

Effect of Age and Elemental Diets on Gastric Emptying in Rats

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ABSTRACT

AIM: Delayed gastric emptying affects the quality of life. We previously showed that elemental tube feeding emptied more rapidly than standard tube feedings and were associated with a decreased risk of aspiration. The current aim was to develop animal model to confirm the human findings and for subsequent use to identify which components were responsible for the phenomena.

METHODS: Gastric emptying was assessed in young (7-11 weeks, $n=8$), adult (9 months, $n=6$) and aged (18 months, $n=8$) male Wistar rats using water, a low fat (0.6%) elemental diet containing glutamine or a standard liquid diet (20% fat). ^{13}C sodium acetate breath tests were used to assess gastric emptying (i.e., as ^{13}C excretion rate as percent of intake/min).

RESULTS: Gastric emptying of the three different diets decreased with age. The gastric emptying rate in aged rats reduced to about two thirds of adult rats and one half of young rats. In young rats and adult rats gastric emptying of the elemental diet and standard liquid diet were similar ($p>0.999$ at 21 min). In contrast, the elemental diet was emptied significantly more rapidly compared with the standard liquid diet in aged rats ($p<0.0001$ at 21 min).

CONCLUSIONS: Gastric emptying in rats was age dependent. In aged rats the low fat elemental diet containing glutamine was emptied significantly more rapidly than a standard liquid diet. The animal model appears suitable for identifying which components are responsible for the differences.

INTRODUCTION

Percutaneous endoscopic gastrostomy (PEG) feeding is commonly used for bedridden elderly patients^[1]. However, tube feedings may be complicated by vomiting and with aspiration resulting in aspiration-induced pneumonia^[2-4]. The prevalence of aspiration pneumonia in such patients is reported to be in the range of 10 to 22% and is often caused by aspiration of refluxed stomach contents^[4]. It is unclear whether, or to what extent, these serious adverse effects of enteral feeding are related to changes in gastric emptying that may occur in bedridden PEG patients. Various methods have been proposed for preventing the risk of aspiration pneumonia^[1-4]. We previously compared gastric emptying of a low fat elemental diet containing glutamine with a standard liquid diet in elderly gastrostomy patients, and showed that the elemental diet emptied more rapidly and was associated with a decreased risk of aspiration pneumonia^[5].

Delayed gastric emptying affects the quality of life of people. Although the elderly with swallowing disorder sometimes require tube feedings, it remains unclear which type of diet is preferred especially in relation to gastric emptying. Previous studies have shown that gastric emptying is delayed in aging rats^[6,7]. This study was designed to compare the effects of elemental and standard liquid diets on gastric emptying in relation to aging using a rat model of young, adults and aged rats.

METHODS

Animals

Male Wistar rats (young, seven to eleven weeks; adult, nine months;

and aged, 18 months) were obtained from Charles River Laboratories Japan, Inc. (Yokohama, Japan). The rats were allowed to acclimatize for at least seven days to the condition in our animal house (12/12 h day/night cycle, the day starting at 10:00AM; temperature 25°C, humidity 60%). All animal experiments were approved by the Animal Care and Use Committee of Institute for Innovation.

Test meals

A commercially available predigested or elemental diet, Elental[®] (1 kcal/mL, Ajinomoto Pharmaceutical Co, Tokyo, Japan) was used consisting of 17.6% protein (provided as amino acids), 79.3% carbohydrates [provided as dextrin (mean molecular weight 900)], 0.60% fat, and 3.36% vitamins, and minerals dissolved in water to yield 1 kcal/mL. The elemental diet also contains 2415 mg of L-glutamine. The standard liquid diet was Ensure liquid[®] (1 kcal/mL, Abbott Japan, Tokyo, Japan), consisting of 18% protein, 20% fat, and 62% carbohydrate. The standard diet does not contain amino acids including L-glutamine.

