The effect of exogenous GLP-1 on food intake is lost in male truncally vagotomized subjects with pyloroplasty

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Am J Physiol Gastrointest Liver Physiol 304: G1117–G1127, 2013. First published April 18, 2013; doi:10.1152/ajpgi.00035.2013.—Rapid degradation of glucagon-like peptide-1 (GLP-1) by dipeptidyl peptidase-4 suggests that endogenous GLP-1 may act locally before being degraded. Signaling via the vagus nerve was investigated in 20 truncally vagotomized subjects with pyloroplasty and 10 matched healthy controls. Subjects received GLP-1 (7-36 amide) or saline infusions during a standardized liquid mixed meal and a subsequent ad libitum meal. Despite no effect on appetite sensations, GLP-1 significantly reduced ad libitum food intake in the control group but had no effect in the vagotomized group. Gastric emptying was accelerated in vagotomized subjects and was decreased by GLP-1 in controls but not in vagotomized subjects. Postprandial glucose levels were reduced by the same percentage in both groups. Peak postprandial GLP-1 levels were approximately fivefold higher in the vagotomized subjects. GLP-1 significantly reduced glucagon secretion in both groups, but levels were approximately twofold higher and were nonsuppressible in the early phase of the meal in vagotomized subjects. Our results demonstrate that vagotomy with pyloroplasty impairs the effects of exogenous GLP-1 on food intake, gastric emptying, and insulin and glucagon secretion, suggesting that intact vagal innervation may be important for GLP-1’s actions.

GLP-1; degradation; DPP-4; vagus nerve; vagotomy; meal test; insulin and glucagon secretion

GLUCAGON-LIKE PEPTIDE-1 (GLP-1) is an intestinal hormone, released into the circulation from endocrine L cells in the small intestine during meal ingestion (31). Moreover, GLP-1 is produced in neurons in the nucleus of the solitary tract. GLP-1 receptors (GLP-1Rs) are widely expressed both in the brain, particularly in areas involved in control of food intake including the arcuate and paraventricular nuclei of the hypothalamus (42), and in the periphery, where they are found in the gastrointestinal tract, pancreatic islets, portal vein, liver, and vagus nerve [reviewed by Holst (30)]. The effects of the hormone include augmentation of glucose-stimulated insulin secretion (incretin effect) (50), inhibition of glucagon secretion (50), delay of gastric emptying (enterogastrone function) (69), and suppression of appetite and food intake (19). Thus GLP-1 administration is associated with reduction of both appetite and subsequent food intake (10, 31) in healthy as well as in obese and diabetic subjects (19, 24, 49). Furthermore, gastric emptying is inhibited (13, 19, 47), limiting propulsion of additional food, which, together with the effects of GLP-1 on the endocrine pancreas (23, 68), results in beneficial conditions for the deposition of already-absorbed nutrients in the liver and periphery. However, the exact mechanisms behind these effects remain to be elucidated. The rapid dipeptidyl peptidase-4 (DPP-4)-mediated degradation and inactivation of GLP-1 (11, 27, 41), suggests that endogenous GLP-1 may act locally, possibly via the vagus nerve, before being degraded. Several findings support the hypothesis of vagal afferent sensing of GLP-1: 1) the GLP-1R gene is expressed in large amounts in the nodose ganglion (44), 2) vagal nerve terminals innervating the portal vein were reported to express GLP-1R (67), and 3) both intraportal and intravenous GLP-1 infusions in rats lead to increased impulse generation in vagal afferent fibers (8, 48). Even though GLP-1Rs have not yet been identified on neurons innervating the intestine, it seems plausible that they may be present on vagal terminals throughout the gastrointestinal tract, considering the extensive GLP-1R gene expression in the nodose ganglion (44). Thus it has been shown in rats that subdiaphragmatic deafferentation prevents anorexia (59) induced by intraperitoneal GLP-1, and ganglionic blockade eliminated the insulinotropic effects of intraportally infused GLP-1 (5). It therefore seems likely that GLP-1 acts locally, possibly via the vagus nerve, prior to degradation.

The present study was designed to evaluate the importance of vagal pathways for the effect of GLP-1 on food intake (primary end point), appetite sensations, and gastric emptying, and, additionally, on meal-related plasma glucose (PG), insulin, and glucagon responses. Subjects with truncal vagotomy and pyloroplasty were evaluated during two double meal tests, with and without GLP-1 infusion, and compared with matched healthy subjects with intact vagal innervation.

MATERIALS AND METHODS

Study Protocol

The study was approved by the Scientific-Ethical Committee of the Capital Region of Denmark (registration no. H-D-2008-050) and by the
Danish Data Protection Agency (journal no. 2008-41-2220). It was registered at www.clinicaltrials.gov (ClinicalTrials.gov ID: NCT01176890) and performed in accordance with the Helsinki declaration II. Written and oral informed consent was obtained from all participants.

