Early phase insulin infusion and muscarinic blockade in obese and lean subjects

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The objectives of the present study were 1) to determine if preabsorptive insulin release contributes to postprandial glucose regulation, independent of a general activation of the PNS, and 2) to determine if there

Teff, Karen L., and Raymond R. Townsend. Early phase insulin infusion and muscarinic blockade in obese and lean subjects. Am. J. Physiol. 277 (Regulatory Integrative Comp. Physiol. 46): R198–R208, 1999.—The effect of early phase insulin on postprandial levels of insulin, C-peptide, glucose, and glucagon was investigated in lean (n = 10) and obese (n = 12) subjects. Subjects underwent four conditions during ingestion of a meal (600 kcal): 1) saline infusion; 2) 10-min insulin infusion simultaneously with meal ingestion (0.24 U bolus, 15 μU·m⁻²·min⁻¹); 3) atropine infusion (0.4 mg/m² bolus, 0.4 mg·m⁻²·h for 4 h); 4) insulin and atropine infusion. Blood samples were taken for 3.5 h. Insulin infusion had no effect on postprandial insulin levels in either population but significantly reduced postprandial glucose in the obese subjects (P < 0.05). Obese subjects with elevated postprandial glucose levels in the presence of muscarinic blockade exhibited a decline in glucose with insulin supplementation. Atropine reduced postprandial insulin levels in both groups, with a greater attenuation in the obese (P < 0.01), but postprandial glucose levels were also significantly reduced, suggesting that atropine inhibited gastric emptying. Thus the effects of muscarinic blockade on postprandial insulin levels cannot be evaluated. These data suggest that insulin supplementation during the preabsorptive time period may contribute to glucose regulation in the obese population.

Despite much interest in the relationship between the sympathetic nervous system and insulin resistance (34), little attention has been paid to the role of the parasympathetic nervous system (PNS) in human glucose regulation. Data derived from animals suggest that the PNS may contribute to the regulation of glucose metabolism. In vitro, acetylcholine has been shown to potentiate glucose-induced insulin release from pancreatic islets (10, 24–57), whereas in vivo, electrical stimulation of the vagus nerve elicits insulin secretion (4, 19) and alters activity of hepatic enzymes involved in glycogen storage and gluconeogenesis (43).

In humans, experiments investigating the role of the PNS in glucose metabolism report conflicting results. Atropine has been shown to inhibit insulin release in some (5, 15) but not all (23, 41) studies. Discordant results are partially a result of the different routes of nutrient administration. Activation of the PNS occurs at the onset of and during food ingestion. In contrast, intravenous administration of nutrients, which bypasses the oral cavity and digestive tract, does not elicit PNS activity. Thus muscarinic blockade attenuates insulin release after oral glucose ingestion but not during intravenous glucose administration (15, 54). However, atropine significantly delays gastric emptying (49a), and therefore the attenuated insulin release observed after atropine administration may be caused by a combined effect of muscarinic blockade on the stomach as well as the B-cell.

Preabsorptive or cephalic phase insulin release is a vagally mediated response initiated by the presence of food in the oral cavity. The response occurs within the first few minutes of food ingestion, peaking at 4 min and returning to baseline at 10 min before nutrient absorption (51, 52). During intragastric nutrient administration, preabsorptive insulin release does not occur, resulting in postprandial hyperglycemia and hyperinsulinemia compared with nutrients administered orally (33, 45). In an earlier study, this laboratory demonstrated that when intragastric glucose was paired with a modified sham feed, in which subjects tasted, chewed, and expectorated food for a 5-min period, glucose tolerance was improved compared with intragastric glucose alone (50). These data suggest that activation of the PNS by food-related oral sensory stimulation enhances glucose metabolism. However, it was not known whether this effect was caused by a general activation of vagal efferent fibers that could potentially influence a number of different processes, including gastric emptying (37), glucose absorption (48), and hepatic glucose storage (43), or caused specifically by the presence of vagally mediated preabsorptive insulin release (31).