Measurement of gastric emptying: Breath test

Gastric emptying was assessed by monitoring breath ¹³CO₂ expiration as an indicator of oxidation of [1-¹³C]acetate (Cambridge Isotope Laboratories Inc, Tewksbury, MA, USA) added to the liquid test meals or water as previously described^[8]. After a 15 h fast each rat was placed in a sealed chamber (how big) with was aspirated at a constant flow rate to collect expired air samples. Then, 20 mL/kg of each liquid meal or water containing 1 mg/mL sodium [1-¹³C]acetate was administered orally using gastric sonde. CO₂ concentrations and ¹³CO₂ enrichments in the gas samples in the exhaust line of the chamber were measured automatically at 7 min intervals using a CO₂ mass spectrometer (ABCA 20106/1; Sercon Ltd., Crewe, Cheshire, UK) interfaced to the exhaust line by an automated gas sampler (GS2012; SENS Corp, Kashiwa, Japan) and a gas chromatograph (GS1000/A, SENS). Rate of ¹³CO₂ production [$R_{CO_2}(t)$, mol/min] at each time point (what) was calculated as follows; $R_{CO_2}(t) (\mu\text{mol}/\text{min}) = C(t) \times E(t) \times F/V/0.8$. where $C(t)$, $E(t)$, represent the CO₂ concentration (mol%) in the gas collected from the exhaust line and ¹³C-enrichments (mol%) of the expired CO₂ at time t , respectively. F and V are constant flow rate of the ventilator (i.e. 1~1.3 L/min) and volume of 1 mole of gas at 23°C and 1 atm (i.e. 24.272 L/mol), respectively. Because some proportion of the ¹³CO₂ produced is incorporated in the TCA-cycle, urea cycle and others, the values were divided by a constant factor of 0.8, which has been derived from the recovery of labeled bicarbonate in another study^[9]. The sum of ¹³CO₂ production (mol) was calculated from the plots of time and the rate of ¹³CO₂ production as the area under the curve of the graph. The rate of ¹³CO₂ production was divided by the ingested amount of sodium [1-¹³C]acetate (mol) and was expressed in percent of intake per minute (%intake/min). All examinations were performed with a one-day washout interval between studies.

Statistical analysis

Data are presented as mean±SD. Statistical tests to compare the measured results for the two groups were as follows: A repeated-measures 2-factor ANOVA with interactions was used to analyze treatment and time effects in rats, and then a Bonferroni correction was applied to comparisons of two groups. Differences were considered significant if the p value was less than 0.05. Statistical analysis was performed by using Prism 6 software (GraphPad Software, Inc., San Diego, CA).

RESULTS

As shown in Figures 1, 2, and 3, gastric emptying (excretion) rate (%intake/min) depended on age of the rats. With regard to water the maximal gastric emptying rate in aged rats ($0.9 \pm 0.2\%$ intake/min) was approximately two thirds of that of adult rats ($1.3 \pm 0.5\%$ intake/min) or about a one half of young rats ($2.0 \pm 0.6\%$ intake/min). For both young rats and adult rats there were no significant differences in gastric emptying between the elemental diet and standard liquid diet. On the other hand in aged rats, the elemental diet emptied significantly more rapidly than the standard diet in aged rats ($p < 0.0001$, $n=8$).

DISCUSSION

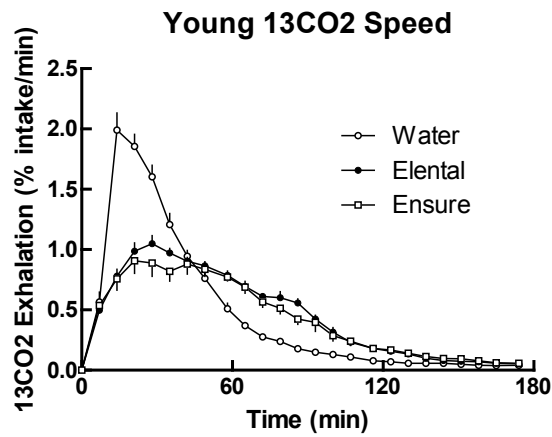
We previously reported that use of an elemental diet was associated with a reduced incidence of aspiration pneumonia compared to standard liquid diets among bedridden patients receiving gastrostomy tube feeding despite the fact that the two liquid diets were administered identically^[5]. Because of the difficulties in studying sick humans, we used an animal model to confirm and extend the original findings and showed that the results in aged rats mirrored the gastric emptying findings in bedridden patients.

It has previously been described that the rate of emptying of liquid diets depended on the amount of liquid or the total energy irrespective of the composition of the diet^[10-13]. In our study the amount and total energy of the test meals were the same and there was no significant differences in gastric emptying between the elemental diet and standard liquid diet in both young and adult rats. However, the elemental diet emptied significantly faster than the standard liquid diet in aged rats. The elemental diet contains free amino acids which can stimulate duodenal receptors and play a role in regulating gastric emptying^[14-16].

Noncaloric water leaves the stomach quickly, whereas solutions of carbohydrates and proteins leave the stomach more slowly but at a similar speed; lipids clear most slowly. Predigested nutrients are assimilated more rapidly so as to spread along a shorter length of the small intestine and thereby activating fewer nutrient sensors. As a result, there is less intestine-gastric inhibitory feedback to slow gastric emptying^[17]. Fatty diets not only empty slowly but also have been reported to be associated with gastroesophageal regurgitation^[18]. One major difference between the standard liquid diet and the elemental diet was the fat content (i.e., 20% vs 0.60%) suggesting that differences in lipid content may be responsible for much of the differences seen in terms of gastric emptying.