Subjects

Eleven subjects who had undergone surgery in the period 1964–1977 (truncal vagotomy and pyloroplasty ad modum Heineke-Mikulicz) for uncomplicated duodenal ulcer were screened. During the screening visit, all underwent a physical examination and blood samples were drawn for measurements of standard hematological and clinical biochemistry parameters. Albumin-to-creatinine ratio was measured in urine samples. Exclusion criteria were diabetes mellitus, overweight [body mass index (BMI) above 30 kg/m²], inflammatory bowel disease, gut resection (except appendectomy), kidney or liver disease (creatinine >150 μM and/or albuminuria; plasma alanine aminotransferase or plasma aspartate aminotransferase above two times normal values) and medication that could not be paused for 12 h. One patient with severe dumping symptoms was excluded. Two patients with recurrent gastric ulcer (one after NSAID treatment) and one with Type 2 diabetes, who was well regulated with only 0.5 mg of glipizide once daily [fasting PG 5.5 mM and glycated hemoglobin A1c (HbA1c) of 4.9%] were included; all calculations and statistical analyses were carried out both with and without exclusion of these three participants and the results were identical. Furthermore, 10 subjects treated in the period 2004–2008 with cardia resection ad modum Ivor Lewis, including truncal vagotomy and pyloroplasty, for esophageal cancer were screened and included. None of these subjects showed any sign of tumor recurrence. HbA1c (%) was slightly lower in patients treated for duodenal ulcer than esophageal cancer, which otherwise showed no differences. Furthermore, the results from this study were similar in the two groups and were therefore analyzed together. All the subjects operated for esophageal cancer had received chemo and radiation therapy postoperatively and were treated with proton pump inhibitors (for prevention of gastroesophageal reflux). Additional medications included (whole vagotomized group): statins (dyslipidemia, N = 3), anticoagulant therapy (dipyridamol or acetylsalicylic acid, N = 3), antihypertensive therapy [thiazides, angiotensin converting enzyme (ACE) inhibitors, calcium antagonists, and/or beta-blockers, N = 3], antithyroid and thyroid hormone substitution (thyroxine and levothyroxine, N = 1), or therapy for benign prostate hypertrophy (alpha-blocker, N = 1). The vagotomy of those treated for duodenal ulcer was at that time considered complete as evidenced by more than 90% reduction in insulin-induced peak acid output 3 mo and 5 yr after the operation (confirmed from existing hospital records for five of the participants in this study). No similar test was performed in the subjects operated for esophageal cancer with cardia resection. However, their type of operation (cardia resection ad modum Ivor Lewis) did involve a truncal vagotomy due to the anatomical proximity of the cancer to the vagal nerve and therefore also included a pyloroplasty (54).

Ten healthy subjects matched to the vagotomized subjects with regard to age, sex, and BMI, all without family history of diabetes, were included and served as controls. Medications in this group included antihypertensive treatment (ACE inhibitors, calcium antagonists, and/or thiazides, N = 3), anticoagulant therapy (acetylsalicylic acid, N = 2), or therapy for benign prostate hypertrophy (alpha-blocker, N = 1).

The truncally vagotomized subjects treated for duodenal ulcer were located by use of old hospital records from throughout Denmark. The truncally vagotomized subjects treated with cardia resection were all treated in the Rigshospital, Copenhagen, Denmark and were identified from the local archive. The healthy control subjects were recruited from lists of subjects who had previously participated in studies and who had given their consent to be contacted again, or from advertising in newspapers or on the internet. Subjects received a letter and a phone call with a request to participate.

All medications were paused at least 12 h prior to examinations. Subject characteristics are shown in Table 1; all participants had normal clinical and biochemical parameters.

Experimental Procedures

Subjects were studied on three occasions including a “sham-feeding” test and two meal tests. On each occasion subjects were studied after a 12-h fast (including abstinence from smoking and drinking) and were instructed to maintain an unchanged lifestyle but to abstain from alcohol and exercise in the 24 h leading up to the experiments and to standardize their food intake on the day before each experiment. Subjects were seated during all experiments. During the sham feeding, one cannula was inserted into an antecubital vein in one arm for collection of arterialized blood samples [cannulated forearm placed in a heating box (50°C) throughout the experiments]. During the meal test, an additional cannula was inserted into the contralateral antecubital vein for GLP-1 or saline infusion.

To evaluate vagal integrity, participants underwent a 15-min chew-and-spit sham feeding (4), with a test meal consisting of a standardized breakfast platter (including eggs, bacon, bread, butter, cheese, marmalade, yogurt, fruits, pancakes, orange juice, and tea or coffee). Participants were observed throughout the sham feeding and were instructed to avoid swallowing any food, drink, or saliva. Blood was drawn at −15, 0, 15, 30, 45, and 60 min. For bedside PG measurement, blood was collected into fluoride tubes and centrifuged immediately (7,400 g for 1 min at room temperature). For analyses of total GLP-1 and pancreatic polypeptide (PP) blood was distributed into chilled tubes containing EDTA.