Exaggerated preabsorptive insulin release has been observed in obese humans (18, 36, 44) and animals (31) compared with their lean counterparts. The increase in preabsorptive insulin is thought to be a reflection of increased PNS activity, exhibited in many animal models of obesity (22, 31, 39). However, in humans there is little evidence supporting an increase in PNS activity at the level of the pancreas (24, 41). Furthermore, we (53) and other investigators (46) have suggested that increases in preabsorptive insulin release in obesity are merely a reflection of increased basal insulin levels. Thus the level of PNS activity and its contribution to preabsorptive and postprandial insulin release in human obesity is still unresolved.

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were differential responses to preabsorptive insulin supplementation between lean and obese individuals. To address these questions, we administered exogenous insulin, infused at a concentration and pattern to mimic preabsorptive insulin release, alone and in the presence of atropine, to normal-weight and obese subjects during ingestion of a mixed-nutrient meal.

**RESEARCH DESIGN AND METHODS**

**Subjects.** Ten normal-weight, lean subjects 20–41 yr of age (mean 27.4 ± 6.2 yr) with body mass indexes (BMI) ranging from 19 to 24 kg/m² (mean 21.9 ± 1.3 kg/m²) and 12 obese subjects 18–57 yr of age (mean 33.3 ± 11.3 yr) with BMI ranging from 30 to 51 kg/m² (mean 36.6 ± 7.4 kg/m²) participated in this study (Table 1). 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 mm Hg were excluded from the study. These studies were approved by the Committee on Studies Involving Human Beings at the University of Pennsylvania.

Experimental protocol. Each subject underwent four experimental conditions that were administered in a random order over a 10-day period. The four conditions were 1) saline infusion starting at 30 min before food ingestion, 2) infusion of insulin simultaneously with onset of meal ingestion (0.24 U bolus, followed by 0.4 mg·m⁻²·min⁻¹ for 10 min), 3) atropine infusion (0.4 mg·m⁻² bolus, followed by 0.4 mg·m⁻²·h⁻¹ for 4 h) starting at 30 min before food ingestion, and 4) atropine infusion starting at 30 min before food ingestion followed by insulin infusion simultaneously with onset of meal ingestion in the same dosages as described above. The objective of the insulin infusion was to mimic neurally mediated insulin release, i.e., a small, rapid increase in insulin that occurs preabsorptively, peaks at 4 min poststimulus, and returns to baseline at 10 min poststimulus. When measured in peripheral blood, the neurally mediated insulin response is typically 25–30% above baseline levels. However, to approximate levels in portal blood, we aimed at achieving plasma insulin levels in the range of 50% above baseline. The dose of atropine has previously been shown to be effective (41) 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 0730, two intravenous lines were inserted. One catheter was inserted into an antecubital vein for infusions. A second catheter was inserted retrograde into a dorsal hand vein on the contralateral side and kept patent by the slow infusion of saline. This hand was heated to between 45 and 55°C to arterialize the venous blood. After a 30-min period of acclimatization, three baseline blood samples were taken at 15-min intervals. If the experimental condition involved atropine infusion, this was started immediately after the first blood draw. The subject was then given the standardized breakfast, which contained 601 kcal, consisting of corn flakes with milk, a banana, a grilled cheese sandwich, and orange juice. The total macronutrient content was 13% protein, 65% carbohydrate, and 22% fat. Subjects ingested the breakfast within 15 min. Simultaneously with food ingestion, blood samples were taken every 2 min for 16 min and then at 15-min intervals for 4 h. If the experimental condition involved insulin infusion, this was initiated simultaneously with the onset of meal ingestion. Heart rate was monitored continuously throughout the experiment while blood pressure was measured every 15 min. Blood was collected in tubes containing EDTA. Transthyretin and leupeptin were added, and the samples were kept on ice for not longer than 1 h. Samples were then centrifuged and stored at −70°C for later assay.

