Effect of hyperglycemia on triggering of transient lower esophageal sphincter relaxations

Qing Zhang,1 Michael Horowitz,2 Rachael Rigda,1 Christopher Rayner,1 Andrew Worynski,1 and Richard H. Holloway1,2

1Department of Gastroenterology, Hepatology and General Medicine, Royal Adelaide Hospital; and 2Department of Medicine, University of Adelaide, Adelaide 5000, South Australia, Australia

Submitted 4 September 2003; accepted in final form 17 December 2003

Zhang, Qing, Michael Horowitz, Rachael Rigda, Christopher Rayner, Andrew Worynski, and Richard H. Holloway. Effect of hyperglycemia on triggering of transient lower esophageal sphincter relaxations. Am J Physiol Gastrointest Liver Physiol 286: G797–G803, 2004; 10.1152/ajpgi.00383.2003.—Acute changes in blood glucose concentration have major effects on gastrointestinal motor function. Patients with diabetes mellitus have an increased prevalence of gastroesophageal reflux. Transient lower esophageal sphincter (LES) relaxation (TLESR) is the most common sphincter mechanism underlying reflux. The aim of this study was to investigate the effect of acute hyperglycemia on triggering of TLESRs evoked by gastric distension in healthy volunteers. TLESRs were stimulated by pressure-controlled and volume-controlled (500 ml) gastric distension using an electronic barostat and performed on separate days. On each day, esophageal manometry was performed in the sitting position during gastric distension for 1 h under euglycemia (5 mM), and either marked hyperglycemia (15 mM) or physiological hyperglycemia (8 mM) in randomized order was maintained by a glucose clamp. Marked hyperglycemia doubled the rate of TLESRs in response to both pressure-controlled [5 (3–10.5, median or interquartile range) to 10 (9.5–14.5) per hour, P < 0.02] and volume-controlled [4 (2.5–7.5) to 10.5 (7–12.5) per hour, P < 0.02] gastric distension but had no effect on basal LES pressure. Physiological hyperglycemia had no effect on the triggering of TLESRs or basal LES pressure. In healthy human subjects, marked hyperglycemia increases the rate of TLESRs. Increase in the rate of TLESRs is independent of proximal gastric wall tension. Mechanisms underlying the effect remain to be determined. Hyperglycemia may be an important factor contributing to the increased esophageal acid exposure in patients with diabetes mellitus.

diabetes mellitus; esophageal motility; gastroesophageal reflux disease

ACUTE CHANGES IN BLOOD GLUCOSE concentration have major effects on gastrointestinal motor function in both normal subjects and patients with diabetes mellitus (49). Marked hyperglycemia (∼15 mM) affects motility in the esophagus (13), stomach (21, 25), gallbladder (14), small intestine (4), colon (56), and anorectum (11). In normal subjects, hyperglycemia has been reported to decrease basal lower esophageal sphincter (LES) pressure and to increase the duration but decrease the velocity of esophageal peristaltic pressure waves (13) as well as increasing the perception of sensations arising from the esophagus (50).

Blood glucose concentration may also serve as a physiological modulator of gastrointestinal motor and sensory functions (49). Increases in blood glucose concentration within the normal postprandial range (8 mM) slow gastric emptying of both solids and liquids (54), and we have shown that such changes in blood glucose concentration also affect esophageal motility and sensation (6).

Esophageal motor dysfunction (12, 26, 42, 45) and esophageal symptoms (55) occur frequently in patients with diabetes mellitus. Abnormalities of peristalsis are common (32). Basal LES pressure has been reported to be normal (26, 35, 45) and decreased (57), but information on transient LES relaxations (TLESRs) is lacking. Abnormalities in esophageal function have been proposed to be a result of irreversible vagal damage (19, 52). However, these studies, in which autonomic nerve function has been formally evaluated, have failed to establish a close relationship between autonomic neuropathy and abnormal esophageal motility (31, 33–35). Furthermore, studies of esophageal motility in patients with diabetes have been performed either without blood glucose monitoring or during hyperglycemia. A role for hyperglycemia in the etiology of upper gastrointestinal symptoms in patients with diabetes mellitus is supported by cross-sectional epidemiological studies (7, 8).