Thereafter, subjects were investigated on two different occasions during infusion of GLP-1 [1.2 pmol·kg⁻¹·min⁻¹; the original, therapeutically most effective dose of GLP-1 (46)] or saline in a randomized, placebo-controlled single-blinded crossover design. At time 0 min, a continuous intravenous infusion of GLP-1 or saline was started and continued throughout the experiment. After 30 min, a standardized liquid mixed meal was served and consumed over 10 min, with participants being encouraged to drink at an even rate during the 10 min. After 240 min, a warm lunch was served with 300 ml chilled water, and subjects were instructed to eat ad libitum until comfortably satisfied. The weight of food consumed was measured. Appetite, well-being, and palatability ratings for the ad libitum meal were made by visual analog scales (VASSs). Arterialized blood was drawn 15, 10, and 0 min before and 15, 30, 45, 60, 75, 90, 105, 120, 150, 180, 210, and 240 min after the meal. For bedside PG measurement, blood was collected into fluoride tubes and centrifuged immediately (7,400 g for 1 min at room temperature). Blood was distributed into chilled tubes containing a

Table 1. Subject characteristics

<table>
<thead>
<tr>
<th></th>
<th>Vagotomized</th>
<th>Controls</th>
<th>P</th>
</tr>
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<tr>
<td>Sex, M/F</td>
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<td>10/0</td>
<td>0.5666</td>
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<tr>
<td>Age, yr</td>
<td>68 (5)</td>
<td>67 (3)</td>
<td>0.2690</td>
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<td>BMI kg/m²</td>
<td>24 (3)</td>
<td>25 (2)</td>
<td>0.4334</td>
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<td>Waist:hip ratio</td>
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<td>Systolic blood pressure, mmHg</td>
<td>146 (20)</td>
<td>142 (15)</td>
<td>0.1868</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>85 (12)</td>
<td>90 (9)</td>
<td>0.0211</td>
</tr>
</tbody>
</table>

Data are mean values with SD in parentheses. BMI, body mass index; PG, plasma glucose; HbA1c, glycated hemoglobin A1c; HOMA, homeostasis model assessment; IR, insulin resistance; %B, beta cell function; %S, insulin sensitivity.
DPP-4 inhibitor (valine pyrrolidide, 0.01 mM final concentration, a gift from Novo Nordisk, Bagsværd, Denmark) and EDTA plus apro- 
inin (500 kIU/ml blood; TrasyloI; Bayer, Leverkusen, Germany) for 
analyses of glucagon, GLP-1, and PP. Blood was collected in dry 
tubes (left 20 min at room temperature for coagulation) for insulin and C-peptide analyses and into lithium-heparin tubes for acetamino 
phen measurements. Blood was centrifuged (for 20 min at 1,200 g and 4°C) and plasma for GLP-1, glucagon, and acetaminophen analyses was 
stored at −20°C, and serum for insulin and C-peptide analyses was 
stored at −80°C.

The standardized liquid mixed meal consisted of 100 g NAN1 
[2,170 kJ: 10% protein (2.8% casein and 6.7% whey), 58% lactose, 
and 28% fat (11.3% saturated, 10% monounsaturated, and 4.7% 
polysaturated); Nestlé Nutrition, Amsterdam, The Netherlands] 
dissolved in 300 ml water; 1.5 g acetaminophen (Panodil, GlaxoSmith 
Kline, Copenhagen, Denmark) was added to the meal. Acetaminophen is 
poorly absorbed from the stomach but rapidly so from the proximal part 
of the small intestine. Its absorption rate can, therefore, be used as an 
direct measurement of gastric emptying (71).

The ad libitum meal consisted of an appetizing but rather homog- 
nenous mixture of minced meat, pasta, corn, carrots, and green peppe 
(37% fat, 13% protein, and 50% carbohydrates) as used in previous 
studies (3, 19, 21). The participants were not allowed to select certain 
food items from the ad libitum meal. The amount of food presented 
and remaining after the ad libitum meal was measured by use of a 
digital scale. Subjects had 30 min to eat until they were comfortably 
satisfied.

Appetite, well-being, and palatability for the ad libitum meal 
ratings were made using 10 cm-VASs with words anchored at each 
end expressing the most positive and the most negative rating. VAS 
was used to assess satiety, hunger, fullness, prospective food con- 
sumption, well-being, nausea, and thirst every 30 min, starting at 0 
min, and for measurement of palatability of the ad libitum meal (visual 
appearance, taste, smell, aftertaste, and overall palatability) imme- 
diately after consumption of the ad libitum meal. On the VAS 10 cm 
equals “I cannot eat another bite” (satiety), “I have never been more 
hungry” (hunger), “I am totally full” (fullness), “I can eat a lot” 
(prospective food consumption), “I feel really good” (well-being), “I 
have never been more nauseated” (nausea), “I have never been more 
thirsty” (thirst), “The meal looks really good” (visual appearance), “The 
meal smells wonderful” (smell), “The taste of the meal is wonderful” 
(taste), “The meal has a strong aftertaste” (aftertaste), and “The meal 
is appetizing” (overall palatability). VAS ratings for appetite sensation 
have been validated previously in a study involving young healthy 
males (20), not in an elderly age group, as studied here. However, VAS 
ratings for appetite sensation have been used previously in elderly 
age group (22). We therefore regard VAS ratings applicable for appetite estimations also in our groups. Notably, the 
groups in this study were matched for age.