Analytic methods. Plasma insulin, C-peptide, and glucagon were measured in duplicate with commercially available double-antibody RIAs purchased from Linco Research (St. Charles, MO). RIAs were performed by the Diabetes Research Center of the University of Pennsylvania. Intra-assay variation for insulin, C-peptide, and glucagon was 4.9%, 2.4%, and 6.8% respectively. The interassay variation was 5.9%, 7.1%, and 8.4% for insulin, C-peptide, and glucagon. Technicians were blind to the conditions of the experiment. Whole blood glucose was monitored immediately after blood withdrawal with a YSI Glucometer (Yellow Springs, OH). In the subset of subjects, the first five normal-weight and five obese subjects participated in the study were analyzed for pancreatic polypeptide (PP) and leptin under saline and atropine conditions. PP and leptin were analyzed by Linco Research (St. Charles, MO).

Statistical analysis. Area under the curve (AUC) was calculated for glucose, insulin, C-peptide, and glucagon with a computerized trapezoidal method (GraphPlot Inplot). Positive and negative AUCs were calculated separately and combined for a net change from baseline values (mean of three baseline values). Repeated-measures ANOVAs were used to determine 1) whether there were significant differences between the two groups, and 2) whether there were significant differences between treatments within groups. When significant group-by-treatment-by-time interactions were found, post hoc analyses were done using a Tukey’s test to determine which treatments were significantly different between and within groups. Independent Student’s t-tests were used to compare baseline values and AUCs after saline infusion between groups. The critical value for significance was P < 0.05.

**RESULTS**

Baseline, preabsorptive, and postprandial levels: saline condition. Subject characteristics of the two popula-
The molar ratio of C-peptide to insulin was not significantly different between the lean and obese subjects \( (F_{1.19} = 1.1, P < 0.32) \), suggesting that hepatic extraction of insulin was not different between the two groups. Postprandial glucagon levels were significantly lower in the obese individuals compared with the normal-weight subjects under control conditions \((654 \pm 2.038\) and \(-1.830 \pm 2.133\) ng·l\(^{-1}\)·245 min\(^{-1}\) for normal-weight and obese subjects, respectively; \(t = 2.78, P < 0.01\); Fig. 4).

Preabsorptive and postprandial periods: muscarinic blockade. Atropine administration significantly blocked insulin and C-peptide release in the preabsorptive time period in lean and obese subjects \((F_{1.20} = 20\) and \(P < 0.002\) for both groups). Glucagon secretion during the preabsorptive time period was also inhibited by atropine in normal-weight subjects \((F_{1.20} = 15, P < 0.001)\).

Atropine administration significantly decreased postprandial plasma insulin levels in both lean and obese subjects \((F_{1.19} = 51, P < 0.00001)\). Mean AUC for insulin measured from 12 to 255 min postingestion decreased from \(42,342 \pm 14,322\) to \(16,554 \pm 12,396\) pmol·l\(^{-1}\)·245 min\(^{-1}\) in the normal-weight subjects. In the obese subjects, mean AUC for insulin decreased from \(111,630 \pm 62,598\) to \(30,666 \pm 6,264\) pmol·l\(^{-1}\)·245 min\(^{-1}\). Significant population-by-treatment interactions were also found \((F_{1.20} = 7.6, P < 0.01)\), indicating that the effect of atropine on insulin secretion was greater in the obese subjects compared with lean subjects. As can be seen from Fig. 1, atropine administration reduced postprandial insulin levels in the obese almost to the range observed in the nonobese subjects. Furthermore, in the obese subjects, unlike in the lean individuals, variability was significantly reduced by atropine administration, suggesting that the large variation in postprandial insulin levels observed in the obese are the result of differences in parasympathetic activity. The effect of atropine on C-peptide paralleled the effects on insulin (Fig. 2). AUCs for C-peptide were significantly reduced in normal-weight \((164.2 \pm 61.2\) to \(48.3 \pm 21.5\) nmol·l\(^{-1}\)·120 min\(^{-1}\)·10 min\(^{-1}\) for saline and atropine, respectively; \(F_{1,20} = 121, P < 0.00001\) and obese subjects \((297.2 \pm 121.5\) to \(109.6 \pm 64.8\) nmol·l\(^{-1}\)·120 min\(^{-1}\)·10 min\(^{-1}\) for saline and atropine, respectively) with a statistically significant population-by-treatment interaction \((F_{1.19} = 7.65, P < 0.01)\). A small increase in the molar ratio of C-peptide to insulin was found after atropine administration \((F_{1.19} = 4.75, P < 0.04)\). However, post hoc analysis revealed that the differences were very minor and only at a few specific time points.

