the data. One of the ways in which one might explain some of these findings is that one of the effects of TPN on the short term is to increase gut blood flow. And one of the things that happens to the bowel in TPN is it serves as a principal area for transamination. If you have a presentation of a lot of substrate such as amino acids to the gut on the blood side, or the basolateral membrane side, then you might expect an increase in alanine, for example, delivered to the cell from the blood flow side and not from the lumen. It is interesting that of all the amino acids that decreased that were studied, alanine and methyl AIB, which measures the system A for alanine, are the most decreased. I have a few questions. The first really is methodologic. As I review your technique and look at the reference of technique, the scraping and the subsequent homogenization to obtain the membranes is similar except for a couple of changes in the way one goes about with the reagents of obtaining isolated enterocytes. It is true the reagents are a little different and the technique is a little different, but how can you be certain that all of the vesicles that you are obtaining are really from the brush border and some of them are not from another part of the cell, namely, the basolateral membrane. I ask that question because if you assume that glutamine supply is the same via the gut and that there is the appropriate amount of glutamine from the blood that you may be getting maintenance of the glutamine transport if some of your membrane vesicles are really not brush border, but they really are basolateral membrane. There is a discrepancy in system B between glutamine and alanine; whereas the transport of system B alanine is decreased, system B for glutamine remains intact. Presumably they are the same carrier and how do you explain that discrepancy? The other problem I have with the relationship between the systems is that in most other systems, system L for leucine and system Y+ for arginine, usually are linked, and if system L is down, arginine and other dibasic amino acids should be up. One of the ways in which one could explain all of these discrepancies is if the model really does not dissect out brush border alone but has a mixed bag of vesicles and I don't know how one would go about it. In the manuscript you spoke about an x18 enrichment, which I believe, but I am not sure that rules out different vesicles. And finally, I would raise the issue of whether glutamine has other uses other than fuel. Taking off in our laboratory, Per- Olof Hasselgren has pursued some of the data that you raised, in last year's presentation, about the discrepancy between increased use of glutamine and a decrease in glutamine synthase in sepsis. It does appear that there are alter-