GLP-1 Infusions

Synthetic GLP-1(7-36 amide) (PolyPeptide Laboratories, Wolfen- 
büttel, Germany) (correctness of structure and purity was assessed by 
mass, sequence, and HPLC analysis) was dissolved in sterilized water 
containing 2% human albumin (Statens Serum Institut, Copenhagen, 
Denmark), thereafter subjected to sterile filtration, and dispensed into 
vials and stored frozen under sterile conditions until the experiment.

Calculations and Statistical Analyses

All statistical analyses were carried out with GraphPad Prism 
version 5.00 for Windows (GraphPad Software, San Diego, CA). 
Results are reported as mean values with standard deviations in 
parentheses. A two-sided P value <0.05 was considered significant. 
Data were tested for normal distribution by D’Agostino-Pearson 
omnisus K2 normality test. Two-way repeated-measures analysis of 
variance (ANOVA), followed by Bonferroni posttests, were applied 
to test for differences in repeatedly measured values between days 
(absolute PG, hormone concentrations, acetaminophen, and VAS). 
Paired t-tests within the groups and unpaired t-tests between groups 
were used for comparisons between single values [between baseline, 
food intake and area under the curve (AUC) values] in data that 
followed a normal distribution. For data that did not follow a normal 
distribution, the significance of difference was tested using the Wil- 
coxon test for paired difference and the Mann-Whitney test for 
unpaired difference. Insulin resistance, as well as steady-state beta cell 
function and insulin sensitivity, the latter expressed in percent of a 
normal reference population, were calculated using the homeostasis 
model assessment (The HOMA2 calculator: http://www.dtu.ox.ac.uk/ 
index.php?maindoc=/homai). AUC and incremental AUC (iAUC; i.e., 
baseline levels subtracted) were calculated by use of the trape- 
zoidal rule. A mean appetite score was calculated (based on VAS 
ratings) by the formula [(hunger + prospective intake + (100 − 
satiety) + (100 − fullness)]/4] (64).

RESULTS

Sham Feeding

Only 19 vagotomized subjects and 9 control subjects participated 
in the sham feeding. The vagotomized subject not participating in the 
sham feeding had a documented negative insulin test at both 3 mo and 5 yr after the operation. Baseline 
values of PP were identical in the vagotomized subjects and control 
subjects [23(23) vs. 21(15) pM, P = 0.8258; Fig. 1A]. PP levels 
increased in 12 of the 19 (63%) vagotomized subjects and 8 of the 9 (89%) control subjects during the 1 h after the 
sham feeding. Examination of the cephalic responses during the 
first 15 min after sham feeding revealed an absence of 
vagally stimulated PP secretion in the vagotomized subjects 
compared with the control subjects [increase of PP secretion in 
percent after sham feeding: 8(56) vs. 64(55)%], P = 0.0195], 
(Fig. 1B). Mean baseline PG values were similar in the vagotom- 
zized subjects and control subjects [5.4(0.5) vs. 5.1(0.4) pM, 
P = 0.1230], and a small rise in PG following sham feeding 
(P < 0.05) was observed in both groups (Fig. 1C). Baseline 
GLP-1 levels were similar in vagotomized subjects and control 
subjects [11.5(6.1) vs. 8.9(2.6) pM, P = 0.2533] and were 
unaltered during sham feeding in both groups (Fig. 1D).
Ad Libitum Meal Including Palatability Rating

GLP-1 reduced ad libitum food intake in controls but had no effect in the vagotomized group [control subjects: 367(115) (saline) vs. 318(92) (GLP-1) g, \(P = 0.0417\); vagotomized subjects: 286(95) (saline) vs. 268(116) (GLP-1) g, \(P = 0.2049\)]. There was no significant difference in food intake between the two groups on any of the experimental days (Fig. 2). The palatability ratings were identical during saline and GLP-1 infusions in both groups (Table 2). Vagotomized subjects rated the visual appeal and smell of the ad libitum meal higher than the control subjects both during saline and GLP-1 infusions and overall palatability higher only during GLP-1 infusions. Otherwise there was no difference between the groups.