### Table 2. Effect of atropine on basal levels of hormones and glucose

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Insulin, pmol·l(^{-1})·10 min(^{-1})</th>
<th>Glucose, mmol·l(^{-1})·10 min(^{-1})</th>
<th>Glucagon, ng·l(^{-1})·10 min(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline Obese</td>
<td>Baseline Obese</td>
<td>Baseline Obese</td>
</tr>
<tr>
<td>Normal Weight</td>
<td>82.8 ± 34.2 80.4 ± 33.6</td>
<td>116.4 ± 49.4 108 ± 58.8</td>
<td>5.8 ± 1.3 5.8 ± 1.3</td>
</tr>
<tr>
<td>Obese</td>
<td>0.34 ± 0.01 0.33 ± 0.09</td>
<td>0.52 ± 0.20 0.53 ± 0.31</td>
<td>7.4 ± 1.7 5.8 ± 1.3</td>
</tr>
<tr>
<td>C-peptide</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal Weight</td>
<td>4.3 ± 0.5 4.2 ± 3.9</td>
<td>4.5 ± 0.5 4.4 ± 3.7</td>
<td>12.5 ± 6.6 9.6 ± 2.9</td>
</tr>
<tr>
<td>Obese</td>
<td>53.4 ± 18.5 46.7 ± 15.9</td>
<td>71.8 ± 21.4 65.6 ± 19.7</td>
<td></td>
</tr>
<tr>
<td>Pepticoly peptide</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal Weight</td>
<td>7.4 ± 1.7 5.8 ± 1.3</td>
<td>12.5 ± 6.6 9.6 ± 2.9</td>
<td></td>
</tr>
<tr>
<td>Obese</td>
<td></td>
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</tr>
</tbody>
</table>

Values are means ± SD. Normal weight, \(n = 10\); obese, \(n = 11\), except for pancreatic polypeptide, where \(n = 5\) for both groups. *\(P < 0.05\); †\(P < 0.01\).

The first 10 min after meal ingestion were considered the preabsorptive time period. Hormones released during this period cannot accurately be labeled as neurally mediated or cephalic phase hormones because subjects were ingesting food at this time, and it is possible that insulin secretion may have been nutrient-stimulated. However, plasma glucose levels were not significantly elevated until 14 min postingestion, and therefore it is highly likely that hormone secretion during the first 10 min was caused by neural release. AUC for preabsorptive insulin release was similar in the normal-weight and obese subjects (Table 3), as were C-peptide values (normal, \(0.40 \pm 0.60\) nmol/l; obese, \(0.65 \pm 0.78\) nmol/l). Preabsorptive glucagon AUC was \(46.0 \pm 55.4\) ng·l\(^{-1}\)·10 min\(^{-1}\) in the normal-weight subjects and \(77.9 \pm 103.6\) ng·l\(^{-1}\)·10 min\(^{-1}\) in the obese individuals, but these values were not significantly different (Table 3).

Postprandial insulin \((t = -3.4, P < 0.002\); Fig. 1) and C-peptide AUCs \((t = -3.14, P < 0.005\); Fig. 2) were significantly greater in the obese subjects compared with normal-weight individuals, but postprandial glucose levels were similar in the two populations (Fig. 3).
No significant population-by-treatment interaction was found.

Glucose levels were also significantly attenuated by muscarinic blockade ($F_{1,20} = 32, P < 0.00001$; Fig. 3). Glucose AUCs were decreased from $172.4 \pm 102.2$ to $47.4 \pm 76.3$ mmol·l$^{-1}$·245 min$^{-1}$ in the normal-weight subjects and from $228.2 \pm 100.2$ to $91.1 \pm 133.7$ mmol·l$^{-1}$·245 min$^{-1}$ in the obese. No significant differences in the degree of attenuation by muscarinic blockade between the normal-weight and obese subjects were found.