Asymptomatic patients with diabetes mellitus have also been reported to have an increased prevalence of abnormal gastroesophageal reflux (41) and excessive esophageal acid exposure (45). Mechanisms underlying these have not been established. TLESRs are the most common sphincter mechanism underlying gastroesophageal reflux in normal subjects and in the majority of patients with reflux disease (44). The major stimulus for triggering TLESRs is gastric distension acting through vagal pathways that are integrated in the brain stem. Hyperglycemia suppresses vagal activity as assessed by a fall in the secretion of pancreatic polypeptide (13) and alterations in cardiovascular reflexes (61). The effect of hyperglycemia on triggering of TLESRs, however, has not been evaluated. The purpose of this study, therefore, was to investigate the effect of both marked and physiological hyperglycemia on triggering of TLESRs evoked by gastric distension in healthy volunteers.

MATERIALS AND METHODS

Subjects. Studies were done in 15 healthy subjects (10 men, 5 women; aged 18–48 yr; median, 24 yr) with a mean body mass index of 24.2 ± 1.0 kg/m². Subjects underwent pressure-controlled or volume-controlled gastric distension during marked hyperglycemia (15 mM) and volume-controlled distension during physiological hyperglycemia (8 mM) All subjects were free of gastrointestinal symptoms and were nonsmokers; none had a history of upper gastrointestinal surgery or was taking medication known to affect gastrointestinal
motility. Each volunteer gave written informed consent, and the study protocol was approved by the Research Ethics Committee of the Royal Adelaide Hospital.

Study protocols. Three types of study were performed 1) pressure-controlled gastric distension during marked hyperglycemia (n = 8), 2) volume-controlled gastric distension during marked hyperglycemia (n = 8), and 3) volume-controlled distension during physiological hyperglycemia (n = 8). Euglycemia (5 mM) was used as the control. Effects of euglycemia and hyperglycemia were assessed in a single-blind fashion on the same day in a randomized sequence. In those subjects who underwent both pressure-controlled and volume-controlled distension, these studies were performed on separate days at least 1 wk apart. In subjects who underwent assessments of both marked and physiological hyperglycemia, these studies were also performed on separate days at least 1 wk apart.

On each study day, subjects were studied after an overnight fast. The manometric assembly with the barostat bag folded around it was passed via an anesthetized nostril into the proximal stomach. The bag was then deflated, and the manometric assembly was positioned so that the sleeve sensor straddled the LES, thereby positioning the barostat bag in the proximal stomach. Subjects were allowed to accommodate to the assembly for 15 min. All studies were performed with the subjects sitting upright in an ergonomic chair, designed to minimize abdominal compression. Intravenous cannulae were inserted into an antecubital vein in each arm, one for administration of glucose or normal saline and the other for obtaining blood samples.

Minimal distending pressure (MDP), defined as the lowest intrabag pressure at which the intrabag volume was 30 ml or more, was determined by increasing the intrabag pressure with stepwise increments of 1 mmHg, each sustained for 60 s (46). In pressure-controlled studies, the distending pressure required to achieve a bag volume of 500 ml was then determined by increasing the bag volume to 500 ml in 100-ml increments, each over 60 s. The pressure detected when intrabag volume was 500 ml was accepted as the target pressure for both euglycemia and hyperglycemia studies in the same subject. Intravenous glucose or saline was then given in randomized order. After the glucose concentration was stabilized at the desired level for at least 30 min, the stomach was distended, and the distension was maintained for 60 min. To achieve pressure-controlled distension, bag pressure was increased stepwise in 2-mmHg increments, each for 60 s, from 0 mmHg to the target pressure obtained from 500-ml volume distension. To achieve volume-controlled distension, intrabag volume was increased stepwise from 0 to 500 ml in 100-ml increments, each for 60 s. Esophageal motility, intrabag pressure, and volume were recorded during gastric distension, and sensations were assessed at 15-min intervals as described below. After 60 min of gastric distension, the bag was deflated completely, the infusion was stopped, and the sleeve sensor. The cylinder introduced or withdrew air from the distending pressure required to achieve a bag volume of 30 ml or more, was determined by increasing the intrabag pressure with stepwise increments of 1 mmHg, each sustained for 60 s (46). In pressure-controlled studies, the distending pressure required to achieve a bag volume of 500 ml was then determined by increasing the bag volume to 500 ml in 100-ml increments, each over 60 s. The pressure detected when intrabag volume was 500 ml was accepted as the target pressure for both euglycemia and hyperglycemia studies in the same subject. Intravenous glucose or saline was then given in randomized order. After the glucose concentration was stabilized at the desired level for at least 30 min, the stomach was distended, and the distension was maintained for 60 min. To achieve pressure-controlled distension, bag pressure was increased stepwise in 2-mmHg increments, each for 60 s, from 0 mmHg to the target pressure obtained from 500-ml volume distension. To achieve volume-controlled distension, intrabag volume was increased stepwise from 0 to 500 ml in 100-ml increments, each for 60 s. Esophageal motility, intrabag pressure, and volume were recorded during gastric distension, and sensations were assessed at 15-min intervals as described below. After 60 min of gastric distension, the bag was deflated completely, the infusion was stopped, and the subjects were allowed to move for a few minutes while the monitoring of the blood glucose concentration was continued. The blood glucose concentration was then restabilized at the alternative level, and gastric distension was repeated. If the hyperglycemic phase was randomized to be the first, the blood glucose level was required to be stable at 5 mM for a minimum of 30 min before the start of the euglycemic phase.