Standardized Liquid Mixed Meal Test

Appetite and well-being sensations. There was no difference between the groups in baseline values of any of the VAS scores, except for a slightly higher well-being score on the day with GLP-1 infusion in the vagotomized subjects. After the liquid meal, decreased ratings of appetite score and increased ratings of satiety and fullness were seen on both days (Fig. 3) in both groups. Furthermore, vagotomized subjects reported decreased ratings of hunger, prospective food consumption, and thirst after the liquid meal. Control subjects reported a higher rating of appetite score and prospective food consumption than the vagotomized subjects. In contrast, the vagotomized subjects reported a higher rating of satiety and fullness than the controls, but there were no differences between the groups in hunger ratings (Table 3). No differences were seen in any of the VAS ratings when comparing saline and GLP-1 infusions. About 60–75 min after the liquid meal, two of the vagotomized subjects experienced nausea, dizziness, and fatigue and had to lie down for about 15–20 min before returning to their habitual state. Ten had to use the restroom one to two times (loose stools) between 1 and 2 h after the liquid meal. These side effects of the standardized liquid mixed meal were equally distributed between the vagotomized subjects treated for duodenal ulcer and esophagus cancer. The symptoms were the same on both experimental days and the subjects were familiar with these symptoms. Despite this, there was no difference in VAS for either nausea or well-being between the vagotomized subjects and control subjects.

Ad Libitum Meal Including Palatability Rating

GLP-1 reduced ad libitum food intake in controls but had no effect in the vagotomized group [control subjects: 367(115) (saline) vs. 318(92) (GLP-1) g, \(P = 0.0417\); vagotomized subjects: 286(95) (saline) vs. 268(116) (GLP-1) g, \(P = 0.2049\)]. There was no significant difference in food intake between the two groups on any of the experimental days (Fig. 2). The palatability ratings were identical during saline and GLP-1 infusions in both groups (Table 2). Vagotomized subjects rated the visual appeal and smell of the ad libitum meal higher than the control subjects both during saline and GLP-1 infusions and overall palatability higher only during GLP-1 infusions. Otherwise there was no difference between the groups.

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Acetaminophen. As illustrated in Fig. 4A, gastric emptying was accelerated in vagotomized subjects compared with controls during the saline infusion [time to peak plasma acetaminophen: 67(36) vs. 152(21) min, \( P = 0.0001 \)]. GLP-1 had no effect on the acetaminophen profile in vagotomized subjects but powerfully suppressed and delayed gastric emptying in control subjects [time to peak plasma acetaminophen: 75(38) vs. 222(16) min, \( P = 0.0001 \)].

Glucose. Fasting PG differed between vagotomized subjects and control subjects on the saline day (Table 4), but not on the GLP-1 day (Fig. 4B). Peak PG concentrations were higher in the vagotomized subjects after saline infusion [9.2(2.7) vs. 6.8(0.6), \( P = 0.0100 \)], and in this group, GLP-1 lowered postprandial glucose levels at time 45–75 min (\( P < 0.05 \)), whereas GLP-1 reduced postprandial PG levels below baseline in control subjects [time 30–105 min (\( P < 0.04 \)). Total AUCs were significantly reduced by GLP-1 in both groups (Table 4). However, the vagotomized subjects had significantly higher total AUCs than the controls during GLP-1 infusion. The reductions in percent during GLP-1 infusion were similar [vagotomized: \(-15.7(10.6) \text{ vs. controls } -13.0(6.9)\% \), \( P = \text{NS} \)].

GLP-1. Fasting GLP-1 levels were similar in the two groups (Table 4 and Fig. 4C). However, peak meal-stimulated endogenous GLP-1 concentrations were higher in vagotomized subjects relative to controls on the saline day [191(97) vs. 35(8) pM, \( P = 0.0011 \) (Fig. 4C)]. During GLP-1 infusions, peak GLP-1 levels were also higher postprandially in the vagotomized group [431(215) vs. 227(90) pM, \( P = 0.0083 \)]. In fact, the meal-induced GLP-1 response on the saline day appeared to be superimposed on the plateau generated by the infusion in the vagotomized subjects. However, in the control subjects there may be a slight feedback inhibition of endogenous GLP-1 secretion during the GLP-1 infusion (Fig. 4C).

PP. There was no difference in fasting PP levels between the 2 experimental days in either group. However, fasting PP was significantly lower in the vagotomized and higher in the control group during the GLP-1 day (Table 2). The PP response to the standard liquid mixed meal was completely unaffected by GLP-1 in the vagotomized group, whereas GLP-1 markedly reduced PP responses in the control subjects (Table 2 and Fig. 4D).

Insulin and C-peptide. Fasting insulin and C-peptide levels were similar in the two groups (Table 4 and Fig. 4, E and F). The vagotomized subjects demonstrated a larger beta cell response after the liquid meal with saline infusion compared with the controls, as evaluated by the AUC for C-peptide (Table 4) and two-way repeated-measures ANOVA, followed by Bonferroni posttests for insulin (significant difference between the groups at time 60–90 min, \( P < 0.01 \)). However, insulin secretion was unaffected by exogenous GLP-1 in vagotomized subjects, whereas it was suppressed in control subjects (Table 4). Both groups displayed similar insulin resistance, steady-state beta cell function, and insulin sensitivity in the fasting state (Table 1).