Glucagon levels were significantly reduced by muscarinic blockade in the normal-weight subjects (654 $\pm$ 2,038 to 2,172$\pm$1,724 $\pm$ 1,126 ng·l$^{-1}$·245 min$^{-1}$ for saline and atropine, respectively; $P < 0.006$). However, in the obese subjects, although the mean AUCs decreased from $1,830 \pm 2,133$ to $2,494 \pm 1,067$ ng·l$^{-1}$·245 min$^{-1}$, post hoc analysis revealed that this decline was not statistically significant. As can be observed in Fig. 4, plasma glucagon levels after meal ingestion under saline conditions were significantly lower in the obese compared with the normal-weight subjects ($654.7 \pm 2,038.8$ vs. $1,830.9 \pm 2,133.4$ ng·l$^{-1}$·245 min$^{-1}$; $t = 2.8, P < 0.01$). Thus although muscarinic blockade still decreased plasma glucagon levels in the obese subjects, the degree of attenuation was significantly less in the obese compared with the normal-weight subjects.

Atropine administration during ingestion of the mixed-nutrient meal completely suppressed PP levels compared with the saline infusion in the subset of lean and obese subjects ($F_{1.8} = 9.6, P < 0.01$). The efficacy of the atropine dose in blocking postsynaptic muscarinic receptors was evident from the total inhibition of PP secretion during ingestion of the mixed-nutrient meal in the subset of both obese and normal-weight subjects (Fig. 5). PP, secreted by the F-cells of the pancreas, is mediated almost exclusively by vagal efferent fibers and is a sensitive and specific index of vagal activation (42). Thus inhibition of PP secretion by the dose of atropine administered in this study suggests there was complete blockade of pancreatic muscarinic receptors and that there was relatively equivalent efficacy of the
muscarinic blockade at the pancreatic level in both populations. Atropine had no effect on plasma leptin levels in either the obese or normal-weight subjects (data not shown).

Heart rate increased by \( \sim 25 \) beats/min after atropine infusion in both the normal-weight and obese subjects (\( F_{1,16} = 39, P < 0.00002 \)), indicating PNS blockade at the level of the heart as well as the pancreas. Blood pressure was significantly increased by atropine, but this effect was primarily a result of the greater increase in the normal-weight subjects. A significant population-by-treatment interaction was found (\( F_{1,16} = 5, P < 0.04 \)). In the obese subjects, blood pressure was not significantly increased compared with the saline condition.

**Preabsorptive insulin supplementation:** with and without muscarinic blockade. Insulin infusion during the preabsorptive time period (0–10 min postingestion) significantly increased plasma insulin levels during this time period (Table 2). Peak levels occurred at 2 (insulin alone) or 4 min (insulin plus atropine) postinfusion and by 10 min postinfusion had returned to baseline levels. During the experimental condition in which insulin alone was infused, the preabsorptive insulin AUC is a result of both exogenous insulin infusion and endogenous insulin release. Therefore, to correctly assess the magnitude of exogenous insulin infusion, one has to compare the experimental condition in which both atropine and insulin were infused to the saline condition, thereby comparing exogenous insulin infusion in the presence of muscarinic blockade with endogenous preabsorptive insulin release. In normal-weight subjects, the preabsorptive insulin AUC during the saline condition was \( 297 \pm 346 \) pmol·l\(^{-1}\)·10 min\(^{-1} \) compared with \( 500.5 \pm 423.6 \) pmol·l\(^{-1}\)·10 min\(^{-1} \) during the atropine and insulin infusion. However, no statistically significant difference was found between the two areas. In obese subjects, preabsorptive insulin AUCs were \( 469.0 \pm 139.9 \) pmol·l\(^{-1}\)·10 min\(^{-1} \) after saline infusion and \( 561.8 \pm 206.4 \) pmol·l\(^{-1}\)·10 min\(^{-1} \) after insulin infusion. No significant differences were found in the magnitude of plasma insulin after insulin infusion between the normal-weight and obese subjects. Our initial objective was to achieve an increase in
plasma insulin ~50% above baseline to mimic a profile of plasma insulin representative of preabsorptive release. This objective was achieved with respect to the normal-weight subjects but was underestimated in the obese subjects. Interestingly, greater effects of preabsorptive insulin infusion on postprandial glucose levels were observed in the obese subjects. No significant decreases were observed in plasma glucose during this time period in either population.