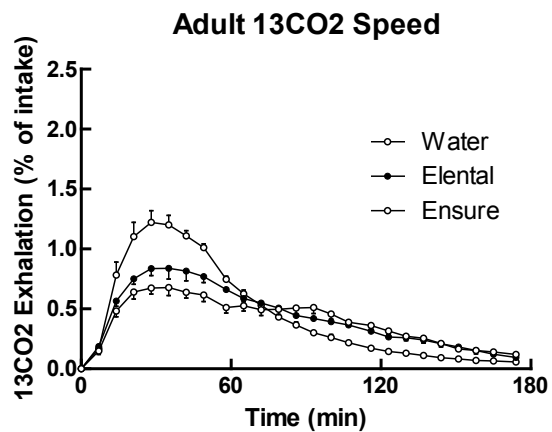
Finally, it has been reported that enrichment with monosodium L-glutamate facilitates gastric emptying of a protein-rich meal suggesting that free glutamate may be helpful in the management of delayed gastric emptying^[19]. The design of this study does not allow us to identify the component or components most responsible for the increase in gastric emptying but accomplished our goal of identifying an animal model to allow subsequent detailed study of the phenomena.

In conclusion, gastric emptying in rats was delayed in aged rats. The low-lipid elemental diet including glutamine significantly facilitated delayed gastric emptying in aged rats compared to the standard liquid diet. The results support our findings that the use of elemental diet tube feedings were associated with more rapid gastric emptying and fewer episodes of aspiration than standard liquid tube feedings among bedridden PEG patients compared to standard tube feedings. The animal model described above will allow the mechanism to be further elucidated.



Time (min)	W vs E	W vs S	E vs S
7	ns	ns	ns
14	****	****	ns
21	****	****	ns
28	****	****	ns
35	*	****	ns
42	ns	ns	ns
49	ns	ns	ns
58	**	**	ns
65	****	***	ns
72	****	**	ns
79	****	**	ns
86	****	*	ns
93	**	*	ns
100	ns	ns	ns
107	ns	ns	ns
116	ns	ns	ns
123	ns	ns	ns
130	ns	ns	ns
137	ns	ns	ns
144	ns	ns	ns
151	ns	ns	ns
158	ns	ns	ns
165	ns	ns	ns
174	ns	ns	ns

Figure 1 Effects of water, the elemental diet or standard liquid diet on gastric emptying in young rats. Curves of ¹³CO₂ excretion (%intake/min) after administration of water and test nutrients. All values are mean ± SD (n= 8).



Time (min)	W vs E	W vs S	E vs S
7	ns	ns	ns
14	*	****	ns
21	****	****	ns
28	****	****	ns
35	****	****	ns
42	****	****	ns
49	**	****	ns
58	ns	**	ns
65	ns	ns	ns
72	ns	ns	ns
79	ns	ns	ns
86	ns	ns	ns
93	ns	*	ns
100	ns	ns	ns
107	ns	ns	ns
116	ns	ns	ns
123	ns	ns	ns
130	ns	ns	ns
137	ns	ns	ns
144	ns	ns	ns
151	ns	ns	ns
158	ns	ns	ns
165	ns	ns	ns
174	ns	ns	ns

Figure 2 Effects of water, the elemental diet or standard liquid diet on gastric emptying in adult rats. Curves of ¹³CO₂ excretion (%intake/min) after administration of water and test nutrients. All values are mean ± SD (n= 6).

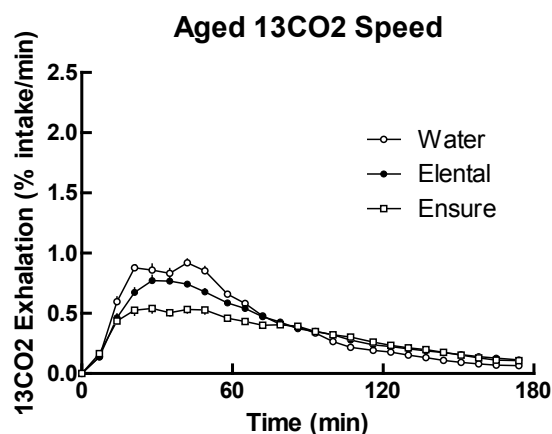


Figure 3 Effects of water, the elemental diet or standard liquid diet on gastric emptying in aged rats. Curves of ^{13}C O $_2$ excretion (%intake/min) after administration of water and test nutrients. All values are mean \pm SD ($n = 8$).

Time (min)	W vs E	W vs S	E vs S
7	ns	ns	ns
14	**	****	ns
21	****	****	****
28	ns	****	****
35	ns	****	****
42	****	****	****
49	****	****	****
58	ns	****	***
65	ns	***	*
72	ns	ns	ns
79	ns	ns	ns
86	ns	ns	ns
93	ns	ns	ns
100	ns	ns	ns
107	ns	ns	ns
116	ns	ns	ns
123	ns	ns	ns
130	ns	ns	ns
137	ns	ns	ns
144	ns	ns	ns
151	ns	ns	ns
158	ns	ns	ns
165	ns	ns	ns
174	ns	ns	ns

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CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests and received no financial support.

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