Glucagon. The vagotomized subjects had significantly lower fasting plasma glucagon levels on the saline day than the controls, and there was a tendency toward the same on the GLP-1 day (Table 4). The vagotomized subjects exhibited hyperglucagonemia in response to the liquid meal during both saline and GLP-1 infusions (Fig. 4G). In contrast to the pronounced suppression of plasma glucagon in the control subjects, exogenous GLP-1 had little effect in the vagotomized group, when assessed during the early phase (AUC0–90 min) of the meal [vagotomized subjects: 1,329(380) (saline) vs. 1,228(447) (GLP-1) pM, \( P = 0.1184 \); control subjects: 933(279) (saline) vs. 618(315) (GLP-1) pM, \( P = 0.0020 \)].

DISCUSSION

Our findings indicate that vagotomy with pyloroplasty is associated with 1) accelerated gastric emptying, 2) altered glucose tolerance, 3) fivefold higher meal-stimulated GLP-1 secretion, 4) increased postprandial insulin and glucagon responses, and 5) an apparently diminished effect of exogenous GLP-1 on meal-related insulin, glucagon, and PP secretion and also on gastric emptying and ad libitum food intake.

### Table 2. Palatability of the ad libitum meal with and without GLP-1 infusion

<table>
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<th></th>
<th>Vagotomized</th>
<th>Controls</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visual appeal</td>
<td></td>
<td></td>
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<tr>
<td>Saline. cm</td>
<td>7.4 (2.5)</td>
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<td>GLP-1. cm</td>
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<tr>
<td>Smell</td>
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<tr>
<td>Saline. cm</td>
<td>7.4 (2.3)</td>
<td>5.8 (1.9)</td>
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<td>GLP-1. cm</td>
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<tr>
<td>Taste</td>
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<tr>
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<td>6.0 (2.1)</td>
<td>0.1993</td>
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<td>GLP-1. cm</td>
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<tr>
<td>Aftertaste</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline. cm</td>
<td>1.5 (1.5)</td>
<td>1.3 (1.4)</td>
<td>0.7723</td>
</tr>
<tr>
<td>GLP-1. cm</td>
<td>1.7 (2.3)</td>
<td>1.3 (0.7)</td>
<td>0.9324</td>
</tr>
<tr>
<td>( P ), saline vs. GLP-1</td>
<td>0.8507</td>
<td>0.9999</td>
<td></td>
</tr>
<tr>
<td>Overall palatability</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline. cm</td>
<td>7.2 (2.2)</td>
<td>5.8 (2.0)</td>
<td>0.1115</td>
</tr>
<tr>
<td>GLP-1. cm</td>
<td>7.2 (3.0)</td>
<td>4.8 (1.9)</td>
<td>0.0111</td>
</tr>
<tr>
<td>( P ), saline vs. GLP-1</td>
<td>0.7531</td>
<td>0.2907</td>
<td></td>
</tr>
</tbody>
</table>

Data are mean values with SD in parentheses from 20 vagotomized subjects with pyloroplasty and 10 control subjects. GLP-1, glucagon-like peptide-1.
It could be a concern that the vagotomies were performed many years earlier than the present study, particularly in the duodenal ulcer patients. Therefore, the subjects underwent a chew-and-spit sham feeding to evaluate their vagal integrity. Meal-induced PP secretion is biphasic, where the early cephalic phase is vagally mediated and the second prolonged phase is not wholly dependent on vagal activity (61–63). The PP secretion immediately after the sham feeding can, therefore, be used as a reliable test for vagal integrity (4). The vagotomized subjects participating in this study were characterized by a lack of the early cephalic PP response, in contrast to the marked response in the control subjects, leading us to believe that their vagotomies were still complete. We also measured PG and GLP-1 before and after sham feeding, as a control for anything being swallowed, and noted a small rise in PG values from baseline at all time points in both groups. It cannot, therefore, be fully excluded that small amounts of food, drink, or saliva were swallowed during sham feeding despite our efforts. However, the rise in PG was very small and observed in both groups, so any nutrient-dependent increase in PP secretion would also be small and equal in both groups. Moreover, it is well established that GLP-1 is secreted in response to nutrients in the intestine (31) and GLP-1 levels were unchanged during sham feeding, supporting the assumption of absence of nutrient-dependent activation of PP secretion.