Infusion of insulin during the preabsorptive period had no effect on postprandial insulin levels in either the normal-weight or obese subjects. In contrast, preabsorptive insulin infusion significantly reduced postprandial glucose levels in the obese subjects but not in the normal-weight individuals (population-by-insulin interaction, $P_{1,20} = 4.0$, $P < 0.05$). Postprandial glucose AUCs in the obese subjects were reduced by 34% ($228.2 \pm 100.2$ vs. $148.7 \pm 86.9$ mmol·l$^{-1}$·245 min$^{-1}$, saline vs. insulin infusion; $P < 0.05$). The pairing of insulin infusion with atropine was not found to be statistically different from atropine alone, although there was a trend toward an attenuation ($91.1 \pm 133.7$ vs. $37.1 \pm 73$. mmol·l$^{-1}$·245 min$^{-1}$, atropine vs. atropine plus insulin; $P < 0.07$).

**DISCUSSION**

The objectives of this study were twofold: one was to determine if the presence of insulin during the preabsorptive time period contributes to glucoregulation independent of a general activation of the PNS, and the second was to determine if there were differential responses to preabsorptive insulin supplementation and muscarinic blockade between obese and lean individuals. To address these objectives, we compared the plasma insulin, C-peptide, glucagon, and glucose profiles in lean and obese subjects after ingestion of a mixed-nutrient meal when insulin was administered during the early phase time period, either alone or paired with muscarinic blockade.

Insulin administered in a concentration and temporal pattern that mimics preabsorptive insulin release...
had no effect on postprandial levels of either glucose or insulin in normal-weight individuals. In contrast, obese individuals exhibited a decrease in postprandial glucose AUC when the insulin was administered alone. When insulin was administered in conjunction with atropine, there was a trend toward a decline, although this effect was not statistically significant. As can be observed in Fig. 6, preabsorptive insulin supplementation decreased glucose AUC in 9 of 12 obese subjects when administered alone (Fig. 6, top). In the presence of muscarinic blockade, 9 of 12 subjects also exhibited a decline in glucose AUC (six of the same subjects; Fig. 6, bottom). In the majority of these subjects, postprandial glucose levels were already dramatically attenuated and further decreases by insulin supplementation were minimal. Of significance, however, is that in the four subjects whose postprandial glucose levels remained elevated despite muscarinic blockade, all four of these subjects exhibited a substantial decrease in postprandial glucose after preabsorptive insulin supplementation. Thus although the combined treatment (atropine plus insulin) was not found to be statistically significant from atropine alone, the data suggest that in those individuals who are less responsive to muscarinic blockade, insulin supplementation is still effective.

Originally, we had predicted that insulin supplementation alone would have no effect on glucose or insulin levels in the lean subjects, as these subjects were expected to have adequate preabsorptive insulin release and therefore maximal rates of glucose uptake as a result of adequate insulin levels. An improvement of glucose tolerance was expected in the obese as a result of a hypothesized attenuated preabsorptive insulin response in this population. Earlier we had found a trend toward attenuated preabsorptive insulin release in the obese, with a negative correlation between BMI and preabsorptive insulin AUC (53). Although this hypothesis was substantiated by the present findings, the results are somewhat surprising considering that some degree of insulin resistance would be expected in the obese subjects, rendering them less sensitive to...
exogenous insulin administration. One possible explanation is that the obese have impaired early insulin release, and preabsorptive insulin supplementation provided an increase in insulin during a time period critical for glucoregulation.

Impaired early phase insulin release has been demonstrated in obese and nonobese subjects with impaired glucose tolerance. Mitrakou et al. (25) have shown that impaired glucose tolerance arises from decreased early insulin release and impaired suppression of glucagon secretion, resulting in a greater delivery of glucose into the peripheral circulation. Other studies have also suggested that early phase insulin release may be critical in the regulation of glucose metabolism (47).