Recording methods. Esophageal motility was recorded with a multilumen manometric assembly, made from a 4.2-mm-diameter silicone rubber extrusion that incorporated a 6-cm sleeve sensor (Dentsleeve, Wayville, SA, Australia) (40). The sleeve sensor monitored LES pressure. Side holes spaced at 3-cm intervals recorded pressures from seven sites along the esophageal body starting at 2 cm above the proximal margin of the LES. A side hole in the pharynx recorded swallowing, and three side holes below the sleeve sensor at 1-cm intervals recorded gastric pressure. The catheter was fixed and maintained in a position so that the midposition of the sleeve sensor was located within the LES. All the side holes were perfused with degassed, distilled water at 0.15 ml/min, and the sleeve sensor was perfused at 0.6 ml/min by a low-compliance pneumohydraulic capillary infusion system. Pressures were sensed by external pressure transducers with output to a multichannel recording system.

Proximal gastric function was measured with an electronic barostat (Distender Series II; G&J Electronics, Willowdale, ON, Canada) that consisted of a rigid cylinder, which introduced or withdrew air from a polyethylene bag positioned in the proximal stomach. Lumina used by the barostat for delivery of air and sensing of pressure within the bag were incorporated within the manometric assembly. The polyethylene bag had a capacity of ~1,100 ml. The proximal portion of the polyethylene bag was tied to the manometric assembly 5 cm distal to the sleeve sensor. The cylinder introduced or withdrew air from the bag at 30 ml/s via an oval channel measuring 1.9 × 2.4 mm internal diameter and 1,570 mm length. Pressure in the bag was sensed via a lumen of 0.6 mm internal diameter that opened directly into the bag.

Data from the pressure transducers and barostat were recorded on a personal computer (model PowerPC 7100; Apple Computer, Cupertino, CA). Manometric data were digitized at 10 Hz using a NBM016 A-D board (National Instruments, Austin, TX). Barostat data were acquired at 1 Hz via a serial interface. A custom-written program (G. Hebbard, Royal Melbourne Hospital, Melbourne, NSW, Australia) using Labview (National Instruments) controlled the barostat and acquired both manometric and barostat data. Data were subsequently imported into Acqknowledge (Biopac Systems, Santa Barbara, CA) for analysis.

Stabilization of blood glucose. The desired blood glucose concentration was achieved and maintained by using a modified glucose clamp method (24). In studies conducted during hyperglycemia, the blood glucose level was increased by an intravenous bolus infusion of 25% glucose. After the bolus infusion, 25% glucose was infused at varying rates guided by blood glucose measurements every 5–10 min by using a portable blood glucose meter (Companion II Glucometer; MediSence, Waltham, MA). Blood glucose levels were maintained within ±10% of the desired level. In studies conducted during euglycemia, an intravenous bolus infusion of normal saline calculated from the body weight was given, followed by a maintenance infusion. In the studies conducted during marked hyperglycemia, including the control, venous blood samples were obtained for measurement of pancreatic polypeptide immediately before and at 20-min intervals after the start of gastric distension. Blood samples were collected into tubes chilled on ice and centrifuged, and plasma was stored at −70°C until assayed.

