Protein absorption depends on load-dependent inhibition of intestinal transit in dogs1-3

Xiao-tuan Zhao, Robert H Miller, Mark A McCamish, Lijie Wang, and Henry C Lin

ABSTRACT Ileal perfusion of protein slows intestinal transit. Because optimal absorption of nutrients requires adequate time in contact with the mucosa, slowed intestinal transit may increase protein absorption by increasing the residence time of nutrients in the small intestine. Although protein supplements are routinely added to enteral feeding to correct protein malnutrition, little information is available on the effect of increasing the load of protein on intestinal transit and the efficiency of protein absorption. In six dogs equipped with duodenal and midintestinal fistulas, intestinal transit and the efficiency of protein absorption (percent age protein absorbed as estimated from the output of midintestinal fistula) were compared during intestinal perfusion with 0-, 50-, 100-, and 200-g/L solutions of a whey-based protein supplement. We found that intestinal transit slowed in a load-dependent fashion (P < 0.05); the amount of protein absorbed within the proximal one-half of the small intestine increased in a load-dependent fashion (P < 0.05) as intestinal transit slowed, and the percentage protein absorbed (reflecting the efficiency of protein absorption) was maintained at a high and nearly constant value of 66.5-72.5% across protein loads of 9-36 g. We conclude that enhanced protein absorption is associated with a load-dependent inhibition of intestinal transit. Am J Clin Nutr 1996;64:319-23.

KEY WORDS Protein, absorption, gastrointestinal motility, small intestine

INTRODUCTION

An important goal of enteral feeding is to correct protein malnutrition (1). To achieve this goal, protein supplements are commonly mixed with enteral formulas to increase the nitrogen supply. The addition of a protein supplement may affect the speed of intestinal transit of the formula and the absorption of proteins. A partial digest of proteins inhibited small intestinal transit when delivered into the ileum (2) and protein absorption increased when intestinal transit was slowed by fat delivered into the distal gut (3). However, the relation between the load of protein supplements, intestinal transit, and absorption of proteins is unknown. Because optimal digestion and absorption of luminal nutrients require adequate time of contact with the mucosa of the small intestine (4), we hypothesize that intestinal transit may be inhibited by protein supplements in a load-dependent fashion and in turn, protein absorption may be increased by controlled transit. To test this hypothesis, we compared intestinal transit and protein absorption in a canine model equipped with duodenal and midintestinal fistulas during perfusion of the whole gut with 0-, 50-, 100-, and 200-g/L solutions of a protein supplement.

METHODS

General experimental design

Intestinal transit (between fistulas) and protein absorption (estimated from output of midintestinal fistula) were compared in dogs equipped with duodenal and midintestinal fistulas while 0-, 50-, 100-, and 200-g/L solutions of protein supplement were perfused into the small intestine. The order of testing followed a randomized schedule.

Animal preparation

The procedures used in this study were approved by the Institutional Animal Care and Use Committee at Cedars-Sinai Medical Center, Los Angeles. Six mongrel dogs were each surgically prepared with two chronic intestinal fistulas. Modified Thomas cannulas were placed into fistulas located ~10 cm (duodenal fistula, distal to bile and pancreatic ducts) and ~160 cm (midintestinal fistula) from the pylorus (5). With the flanges of the cannula resting against the inner surface of the intestinal wall, the cannulas were fixed against rotation. Just distal to the fistula, a length of Tygon tubing (polyvinyl chloride; Norton Performance Plastics Corporation, Akron, OH) with a diameter of 2 mm was looped around the intestine and fixed by suture through the visceral peritoneum to the intestinal wall. The length of tubing used was individualized to be as short as possible without a tightening effect on the lumen. This provided a stent against which an inflated Foley balloon could be pulled to provide a water-tight seal. All dogs were given a recovery period of 4 wk and underwent testing only after normal feeding behaviors were reestablished postoperatively. This procedure resulted in good survival and the six dogs

1 From the Department of Medicine, Cedars-Sinai Research Institute, Cedars-Sinai Medical Center, Los Angeles; School of Medicine, University of California, Los Angeles; and Ross Products Division, Abbott Laboratories, Columbus, OH.
2 Supported in part by Ross Products Division, Abbott Laboratories.
3 Address reprint requests to HC Lin, Cedars-Sinai Medical Center, 8700 Beverly Boulevard, Los Angeles, CA 90048-1869. E-mail: LINH@CSMC.EDU.

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remained healthy with stable body weights and unaffected demeanor for > 12 mo of observation.

