Muscarinic blockade inhibits gastric emptying of mixed-nutrient meal: effects of weight and gender

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Muscarinic blockade inhibits gastric emptying of mixed-nutrient meal: effects of weight and gender. Am. J. Physiol. 276 (Regulatory Integrative Comp. Physiol. 45): R707–R714, 1999.—We compared the vagal contribution to gastric emptying in lean and obese subjects by monitoring gastric emptying of a meal during muscarinic blockade. Lean (n = 6) and obese subjects (n = 6) underwent two treatments: 1) saline infusion and 2) atropine infusion [0.4 mg/m2 bolus, 0.4 mg·(m2)−1·h−1] for 2 h, initiated 30 min before ingestion of a 600-kcal breakfast (64% carbohydrate, 23% fat, 13% protein) composed of orange juice (labeled with Indium-111), egg sandwich (labeled with Technetium-99m), cereal, milk, and banana. Anterior and posterior images were taken every 90 s for 90 min using a dual-headed camera. Atropine significantly delayed emptying of both solid (P < 0.007) and liquid (P < 0.002). Obese subjects exhibited a greater delay in liquid emptying during muscarinic blockade compared with lean subjects (P < 0.02). Female subjects exhibited a slower rate of gastric emptying and were less sensitive to atropine. These data suggest that obese subjects exhibit altered gastric cholinergic activity compared with lean subjects and that gender differences in gastric emptying rate may be due to differences in autonomic tone.

GASTROINTESTINAL SIGNALS have been proposed as mechanisms regulating satiety and meal termination (32). Changes in the responsiveness of mechanical receptors controlling gastric distension or the altered release of neurotransmitters or gut peptides that activate sensory afferent fibers could ultimately influence rates of gastric emptying, postprandial satiety, and subsequent food intake. Thus abnormalities in gastric processes have been postulated as an underlying cause in the development of obesity (23, 32, 37, 54). In humans, the data demonstrating altered rates of gastric emptying in obese compared with lean individuals are highly contradictory. Although some studies have shown increased rates of gastric emptying in obese subjects (18, 52, 53, 55), others have demonstrated decreased rates (10, 22, 31) or reported no differences between obese and lean subjects (16, 24, 46). Discrepancies between results are most likely a consequence of the different methodologies used for the measurement of gastric emptying and the varied nutrient content, size, and composition of the test meals. However, most experiments have not been designed to reveal hypothesized differences in the physiological mechanisms mediating gastric emptying.

One mechanism that may account for differences in gastric emptying rates between obese and normal-weight individuals is the degree of efferent vagal activity. The vagus nerve plays a critical role in the regulation of gastrointestinal processes and gastric emptying. Vagal afferents innervate the gastrointestinal lumen and the circular smooth muscle fibers of the gastric wall. The afferents receive information concerning the degree of gastric distension, nutrient composition, and size of the ingested meal. Vagal afferents terminate within the central nervous system, ultimately activating vagal efferent fibers that are involved in maintaining gastric tone (43) and the elicitation of gastric and exocrine pancreatic secretions involved in nutrient digestion (40, 53).

Vagal efferent fibers also modulate insulin and glucagon secretion from the endocrine pancreas (1, 8, 33, 57). Increased vagal activity has been observed in a number of animal models of obesity, including rats lesioned in the ventromedial hypothalamus (40), the obese Zucker rat (44), and the ob/ob mouse (17). These animals exhibit hyperinsulinemia and hyperphagia, leading investigators to propose increased parasympathetic nervous system (PNS) activity as a mechanism contributing to obesity (26, 45). The human data supporting increased PNS activity in obesity are, like the data on gastric emptying, highly contradictory. Studies examining the effect of atropine, a muscarinic antagonist, on insulin secretion in obese and lean subjects have reported greater attenuation of insulin release in obese subjects, suggesting increased PNS activity in this population (13, 51). However, because of reported effects of atropine on gastric emptying (3, 9, 12, 49), it has been difficult to determine if the observed decrease in insulin release after atropine is a result of direct inhibition of vagal efferent activity at the level of the islet cell or due to a retention of nutrients in the stomach. The objectives of the present study were 1) to compare differences in the vagal contribution to gastric emptying in lean and obese subjects and 2) to determine the magnitude of effect of atropine on gastric emptying of a mixed-nutrient meal. To achieve these objectives, we compared the effects of saline versus atropine infusion on gastric emptying using a dual-radiolabeled method that allows for the simultaneous measurement of the solid and liquid components of a meal in lean and obese male and female subjects.
METHODS