Assessment of symptoms and sensations. Upper gastrointestinal symptoms were assessed by questionnaires. Sensations of abdominal bloating, abdominal discomfort, fullness, nausea, hunger, and desire to eat were measured by using a 100-mm visual analog scale 15 min before and at 15-min intervals after the start of gastric distension (47). Data analysis. Basal LES pressure was measured at end expiration relative to gastric pressure. Mean LES pressure was calculated for each 5-min period during gastric distension for each subject as well as an overall grand mean for the entire 60-min period. TLESRs were defined and counted separately for each subject over the 60-min gastric distension according to established criteria (30).

Bag volume in pressure-controlled distension and intrabag pressure in volume-controlled distension were measured by averaging the recordings obtained during the last minute of 5-min intervals during the gastric distension. Measured volume was corrected for the effects of air compressibility using an experimentally derived constant that also included a component related to internal compliance of the barostat unit. Pressures were expressed as millimeters of mercury above MDP.

Plasma levels of pancreatic polypeptide were measured at 0, 20, 40, and 60 min after the start of the distension by radioimmunoassay. Data from the four measurement points were averaged for each subject.

Statistical analysis. Numbers of TLESRs were compared by using Wilcoxon signed-rank test and expressed as median (interquartile
range). All the other data are presented as means ± SE. Basal LES pressure, intrabag pressure and volume, pancreatic polypeptide levels, and sensation scores were compared by using repeated-measures ANOVA (SuperAnova; Abacus Concepts, Berkeley, CA). Basal LES pressures during pressure-controlled and volume-controlled distensions were analyzed by using the paired Student’s t-test. A P value of <0.05 was accepted as indicating statistical significance.

RESULTS

The study protocol was well tolerated by all subjects. Blood glucose levels closely approximated the desired levels in all experiments.

Effects of marked hyperglycemia. Basal LES pressure remained stable during the period of distension. Overall mean basal LES pressure during hyperglycemia was similar to that during euglycemia for both pressure-controlled (12.9 ± 4.1 vs. 15.7 ± 5.7 mmHg) and volume-controlled distensions (8.0 ± 1.3 vs. 9.2 ± 1.7 mmHg) (Fig. 1).

Marked hyperglycemia increased the rate of TLESRs in all subjects during both pressure-controlled and volume-controlled distension (Fig. 2). The number of TLESRs increased from 5 (3–10.5) to 10 (9.5–14.5) (P < 0.02) during pressure-controlled distension and from 4 (2.5–7.5) to 10.5 (7–12.5) (P < 0.02) during volume-controlled distension.

Hyperglycemia relaxed the proximal stomach. With pressure-controlled distension, intrabag volumes were significantly greater during hyperglycemia (609.5 ± 41.0 ml) than euglycemia (497.0 ± 53.9 ml, P < 0.02). With volume-controlled distensions, intrabag pressures were significantly lower during hyperglycemia (3.4 ± 0.9 mmHg) than during euglycemia (4.1 ± 0.8 mmHg, P < 0.02) (Fig. 3).

Hyperglycemia had no effect on gastrointestinal sensation. The sensation scores for abdominal bloating, abdominal discomfort, fullness, nausea, hunger, and desire to eat were similar between hyperglycemia and euglycemia during both pressure-controlled and volume-controlled distensions (data not shown).

Pancreatic polypeptide levels were significantly lower during hyperglycemia when compared with euglycemia for both

---

Fig. 1. Effect of marked hyperglycemia (15 mM) on basal lower esophageal sphincter (LES) pressure during pressure-controlled gastric distension (A) and volume-controlled gastric distension (B).

Fig. 2. Effect of marked hyperglycemia (15 mM) on the rate of transient LES relaxations during pressure-controlled gastric distension (A) and volume-controlled gastric distension (B). Each point represents data for an individual subject. The horizontal bars indicate median values. *P < 0.02.
pressure-controlled (26.5 ± 0.8 vs. 77.2 ± 1.3 pmol/l, \( P < 0.05 \)) and volume-controlled distensions (31.1 ± 0.7 vs. 75.2 ± 1.2 pmol/l, \( P < 0.03 \)).