**Protein solution**

A test solution consisting of 0 (control), 50, 100, or 200 g/L whey protein supplement (ProMod; Ross Laboratories, Columbus, OH) mixed with varying amounts of mannitol with a final osmolality of 430 mOsm was perfused into the small intestine of each dog at 2 mL/min for 120 min. Mannitol (430 mOsm) alone was used as the control solution. Thus, all perfusates were matched in osmolality. The whey protein supplement consisted of 75% whey protein, 13% carbohydrate, and 12% fat by weight (6). Therefore, the protein load was 0, 37.5, 75, and 150 g/L for each concentration of the protein solution.

**Experimental preparations**

Dogs were deprived of food but not water for 18 h before experiments. Thirty minutes before the start of each experiment, the two intestinal cannulas were uncorked. A cork that was pierced through the center with a blunted needle was placed into the duodenal cannula and a Foley catheter was placed into the distal limb of the midintestinal cannula. A water-tight seal was achieved at the midintestinal fistula by inflating the catheter’s balloon with ~8 mL water and cinching the balloon up against the polyvinyl chloride ring (5). All chyme reaching the midintestinal fistula was diverted to provide accurate sampling of the luminal contents by occluding the lumen. To begin each experiment, the test solution was perfused at 2 mL/min for 120 min via the needle positioned in the cork in the duodenal cannula. Corresponding to the 0-, 50-, 100-, and 200-g/L protein supplement solutions, a total of 0, 9, 18, or 36 g protein was delivered into the small intestine over the perfusion period. This perfusion method allowed the perfusate to be mixed with the endogenous biliary and pancreatic secretions within the intestinal lumen. To allow the perfusate access to the whole gut, the output of the midintestinal fistula was returned to the distal gut via the Foley catheter positioned in the distal limb of the fistula. A pumping system driven by a light sensor synchronized to the outflow from the midintestinal fistula was used to return the output and to collect samples (5). This sensor-driven system responded to a surge of flow from the midintestinal fistula by turning a two-headed pump (Masterflex; Cole-Palmer, Chicago) automatically on and off. The output was returned to the distal limb of the midintestinal fistula by a large pump head so that the bulk of chyme traversed the bowel as it normally would have, while a second small pump head pumped a fraction of total flow (7%) into a collecting tube for later assay.

**Measurement of intestinal transit**

Sixty minutes after the start of the perfusion (to allow time for full activation of inhibitory feedback) (7), ∼740 kBq 99mTc chelated to diethyl triamine pentaacetic acid (8) was delivered as a bolus into the test segment to begin measurement of intestinal transit. Intestinal transit across the proximal one-half of the small intestine was measured by counting the radioactivity of 1-mL samples collected from the output of the midintestinal fistula every 10 min for 60 min. With use of a matched dose of 99mTc to represent the original delivered bolus, the radioactivity delivered into the small intestine (9, 10) and the radioactivity in the recovered fistulous output were measured in a gamma counter. After all counts were corrected to time zero, intestinal transit was calculated as the cumulative percentage recovery of 99mTc over 60 min.

**Protein assay**

Samples of collected fistulous output were analyzed by the Lowry method (11) for protein concentration (Protein Assay Kit; Sigma, St Louis). The amount of protein recovered (g) = concentration of protein (kg/L) × fistulous output volume (mL). The original protein solutions (50, 100, or 200 g/L) were used as the standards for protein analysis in each experiment.

**Analysis of protein absorption**

The amount of protein absorbed was estimated from the following equations:

\[
\text{Estimated amount of protein absorbed (g)} = \text{maximal amount protein recoverable} - \text{amount protein recovered}
\]

(1)

Maximal amount of protein recoverable (g)

\[
= \text{amount protein delivered (g)} \times \text{cumulative percentage recovery } 99mTc
\]

(2)

Percentage protein absorbed

\[
= \frac{[\text{estimated amount of protein absorbed (g)}]}{\text{maximal amount of protein recoverable (g)}} \times 100
\]

(3)

The maximal amount of protein recoverable is the amount of protein that would be expected to reach the midintestinal fistula if there were no absorption. This value is calculated as the product of the amount of protein delivered and the cumulative percentage recovery of 99mTc (to adjust for intestinal transit). Because the percentage protein absorbed depends on the maximal amount of protein recoverable as its denominator, the percentage protein absorbed is a value that incorporates the effect of regulated intestinal transit.