Subjects. Six normal-weight subjects (3 males and 3 females) aged 20–38 years of age (mean 26.5 ± 7.0 yr) with body mass indexes (BMI) ranging from 19.5 to 23 kg/m² (mean 21.6 ± 1.2 kg/m²) and six obese subjects (3 males and 3 females) aged 18–43 years of age (mean 26.3 ± 10.8 yr) with BMI ranging from 30 to 46 kg/m² (mean 40.6 ± 6.2 kg/m²) participated in this study. After a telephone interview to assess eligibility, subjects underwent a physical examination, including an electrocardiogram and a medical history to ensure they had no chronic illnesses, abnormal heart rhythms, hypertension, or family history of diabetes or hypertension. A blood sample was drawn after an overnight fast. Subjects whose fasting blood glucose was > 90 mg/dl or whose blood pressure was > 140/90 mmHg were excluded from the study. These studies were approved by the Committee on Studies Involving Human Beings at the University of Pennsylvania and the Radiation Committee of the Hospital of the University of Pennsylvania.

Experimental protocol. Each subject underwent two experimental conditions that were administered in a random order over a 1-wk period. The two conditions were 1) saline infusion starting 30 min before food ingestion and 2) atropine infusion (0.4-mg/m² bolus followed by 0.4 mg·(m²)⁻¹·h⁻¹ for 2 h) starting 30 min before food ingestion. The dose of atropine has previously been shown to be effective (48) as indicated by increases in heart rate in the range of 25–30 beats/min.

On the evenings before the experimental days, subjects entered the General Clinical Research Center at 1700. Subjects were given dinner at 1800 and a snack at 2000, after which they remained fasted until the following morning. At 900, an intravenous catheter was inserted into an antecubital vein for infusion of either saline or atropine. At 930, baseline heart rate and blood pressure were taken and then measured every 15 min for the duration of the experiment. After the baseline measurements, the infusion was initiated. Thirty minutes after the infusion, subjects were given a mixed-nutrient meal to ingest within 10 min. Immediately after completing the meal, subjects were asked to recline on the platform of the scintillation counter and remain there for 90 min.

Measurement of gastric emptying. Gastric emptying was measured using a double-isotope scintigraphic technique that allows for simultaneous measurement of the emptying of both the solid and liquid components of the meal. Subjects were given a standardized breakfast that contained 602 kcal, consisting of cornflakes with milk, a banana, a scrambled egg sandwich, and orange juice. The scrambled egg was labeled with 500 µCi of Technetium-99m sulfur colloid. The orange juice was labeled with 100 µCi Indium-111-diethylenetriaminepentaacetic acid. The total macronutrient content was 13% protein, 64% carbohydrate, and 23% fat. Subjects were permitted 10 min to ingest the meal but were asked to drink the orange juice last. Immediately after food ingestion, subjects were positioned in a supine position on the platform of the scintillation counter and remained in place for the duration of the study. Anterior and posterior images were taken every 90 s for a period of 90 min by a dual-headed camera interfaced with a dedicated computer for simultaneous acquisition in two energy channels. Twenty-percent energy windows with peaks set at 140 keV and 243 keV for the Technetium and Indium, respectively, were used.

Data and statistical analysis. Images were corrected for patient movement, and the regions of interest for the anterior and posterior views of the stomach were defined for each individual’s scintigram. Data were corrected for radionuclide decay and Compton scatter. The geometric mean of the decay-corrected anterior and posterior counts at each time interval was calculated to correct for tissue attenuation. Because of the slow rate of gastric emptying of the mixed-nutrient meal under saline condition, which was further delayed by the administration of atropine, it was not considered appropriate to calculate the lag time or half-time of gastric emptying because the extent of extrapolation would be too great. Therefore, gastric emptying is expressed as a percent of maximum counts at time 0 (mean ± SE).

Repeated-measures ANOVA was used to determine if there were significant population or gender × treatment interactions. Post hoc analysis was done using Tukey’s test. In exploring the treatment × weight × gender interactions in which there were only three subjects per group, a paired Student’s t-test was used to determine differences between treatments within a group. A P value of <0.05 was considered statistically significant.