Effects of physiological hyperglycemia. Because both pressure-controlled distensions and volume-controlled distensions had similar effects on the rate of TLESRs during marked hyperglycemia studies, we used volume-controlled distensions for studies during physiological hyperglycemia. Physiological hyperglycemia had no effect on basal LES pressure (11.7 ± 1.1 vs. 11.6 ± 1.1 mmHg) or the rate of TLESRs [9.5 (7–13.5) vs. 8.5 (7–12)] (Fig. 4). There was also no significant effect on proximal gastric tone; intrabag pressures during physiological hyperglycemia (3.8 ± 0.4 mmHg) were comparable with those during euglycemia (3.9 ± 0.5 mmHg, \( P > 0.5 \)). There were no significant differences in the sensation scores for abdominal bloating, abdominal discomfort, fullness, nausea, hunger, and desire to eat between euglycemia and physiological hyperglycemia.

DISCUSSION

Hyperglycemia has been shown previously to have significant effects on basal LES pressure, esophageal peristalsis, and sensation. In the present study, we extended these observations to investigate the effects of hyperglycemia on triggering of TLESRs, the major mechanism of gastroesophageal reflux. Our findings show that, in healthy volunteers, marked hyperglycemia doubles the rate of TLESRs triggered in response to gastric distension, an effect that is independent of whether the distension stimulus is pressure or volume controlled.

TLESRs are the major mechanisms of reflux in normal subjects and the majority of patients with reflux disease (17, 48). The primary stimulus is gastric distension. The rate of TLESRs may, however, be modulated by a number of factors, including meals, CCK, posture, and sleep (44). Hyperglycemia has substantial effects on gastrointestinal motor function (49). Marked hyperglycemia (≈15 mM) suppresses gastric antral motility and increases pyloric motility (21), decreases proximal gastric tone (25), slows gastric emptying (43), attenuates the gallbladder contractile response to CCK (14), and inhibits small intestinal (4) and colonic motility (56). In normal subjects, hyperglycemia has been reported to influence basal LES pressure and esophageal peristalsis (13) and to increase esophageal sensation (50). We have now established that acute hyperglycemia facilitates the triggering of TLESRs.

Hyperglycemia has also been reported to decrease basal LES pressure (13). It is also possible, therefore, that hyperglycemia could increase the rate of reflux episodes through reductions in basal LES pressure. However, we did not detect such an effect in the present study. The reasons for this apparent discrepancy are not clear. It is possible that methodological differences between our study and the previous studies might have influenced the findings. We used continuous monitoring of LES pressure throughout the study rather than pull-through sampling at discrete time-points and recorded LES pressure during continuous gastric distension as opposed to an empty, fasted stomach.

**Fig. 4.** Effect of physiological hyperglycemia (8 mM) on the rate of transient LES relaxations.

**Fig. 3.** Effect of hyperglycemia (15 mM) on proximal gastric tone. A: volume in the barostat bag during pressure-controlled distension. B: pressure in the bag during volume-controlled distension. MDP, minimum distending pressure.
Mechanisms mediating the effect of hyperglycemia on the triggering of TLESRs are uncertain and have not been specifically addressed in this study. Nevertheless a number of possibilities merit consideration. First, hyperglycemia might directly influence vagal activity. The vagus nerve carries both the afferent and efferent components of the TLESR response to gastric distension. In normal subjects, hyperglycemia decreases vagal cholinergic activity as assessed indirectly by decreased secretion of pancreatic polypeptide (15–15) and alteration in cardiovascular reflexes (61). We observed a similar decrease in plasma pancreatic polypeptide level. On this basis, we hypothesized that hyperglycemia might reduce the rate of TLESRs, whereas the opposite was the case. This apparent discrepancy suggests that either the effects on the vagal pathways for mediating release of pancreatic polypeptide are independent of any potential effects on the vagal pathways for control of TLESRs, that hyperglycemia exerts its effect via nonvagal mechanisms that are sufficient to override any inhibitory action on vagal function, or that pancreatic polypeptide may also not be a wholly reliable indicator of vagal tone.