**Analysis of data**

Intestinal transit results were compared by using the area under the curve (AUC) representing the cumulative percentage recovery of 99mTc over 60 min. The distribution of AUCs is skewed to the right with larger variances under conditions with higher measures (this is typical of area measurements). Because standard statistical tests assume that data are normal in distribution and that variances are homogeneous, the skewing of intestinal transit AUCs was reduced by taking the square roots of the areas ( √AUC) (12), where 0 is no recovery by 60 min and 74.16 is theoretical, complete, and instantaneous recovery by time 0 (rapid transit). Intestinal transit and protein absorption (g) were then compared by using one-way repeated-measures analysis of variance. The computer program used was BMDP 2V (13).
FIGURE 1. Intestinal transit with protein supplement at concentrations of 0 (control), 50, 100, and 200 g/L as presented by the cumulative percentage recovery of $^{99m}$Tc from the output of midintestinal fistula during the last 60 min of a 120-min perfusion. Data are means.

RESULTS

Intestinal transit

Intestinal transit during perfusion with a 0-, 50-, 100-, or 200-g/L solution of whey protein supplement is illustrated in Figure 1 as the cumulative percentage recovery of $^{99m}$Tc over 60 min. Intestinal transit was inhibited by protein supplement in a load-dependent fashion ($P < 0.05$) (Table 1). The mean cumulative percentage recovery of $^{99m}$Tc decreased from 98.1% to 35.5% when the concentration of protein supplement was increased from 0 (control) to 200 g/L. Correspondingly, the $\sqrt{\text{AUC}}$ values ($\pm$ SEM) decreased in a load-dependent fashion from 57.3 ± 3.9 (control) to 22.6 ± 9.9 (200 g/L).

Protein absorption

Data on protein absorption is shown in Table 2. We found that the estimated amount of protein absorbed ($\bar{x}$ ± SE) by the proximal one-half of the small intestine increased in a load-dependent fashion when the amount of protein delivered into the small intestine increased from 9 (4.9 ± 0.6 g) to 36 g (9.8 ± 3.0 g) ($P < 0.05$). Correspondingly, the percentage protein absorbed was maintained at a high and nearly constant range from 66.5 ± 12.7% to 72.5 ± 4.5% across the range of protein load tested. Because the percentage protein absorbed (calculated as the ratio of estimated amount absorbed and maximal amount recoverable) would be 100 if the estimated amount of protein absorbed equalled the maximal amount of protein recoverable (represented by the line of identity in Figure 2), the percentage protein absorbed may be considered an index of the efficiency of protein absorption. The observed efficiency of protein absorption is then reflected by the deviation of the data from the line of identity (best-fit line in Figure 2). Because the estimated amount of protein absorbed closely matched the maximal amount of protein recoverable, we found that protein absorption was highly efficient across the range of protein loads tested. The efficiency of protein absorption was maintained at a high and nearly constant rate because of the relation between intestinal transit (1/AUC) and protein absorption (estimated amount of protein absorbed) as shown in Figure 3. We found that when the protein load was increased, the mean estimated amount of protein absorbed increased as intestinal transit was inhibited in a load-dependent fashion (smaller mean $1/AUC$).

DISCUSSION

In this canine study we found that intestinal transit slowed in a load-dependent fashion with a protein supplement, protein absorption (estimated amount of protein absorbed) increased in a load-dependent fashion, and the efficiency of protein absorption as represented by percentage protein absorbed by the proximal one-half of small intestine was maintained at a high and nearly constant rate of 66.5–72.5% when the protein load was increased from 9 to 36 g. These results indicate that the efficiency of protein absorption is maintained by the small intestine across a large range of protein loads when intestinal transit is slowed in a load-dependent fashion. Load-dependent inhibition of intestinal transit may increase protein absorption by increasing the duration of residence of the nutrients in the

### Table 1

<table>
<thead>
<tr>
<th>Protein supplement</th>
<th>$\sqrt{\text{AUC}}$</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 g/L</td>
<td>57.3 ± 3.9</td>
<td>98.1 ± 6.9</td>
</tr>
<tr>
<td>50 g/L</td>
<td>37.2 ± 3.5</td>
<td>75.5 ± 8.2</td>
</tr>
<tr>
<td>100 g/L</td>
<td>32.3 ± 6.0</td>
<td>50.3 ± 8.5</td>
</tr>
<tr>
<td>200 g/L</td>
<td>22.6 ± 9.9</td>
<td>35.5 ± 9.9</td>
</tr>
</tbody>
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$1/ \bar{x} \pm \text{SEM. } \sqrt{\text{AUC}} = \text{square root of the area under the curve presented by the cumulative percentage recovery of } ^{99m}\text{Tc over 60 min; the } \sqrt{\text{AUC}} \text{ values varied from 0 to 74.16 (higher value means faster intestinal transit). Recovery is the cumulative percentage recovery of } ^{99m}\text{Tc over 60 min.}$