RESULTS

In lean subjects under control conditions (saline infusion), ~55% of the liquid component of the meal was emptied by 90 min postingestion. An almost identical rate of emptying was observed in the obese subjects during saline infusion. In contrast, only 34% of the solid had left the stomach by 90 min in the lean subjects. Obese subjects exhibited a slightly faster rate of solid emptying (45% remaining), but this was not found to be statistically significant (Table 1).

Atropine significantly delayed the rate of liquid emptying in both the lean and obese subjects (Fig. 1; F,7,63 = 21, P < 0.002). In the lean subjects, atropine significantly slowed gastric emptying at 50, 60, 70, 80, and 90 min postingestion compared with the saline condition (P < 0.01 at all time points). After atropine administration, obese subjects exhibited significantly greater retention of liquid at 20, 30, 40, 50, 60, 70, 80, and 90 min postingestion compared with saline (P < 0.001, except for t = 20 min, P < 0.03). Significant population × treatment × time interactions were also found (F,7,63 = 2.4, P < 0.02). Obese subjects exhibited a significantly greater percent of maximum counts from lean subjects at 60, 70, 80, and 90 min postingestion during the atropine condition (P < 0.001 at all time points; Fig. 1A).

Atropine also significantly impaired the emptying of the solid components of the meal in both lean and obese subjects (Fig. 1B; treatment effect, F = 12.6, P < 0.007). In the lean subjects, significant differences between the saline and atropine conditions were only found at the 80- and 90-min postingestion time points (P < 0.03 at both times). In the obese subjects, the percent of Table 1. Percent counts remaining at 90 min postingestion

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<td>Lean</td>
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<td>Obese</td>
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Values are means ± SE; n = 6. *P < 0.02, †P < 0.004 saline vs. atropine; ‡P < 0.02 lean vs. obese.
maximum counts was significantly greater at 50, 60, 70, 80, and 90 min postingestion compared with saline treatment ($P < 0.0001$ at all time points except at 50 min, at which $P < 0.01$; Fig. 1B). Although a significant population $\times$ treatment $\times$ time interaction was found ($F = 2.46, P < 0.02$) for emptying of the solid component of the meal, post hoc analysis did not reveal any significant differences between the obese and lean subjects under either saline or atropine conditions. However, if the individual differences between percent counts at 90 min under the two conditions were compared, lean subjects averaged a $45 \pm 52\%$ difference in counts, whereas subjects exhibited a $82 \pm 58\%$ difference.

Atropine significantly increased heart rate ($F_{10,80} = 46.4; P < 0.00005$) in both the lean and obese subjects, with no significant differences in the magnitude of response between the populations. The mean increase in heart rate over the testing period was $20.9 \pm 2.6$ beats/min for the lean subjects and $21.8 \pm 3.8$ beats/min for the obese subjects, suggesting that the drug was equally vagolytic in both populations.

Because of previous reports of different gastric emptying rates between genders, statistical analysis was done comparing emptying of liquids and solids between the male and female subjects, independent of weight. Using this grouping, highly significant differences were observed between the rates of liquid emptying for male and female subjects (Fig. 2A), but no significant differences in the emptying of the solid component of the meal were found (Fig. 2B). Significant gender $\times$ treatment ($F_{22,8} = 12.2, P < 0.008$) as well as gender $\times$ treatment $\times$ time interactions were found ($F_{22,8} = 2.4, P < 0.02$) for liquid emptying. However, because the original objective of the experiment was to examine differences in emptying rates between lean and obese subjects and because significant differences were found, it was necessary to examine if there were significant weight $\times$ gender interactions for emptying of the liquid component of the meal. When grouped by sex and weight, no significant weight $\times$ gender $\times$ treatment interactions were found.
interaction was found due to the very small number of subjects in each group (n = 3). However, visual examination of the rates of liquid emptying among the four groups revealed some interesting differences (Fig. 3). Emptying of the liquid component of the meal was slower in both the lean (F,36 = 3.97, P < 0.001) and obese females (F,36 = 2.18, P < 0.03) compared with the males when multivariate ANOVA was conducted within weight categories, i.e., comparing lean males to lean females and obese males to obese females. As can be observed in Fig. 3, atropine decreased the rate of liquid emptying in the lean males, obese males, and obese females (significant differences using paired Student’s t-tests at all time points beyond 10 min postingestion except the obese women, whereby the first significant time point was at 50 min postingestion). However, atropine had no effect on liquid emptying in the lean females at any time point. In contrast, atropine did delay solid emptying in all four subgroups of subjects, even the lean females (Fig. 4). To verify that the lack of effect of atropine on liquid emptying was not a result of one discrepant individual, we show in Fig. 5 the rate of liquid emptying of each of the lean females. The mean increase in heart rate for these three individuals shown in the insets verifies that although the atropine administration had no effect on the rate of liquid emptying, the drug was still effective at the level of the heart.