A second possibility is that hyperglycemia could increase the degree of gastric distension, the major trigger for TLESRs. We have reported that hyperglycemia increases relaxation of the proximal stomach (25). This effect was confirmed in the present study by the observation that hyperglycemia increased bag volume during pressure-controlled distension and decreased bag pressure during volume-controlled distension. During pressure-controlled distension, therefore, the distension stimulus would be greater, which might increase the rate of TLESRs. Recent data on this effect conflict with one study reporting no effect (10) and another reporting an increase in TLESRs (9). During volume-controlled distension, however, given the reduction in bag pressure, and thereby wall tension, one might expect the stimulus to be smaller during hyperglycemia. Yet hyperglycemia increased triggering of TLESRs during both methods of gastric distension. Thus alterations in proximal gastric motility do not appear to be the major mechanism by which hyperglycemia stimulates triggering of TLESRs.

Hyperglycemia affects the brain stem vagal nuclei that are pivotal in the control of TLESRs. Afferent vagal signals from the proximal stomach are integrated in the vagal nuclei from which the vagal motor output appears to be controlled by a pattern generator (44). This area appears to be the principal site of action for many pharmacological agents that inhibit triggering of TLESRs (27). In animal studies, glucose-responsive neurons that respond to physiological as well as pathological changes in extracellular glucose concentration have been identified within the area postrema, the nucleus tractus solitarius (NTS), and the dorsal motor nucleus of the vagus (DMV) (51). Convergence of afferent input from peripheral hepatic glucose sensors with vagal afferents in the NTS, from gastric mechanoreceptors that respond to gastric distension, and with neurons within the DMV that innervate the stomach has also been demonstrated in rats (1). However, in rodents, the effects of glucose injection into the dorsal vagal complex on gastric motility appear to be largely inhibitory, rather than the apparently excitatory effects on TLESRs, and may be the result of effects on the NTS rather than the DMV (20, 53, 58).

It would be expected that plasma insulin levels would increase markedly during hyperglycemia in healthy subjects. Some studies (18, 38, 60) have suggested that insulin may affect gastrointestinal motor function. However, most studies have shown no effect (11, 36, 37, 56), and hyperglycemia slows gastric emptying in type 1 diabetics who have no endogenous insulin secretion (22). Nevertheless, an effect of hyperinsulinemia cannot be totally excluded at this stage, and studies in patients with type 1 diabetes are needed to address this issue specifically.

Meals are well documented to increase the rates of TLESRs (29). The postprandial increase in the rate of TLESRs is an important factor underlying the increase in gastroesophageal reflux after meals. The mechanism by which meals increase the rate of TLESRs is probably multifactorial and includes gastric distension (28) as well as the release of peptides such as CCK (59, 62). However, blood glucose also increases after meals, and such increases in blood glucose concentration within the normal postprandial range have been shown to affect esophageal (6), gastric (3, 23), and gallbladder (14) motility. These observations suggest that the blood glucose concentration may act as a physiological modulator of gastrointestinal function and potentially contribute to the postprandial increase in TLESRs. This latter notion is not supported by our finding that increasing the blood glucose concentration from 4 to 8 mM did not affect the triggering of TLESRs. However, our studies were done in the fasted state using balloon distension of the stomach to trigger TLESRs. Although this is an accepted stimulus (2, 5, 39), it does not entirely reflect the postprandial state.

The effects of hyperglycemia on triggering of TLESRs may be an important factor underlying the high rate of excessive gastroesophageal reflux in patients with diabetes mellitus (41, 45). Mechanisms of reflux in patients with diabetes mellitus have not been defined. However, the majority of diabetic patients appear to have basal LES pressure within the normal range (26, 35, 45). Because normal subjects and patients with reflux disease have normal basal LES pressure reflux predominantly during TLESRs (16, 17), it is probable that most reflux events in patients with diabetes mellitus also occur during TLESRs. Our data suggest that increased triggering of TLESRs due to poor glycemic control might be a contributing factor underlying excessive reflux in these patients.

In summary, we have demonstrated for the first time that in healthy human subjects, marked hyperglycemia, but not physiological variations of blood glucose level, increases the rate of TLESRs. The increased rate of TLESRs is independent of proximal gastric wall tension. Mechanisms underlying the effect remain to be determined, but direct stimulation of glucose on the glucose-responsive cells both in the central nervous system and peripheral tissues may play an important role.

REFERENCES

G802