No differences were seen in appetite sensations in either group of subjects when comparing saline and GLP-1 infusions. This was unexpected, since GLP-1 repeatedly has been shown to inhibit sensations of appetite (19, 24, 49). VAS recordings of appetite are not particularly sensitive, but according to Flint et al. (20) our number of subjects should have been sufficient to detect clinically relevant differences. However, the technique has not been validated in an elderly age group, as studied here. Moreover, Gutzwiller and colleagues (26) previously reported that only high doses of GLP-1 infusion (1.5 pmol·kg⁻¹·min⁻¹) resulted in a statistically significant difference in the sensation of (only) hunger, whereas lower doses (0.375, 0.75, and 1.5 pmol·kg⁻¹·min⁻¹) still inhibited food intake. In our study, we only administered 1.2 pmol·kg⁻¹·min⁻¹ of GLP-1, possibly explaining the missing effect of GLP-1 on appetite. Nevertheless, GLP-1 had no effect ad libitum food intake in the

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Fig. 3. Subjective appetite ratings of appetite (A), hunger (B), prospective food intake (C), satiety (D), fullness (E), thirst (F), well-being (G), and nausea (H) scores from visual analog scales before and after a mixed meal with concomitant saline infusion (open symbols) or GLP-1 infusion (solid symbols) in vagotomized subjects (circles) and control subjects (squares). Infusions were started at 0 min and lasted throughout the study. The standardized liquid mixed meal was served at 30 min and was consumed during 10 min. Data are mean values and standard deviation from 20 vagotomized subjects with pyloroplasty and 10 control subjects.
GLP-1 infusion and after a mixed meal (area under curve) with and without deafferentation (59). Similarly, vagal sensory ablation using total subdiaphragmatic vagotomy (1) and selective vagal intraperitoneal GLP-1 on food intake in rodents was lost after vagotomized subjects, whereas there was a significant reduction in the control group. In accordance with this, the effect of intraperitoneal GLP-1 on food intake in rodents was lost after both total subdiaphragmatic vagotomy (1) and selective vagal deafferentation (59). Similarly, vagal sensory ablation using capsaicin treatment in mice abolished the inhibitory effect of the GLP-1 agonist exendin-4 (65). All of these observations support a role for sensory afferents in mediating effect of GLP-1 on food intake. There are also other possible mechanisms of action for GLP-1, especially with respect to the effects on appetite, food intake, and the adverse reaction nausea, which can be triggered by large doses of GLP-1 (57). It has been demonstrated in rodents that there are GLP-1Rs in the subfornical organ and the area postrema accessible to peripherally circulating GLP-1 (51). In addition, studies in rats have clearly demonstrated that inhibition of food intake can be elicited by intravenous administration of GLP-1 and this effect is not affected by subdiaphragmatic deafferentation (59). The effect of vagotomy observed here, however, supports a role for vagal mediation of the effect of GLP-1 on food intake in humans.

Nauck et al. (45) showed that the effects of GLP-1 on gastric emptying as estimated by using a two-meal protocol shows rapid tachyphylaxis in humans, and, since there was also a marked reduction in PP levels (used as a marker for vagal activity) after the first but not the second meal, they suggested that the tachyphylaxis happened at the level of vagal activation. This might suggest that other effects of GLP-1 may also be subject to rapid tachyphylaxis, perhaps at the level of vagal activation. However, GLP-1-induced improvements in metabolic control and body weight are maintained during long-term treatment with both GLP-1 and long-acting GLP-1 receptor analogs (32, 56, 72), indicating that, except for inhibition of gastric emptying, the effects of GLP-1 do not show tachyphylaxis. Moreover, other studies involving several hours of GLP-1 infusion to concentrations comparable to those used in this study (3, 19, 25, 26) have demonstrated maintained reduction of food intake at a subsequent ad libitum meal. Another explanation for the lack of effect in the vagotomized group might be the age of the participants. A study by Gregersen et al. (22) indicated decreasing postprandial hunger and desire to eat with increasing age. However, our control subjects were carefully matched for age. To obtain an independent measure of vagal activity, we measured PP responses to the standardized liquid mixed meal and found that the PP responses were identical during the two experimental conditions in the vagotomized group, indicating that GLP-1 did not affect PP secretion in this group. In contrast, the PP response was dramatically reduced during GLP-1 infusion in the control subjects. Although the postprandial PP responses may not be entirely vagally driven (although clearly dependent on cholinergic muscarinic mechanisms) as discussed above and may be related to differences in gastric emptying, as also shown in patients with Type 1 diabetes (16, 17), it remains highly likely that GLP-1 reduces the efferent, vagally mediated signals for PP secretion (61). The PP data, therefore, strongly support the concept that GLP-1 regulation of postprandial PP secretion and appetite involves the vagus nerve.

Accelerated gastric emptying, particularly for liquids, is a known consequence of the pyloroplasty performed in connection with vagotomy (37, 53) and, as expected, gastric emptying, evaluated by use of acetaminophen, was faster in the vagotomized subjects than in the controls. Gastric emptying was not affected by exogenous GLP-1 in these subjects. This would be consistent with a study by Imeryüz et al. (35) in rats, in which capsaicin-induced block of vagal sensory afferents abolished the inhibitory effect of GLP-1 on gastric emptying.