Inhibition of early insulin release by somatostatin infusion results in hyperglycemia and hyperinsulinemia in normal volunteers (9). Conversely, supplementation of insulin coinciding with meal ingestion has been shown to improve glucose tolerance in insulin-dependent (21) and -independent diabetics (8) compared with the effect of insulin infusion during a later time period. Although these studies imply that the temporal patterning of insulin release, i.e., the presence of insulin during the early phase period, is critical in controlling postprandial glucose levels, they do not provide evidence for impaired early phase insulin in glucose-tolerant obese individuals. However, because glucose tolerance was not evaluated, we do not know if the obese subjects had decreased early insulin secretion or higher glucose levels during the early phase time period, variables that cannot be properly evaluated under conditions of mixed-nutrient ingestion.

Postprandial levels of plasma insulin were found to be markedly attenuated by atropine in both the lean and obese subjects. The magnitude of inhibition of insulin release was significantly greater than that previously reported (5, 15) and suggested that the effect of muscarinic blockade on plasma insulin was the result of multiple factors and not solely of a direct inhibition on insulin release. One contributing factor may have been the inhibition of the insulin secretagogue, glucagon-like peptide-1, which was recently shown to be under direct cholinergic control (3) and has significant effects on gastric emptying (29). Although vagal nerve stimulation has been shown to increase gastric blood flow and gastric dilatation to gastric arterioles, this effect is not mediated by muscarinic receptors (27, 56) and is unlikely to play a role in the effects observed in the present study. The concomitant decrease in glucose levels observed in this experiment, in contrast with earlier reports in which plasma glucose was unaltered (15) or only slightly lowered (5, 28, 32), indicates that atropine was lowering plasma glucose levels by delaying gastric emptying (20) or inhibiting intestinal glucose absorption (48). In fact, in a recent study, we found that atropine dramatically delayed gastric emptying of a meal, identical in macronutrient content as administered in this study (49a). Thus we cannot interpret the extent of the effects of muscarinic blockade on insulin release.

Two findings independent of the confounding effect of gastric emptying are worth noting. First, the large variability in postprandial insulin levels observed in obese subjects was substantially reduced by atropine, indicating that differences in PNS activity (whether at the level of the pancreas or the stomach) contribute to the variability in postprandial insulin levels observed between obese individuals. Second, muscarinic blockade had no effect on basal insulin levels in either the lean or obese subjects. This finding argues against the hypothesis that increased parasympathetic tone contributes to the hyperinsulinemia of obesity (17, 38). In contrast, atropine significantly decreased basal glucagon levels in both the lean and normal-weight subjects, supporting findings of other investigators that show that muscarinic antagonists (6) or agonists (7, 14) can alter plasma glucagon levels under basal conditions. Thus the role of the PNS in the regulation of plasma glucagon appears to be substantially different from that of insulin.

Obese subjects exhibited significantly lower plasma glucagon levels after ingestion of the mixed-nutrient meal compared with the normal-weight subjects. In fact, the glucagon profile in the obese subjects under saline conditions resembled that of the lean subjects under muscarinic blockade. The attenuated glucagon response in the obese subjects may be caused by the inhibitory effect of postprandial hyperinsulinemia (40). However, postprandial insulin levels in the obese subjects were not elevated relative to basal (data not
shown), suggesting that the absolute hyperinsulinemia was compensatory to insulin resistance. In the obese, the decrease in plasma glucagon levels during the preabsorptive and postprandial time periods after muscarinic blockade was not statistically significant. The inability of plasma glucagon to reach baseline levels and the decreased sensitivity of muscarinic blockade in the obese suggests that the alpha cell in obese individuals may be unresponsive to PNS activation during food ingestion.