DISCUSSION

The results of the present study suggest that obese individuals exhibit increased vagal efferent activity at the level of the stomach compared with lean subjects. During the saline condition, no statistically significant differences were found between the lean and obese subjects in the gastric emptying of either the solid or liquid component. Obese subjects exhibited a slight trend toward an increased rate of solid emptying. The increased sensitivity of the obese subjects to muscarinic blockade was observed with the emptying of the liquid component of the meal. Considering the slow rate of solid emptying of the mixed-nutrient meal after atropine administration (<50% of maximum counts were emptied by 90 min), greater differences in the rate of solid emptying between the lean and the obese subjects may have been observed if the imaging had taken place over a longer time period. Furthermore, if the subjects had been sitting erect, significant differences in solid emptying may have been expected because posture has been shown to have a greater effect on solid compared with liquid emptying when vagotomized subjects were compared with normals (19). However, because of the effects of atropine on the central nervous system, it was not considered appropriate to have the subjects sitting and standing repeatedly.

To our knowledge, differences in vagal efferent activity at the level of the stomach between lean and obese humans have not been demonstrated. The increased effects of atropine on gastric emptying in the obese subjects may be due to an increase in gastric cholinergic activity. Inasmuch as both genetic and experimental animal models of obesity have been shown to exhibit increased PNS activity at the level of the pancreas (17, 29, 40, 44), increased vagal activity may also be present at the gastric level in human obesity. Because the rates of gastric emptying between the lean and obese subjects were comparable under saline conditions, the altered sensitivity to muscarinic blockade in the obese subjects may be a compensatory response to maintain gastric emptying at a normal rate. The initiating factor

Fig. 3. Gastric emptying of liquid component of meal when subjects are divided by gender and weight. A: lean males; B: obese males; C: lean females; D: obese females. Solid line represents saline infusion, and dashed line represents atropine infusion. Values are means ± SE (n = 3 for each group).
responsible for the hypothesized compensatory response is not known, although increased vagal afferent activity as a result of prolonged gastric distension due to increased food consumption could be one possibility.

The other major finding of the present study is that there is differential sensitivity to the effect of muscarinic blockade on gastric emptying of liquids between male and female subjects. We found that atropine had minimal effects on the emptying of liquids in females compared with the very significant effects observed in the male subjects. The gender differences in gastric emptying rate appear to be primarily due to lean females, who emptied the liquid component of the meal slower than the lean males and, remarkably, were insensitive to atropine administration. Thus, in this very limited group, there is minimal vagal efferent activity contributing to liquid emptying in the lean females (Fig. 3). In contrast, a delay in solid emptying after atropine was observed in all groups, even the lean females (Fig. 4). These data are surprising considering the importance of the vagus nerve in receptive relaxation and accommodation (27) and the known increased rate of liquid emptying after vagotomy (7). Thus there appears to be a differential degree of vagal efferent activity contributing to liquid and solid emptying in lean females.

A decrease in vagal activity may contribute to the previously reported decreased rate of gastric emptying in women (21, 24, 28). Gender differences in gastric emptying rate have been assumed to be a consequence of sex hormones, because menstrual cycle phase (39) and hormone replacement therapy have been shown to influence the rate of gastric emptying. However, Hutson et al. (24) demonstrated that although postmenopausal women emptied liquids more rapidly than premenopausal women or postmenopausal women on hormone replacement therapy, the rate of liquid emptying was still slower compared with men. These data suggest that variables other than sex hormones may contribute to the reported gender differences in gastric emptying rate. In fact, interactions between vagal fibers and female sex hormones have been reported. Subdiaphragmatic vagotomy in rats has been shown to decrease the number of estrogen receptors in the nucleus of the solitary tract, the major area receiving vagal afferent input (14). Furthermore, the gastric vagus has specifically been implicated in suppressing pulsatile luteinizing hormone release during fasting in ovariectomized estradiol-supplemented rats (6). Thus we would hypothesize that autonomic tone, specifically vagal activity, may be one factor that contributes to gender differences in gastric emptying rate. However, the present study did not control for menstrual cycle, and this factor combined with the very limited sample size may have contributed to the differences observed between the lean and obese women. Further research is required to verify if these intriguing preliminary data are representative of a larger population.