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### Table 2

<table>
<thead>
<tr>
<th>Protein supplement and amount delivered</th>
<th>Maximal amount recoverable</th>
<th>Amount recovered</th>
<th>Estimated amount absorbed</th>
<th>Percentage protein absorbed</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 g/L, 9 g delivered</td>
<td>g</td>
<td>g</td>
<td>g</td>
<td>%</td>
</tr>
<tr>
<td>10 g/L, 18 g delivered</td>
<td>6.8 ± 0.7</td>
<td>1.9 ± 0.4</td>
<td>4.9 ± 0.6</td>
<td>72.5 ± 4.5</td>
</tr>
<tr>
<td>20 g/L, 36 g delivered</td>
<td>9.1 ± 1.5</td>
<td>2.9 ± 0.8</td>
<td>6.2 ± 1.3</td>
<td>67.0 ± 9.5</td>
</tr>
<tr>
<td></td>
<td>12.8 ± 3.6</td>
<td>3.0 ± 0.7</td>
<td>9.8 ± 3.0</td>
<td>66.5 ± 12.7</td>
</tr>
</tbody>
</table>

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$1/ \bar{x} \pm \text{SEM. Amount delivered = amount of protein perfused into small intestine; maximal amount recoverable = amount of protein delivered } \times \text{cumulative percentage recovery of } ^{99m}\text{Tc; estimated amount absorbed = maximal amount of protein recoverable } - \text{amount of protein recovered; percentage protein absorbed } = \text{(estimated amount of protein absorbed/maximal amount of protein recoverable) } \times 100.$
small intestine, and thereby the amount of time available for digestion and absorption of the luminal content.

Using this approach, we found that the estimated amount of protein absorbed by the proximal one-half of the small intestine increased in a load-dependent fashion as intestinal transit slowed. These findings suggest that the efficiency of protein absorption was optimized by controlled intestinal transit so that time available for digestion and absorption could be increased when the small intestine is exposed to a greater load of protein. Under this control system, as the load of protein entering the duodenum exceeded the absorptive capacity of the proximal small intestine, protein would spill further distally along the small intestine to trigger greater inhibition of intestinal transit (14–17). By prolonging the duration of contact between the nutrient and the absorptive mucosa, protein absorption (estimated amount of protein absorbed) increased. As a result, the efficiency of protein absorption (percentage protein absorbed) was maintained across a wide range of protein loads.

Protein absorption was estimated from the difference between the amount of protein recovered from the output of the midintestinal fistula and the maximal amount of protein recoverable. Although this estimate does not provide for the exact amount of protein absorbed by the proximal one-half of the small intestine, it is useful for comparing intestinal transit and protein absorption across varying loads of protein.

Steady-state perfusion has been criticized for not representing the surge and flow of normal gastrointestinal transit (18, 19). However, the steady state perfusion method is ideally suited for comparing intestinal transit and protein absorption during delivery conditions similar to tube feeding into the small intestine. The protein loads used in this study were selected to be within the range of exposure commonly encountered during enteral feeding. With a daily protein requirement a 1.5 g/kg body wt, a 70-kg person would require 105 g protein/d. If enteral feeding were delivered for 12 h of a 24-h period (a common situation because tube feeding is often discontinued temporarily for a variety of reason), the protein load that must be delivered would be 17.5 g for each 120-min period (similar to the amount delivered with the 100-g/L test solution). We have also preserved normal digestive physiology by perfusing the protein supplement solution via the needle inserted into the corks in the duodenal fistula. The delivered protein was then allowed to mix with pancreatic and biliary secretions so that normal digestion and absorption proceeded in a fashion similar to that during tube feeding into the small intestine.

Holgate and Read (18) showed that absorption of nutrients decreased when intestinal transit was accelerated by lactulose, metoclopramide, and magnesium sulfate. Conversely, Hugé et al. (3) reported that absorption of nutrients increased when intestinal transit was slowed by perfusion of lipids into the distal small intestine. In this study we extended these observations by showing that the efficiency of protein absorption across varying loads of protein depended on the relation between load of nutrient and intestinal transit. These results suggest that during enteral feeding, the small intestine optimizes protein absorption by generating load-dependent inhibition of intestinal transit.

REFERENCES