In summary, we have demonstrated that supplementation of insulin during the preabsorptive period lowers postprandial glucose levels in obese subjects independently of changes in plasma insulin. Muscarinic blockade significantly reduced postprandial insulin levels in both normal-weight and obese individuals, with greater attenuation in the obese subjects. However, concomitant reductions in plasma glucose suggest confounding effects of atropine on gastric emptying and indicate that atropine is not an appropriate agent for assessing the effects of muscarinic blockade on hormonal release during food ingestion. In conclusion, these data suggest that obese individuals may release inadequate insulin during the preabsorptive period. Future studies should compare different times of insulin supplementation to determine if a critical window exists for insulin supplementation to improve glucose tolerance and evaluate the effects of preabsorptive insulin supplementation in glucose-intolerant individuals.

Perspectives

The present experiment leaves two important questions unanswered. One concerns the potential mechanism by which preabsorptive insulin release contributes to postprandial glucose regulation. In this study, we administered insulin peripherally during a time period in which preabsorptive insulin is released. However, under normal physiological conditions preabsorptive insulin is released into the portal vein, and therefore the liver would be the tissue receiving the highest concentration of insulin over a brief time period. The importance of portal nutrient delivery in the regulation of hepatic glucose uptake has been extensively investigated (1, 12, 16, 26, 30).

Recently, neural factors, particularly acetylcholine, have been proposed as key regulators of hepatic glucose metabolism (2, 11). Furthermore, a role for acetylcholine in peripheral glucose metabolism is suggested by experiments demonstrating that sectioning the anterior hepatic nerve plexus or the administration of intraportal atropine leads to insulin resistance with a decrease in glucose tolerance (35), possibly caused by a decrease in net hepatic glucose uptake. In the only human experiment to address this question, Boyle et al. (7) showed that hepatic glucose production was reduced by 25% in human subjects when betahanechol was administered during an islet clamp with somatostatin.

At the onset of food ingestion, the PNS is activated, eliciting acetylcholine release at multiple tissue sites, including the pancreas and the liver. Vagal efferent activity at the level of the pancreas stimulates neurally mediated insulin and glucagon release, which results in significant increases of both hormones in the portal vein. We would hypothesize that the combined insulin-acetylcholine signal is involved in the regulation of hepatic glucose metabolism, whereas the increased glucagon release maintains plasma glucose levels that would otherwise drop as a result of the increase in insulin. Furthermore, the preabsorptive insulin rise may decrease free fatty acid levels, which in turn may effect hepatic glucose output (35). Thus we would propose that the physiological significance of neurally mediated insulin release is that, in combination with acetylcholine, it is part of the portal signal that contributes to the regulation of glucose metabolism and that this signal must occur before nutrient absorption. Experiments investigating the time course of the portal signal demonstrate that the maximal rate of net hepatic glucose uptake occurs by 15 min, a period of time consistent with preabsorptive insulin release (30).

The other question, which was not addressed by the experimental design of this study, concerns the magnitude of contribution of the PNS to the hyperinsulinemia of obesity. In the present study, obese subjects exhibited a significantly greater attenuation of insulin release by atropine compared with lean subjects. Ostensibly, these data would suggest increased PNS-mediated insulin release in response to food ingestion in the obese, although this conclusion cannot be drawn because of the effect of atropine on gastric emptying.

Animal models of obesity, such as ventromedial hypothalamus-lesioned rats, Zucker rats (17, 38), and ob/ob mice (14) exhibit hyperinsulinemia that can be attenuated by atropine or vagotomy, suggesting mediation by the PNS. However, in humans, some studies show no differential effects of muscarinic blockade or activation on postprandial insulin levels between obese and normal-weight subjects (23, 41), whereas others report greater sensitivity in the obese (5, 13, 49). The inability to reconcile this issue lies in a number of factors: the inherent difference in the magnitude of insulin release in response to a stimulus between lean and obese subjects, which renders interpretation of the findings difficult, and the confounding effect of nonspecific muscarinic agents on gastric emptying. In fact, we have recently demonstrated that atropine has a dramatic effect on gastric emptying. We also found that obese subjects were more sensitive to the effects of muscarinic blockade, suggesting the possibility of increased vagal efferent activity at the level of the stomach in obese individuals (49a). However, the contribution of increased vagal efferent activity in the hyperinsulinemia of human obesity remains to be determined.

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