Atropine was found to almost completely inhibit the emptying of nutrients from the stomach. Gut peptides, including glucagon-like peptide (38) and cholecystokinin (4, 42) inhibit gastric emptying by activation of vagal afferent fibers. An inhibition of peptidergic mediation of gastric emptying by these compounds should increase the rate of emptying, an effect not observed in this study. Vagal nerve stimulation has been shown to increase gastric blood flow and gastric dilatation of gastric arterioles that could ultimately influence gas-
tric emptying. However, studies demonstrate that these effects are not mediated by muscarinic receptors (34, 56). Thus the effect of atropine on gastric emptying is most likely a direct result of blocking gastric muscarinic receptor availability to released acetylcholine during meal ingestion. Despite numerous reports demonstrating atropine to significantly retard gastric emptying (4, 5, 11, 12, 15, 41, 47), metabolic studies have often used atropine and other cholinergic antagonists as pharmacological tools for assessing cholinergic activity and its role in modulating insulin release (2, 20, 30, 35). In light of the present study that used contemporary radiographic techniques and found the retention of gastric contents by atropine to be very significant, this would suggest that the use of atropine combined with meal ingestion is not appropriate for studies investigating the effects of the vagus nerve on insulin release.

In summary, we have demonstrated that gastric emptying in obese individuals is more sensitive to muscarinic blockade compared with lean individuals, suggesting that there is altered cholinergic activity at the level of the stomach in obese individuals. Because the rates of gastric emptying were similar between the two populations under saline conditions, the increased sensitivity may be a compensatory mechanism to maintain gastric emptying at normal rate. In addition, gender differences in sensitivity to muscarinic blockade are also evident, indicating that women may have less vagal efferent activity contributing to gastric emptying of liquids. This may account for the decreased rates of gastric emptying reported in women. It is postulated that interactions between autonomic tone and female sex hormones may contribute to gender differences in gastric emptying rates, but further research is required to verify this hypothesis.

Perspectives

In a previous study, we investigated the effects of muscarinic blockade on postprandial hormone release after ingestion of a mixed-nutrient meal in lean and obese subjects. The purpose of this study was to determine the contribution of the PNS to the hyperinsulinemia associated with obesity. We found, as in the present study, that obese subjects were more sensitive to muscarinic blockade compared with lean individuals; i.e., we observed a greater inhibition of insulin release in the obese subjects. Because of a concomitant attenuation of plasma glucose levels, we were not able to conclude that the decrease in plasma insulin was due to the direct inhibition of insulin release by muscarinic blockade. Instead, it seemed likely that atropine was delaying gastric emptying. To confirm and extend these findings, we conducted the present study to examine the effect of atropine on gastric emptying of a mixed-nutrient meal using a meal identical in macronutrient composition and caloric content as in the earlier study. The data from the present study demonstrate a highly significant effect of atropine on gastric emptying but also suggest that there is altered vagal efferent activity at the gastric level. It is of interest to note that

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Fig. 5. Gastric emptying of liquid component of meal in 3 lean female subjects (A, B, and C). Solid line represents saline infusion, and dashed line represents atropine infusion. Insets represent mean heart rate averaged over experimental trial during saline (left) and atropine infusion (right). Atropine administration did not significantly influence gastric emptying rate in these 3 subjects.
although there was a greater delay in liquid emptying observed in the obese subjects in the present study, postprandial glucose levels during atropine administration were not significantly different between the two populations in the previous study. Thus, despite the greater delay in gastric emptying, levels of glucose in the peripheral circulation were similar, suggesting that the glucose was not being cleared at the same rate in the obese subjects. Furthermore, it would appear that the observed differences between the lean and obese subjects in the attenuation of postprandial insulin release during muscarinic blockade were independent of the effect of atropine on gastric emptying and were due to differences in vagal efferent activity at the level of the pancreas. Thus our interpretation of the two studies would be that there is altered vagal efferent activity at both the gastric and pancreatic level in obesity.

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MUSCARINIC BLOCKADE INHIBITS GASTRIC EMPTYING