### Table 3. Appetite and wellness sensations before (baseline) and after a mixed meal (area under curve) with and without GLP-1 infusion

<table>
<thead>
<tr>
<th>Sensation</th>
<th>Vagotomized</th>
<th>Controls</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appetite score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline_Saline</td>
<td>5.0 (2.0)</td>
<td>5.0 (1.2)</td>
<td>0.7373</td>
</tr>
<tr>
<td>Baseline GLP-1</td>
<td>5.0 (2.1)</td>
<td>5.0 (1.9)</td>
<td>0.4858</td>
</tr>
<tr>
<td>P, saline vs. GLP-1</td>
<td>0.9101</td>
<td>0.5237</td>
<td></td>
</tr>
<tr>
<td>tAUC_Saline cm/min</td>
<td>1,174 (448)</td>
<td>1,215 (363)</td>
<td>0.0189</td>
</tr>
<tr>
<td>tAUC GLP-1 cm/min</td>
<td>1,172 (394)</td>
<td>1,213 (349)</td>
<td>0.0097</td>
</tr>
<tr>
<td>P, saline vs. GLP-1</td>
<td>0.6548</td>
<td>0.6607</td>
<td></td>
</tr>
<tr>
<td>Hunger</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline_Saline</td>
<td>3.6 (2.3)</td>
<td>2.7 (2.2)</td>
<td>0.8946</td>
</tr>
<tr>
<td>Baseline GLP-1</td>
<td>3.5 (2.9)</td>
<td>4.0 (2.4)</td>
<td>0.6485</td>
</tr>
<tr>
<td>P, saline vs. GLP-1</td>
<td>0.7744</td>
<td>0.0669</td>
<td></td>
</tr>
<tr>
<td>tAUC_Saline cm/min</td>
<td>822 (473)</td>
<td>1,094 (422)</td>
<td>0.1348</td>
</tr>
<tr>
<td>tAUC GLP-1 cm/min</td>
<td>771 (402)</td>
<td>1,109 (467)</td>
<td>0.0529</td>
</tr>
<tr>
<td>P, saline vs. GLP-1</td>
<td>0.4982</td>
<td>0.8540</td>
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<tr>
<td>Prospective food intake</td>
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<td></td>
</tr>
<tr>
<td>Baseline_Saline</td>
<td>4.0 (2.1)</td>
<td>5.3 (1.7)</td>
<td>0.0940</td>
</tr>
<tr>
<td>Baseline GLP-1</td>
<td>4.2 (2.3)</td>
<td>4.8 (2.1)</td>
<td>0.4613</td>
</tr>
<tr>
<td>P, saline vs. GLP-1</td>
<td>0.7058</td>
<td>0.5653</td>
<td></td>
</tr>
<tr>
<td>tAUC_Saline cm/min</td>
<td>838 (472)</td>
<td>1,307 (444)</td>
<td>0.0141</td>
</tr>
<tr>
<td>tAUC GLP-1 cm/min</td>
<td>851 (436)</td>
<td>1,247 (409)</td>
<td>0.0235</td>
</tr>
<tr>
<td>P, saline vs. GLP-1</td>
<td>0.8430</td>
<td>0.3995</td>
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<tr>
<td>Satisfaction</td>
<td></td>
<td></td>
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<tr>
<td>Baseline_Saline</td>
<td>4.7 (2.8)</td>
<td>4.2 (1.3)</td>
<td>0.8503</td>
</tr>
<tr>
<td>Baseline GLP-1</td>
<td>5.0 (2.6)</td>
<td>3.8 (2.0)</td>
<td>0.9199</td>
</tr>
<tr>
<td>P, saline vs. GLP-1</td>
<td>0.4691</td>
<td>0.3594</td>
<td></td>
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<tr>
<td>tAUC_Saline cm/min</td>
<td>1,359 (475)</td>
<td>968 (395)</td>
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<tr>
<td>tAUC GLP-1 cm/min</td>
<td>1,400 (438)</td>
<td>933 (354)</td>
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<tr>
<td>P, saline vs. GLP-1</td>
<td>0.3296</td>
<td>0.4810</td>
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</tr>
<tr>
<td>Fullness</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline_Saline</td>
<td>3.5 (2.6)</td>
<td>3.4 (1.7)</td>
<td>0.9395</td>
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<tr>
<td>Baseline GLP-1</td>
<td>3.4 (2.4)</td>
<td>3.5 (2.3)</td>
<td>0.9572</td>
</tr>
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<td>P, saline vs. GLP-1</td>
<td>0.8798</td>
<td>0.9105</td>
<td></td>
</tr>
<tr>
<td>tAUC_Saline cm/min</td>
<td>1,345 (498)</td>
<td>847 (300)</td>
<td>0.0072</td>
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<tr>
<td>tAUC GLP-1 cm/min</td>
<td>1,356 (452)</td>
<td>917 (327)</td>
<td>0.0110</td>
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<tr>
<td>P, saline vs. GLP-1</td>
<td>0.8176</td>
<td>0.0889</td>
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<tr>
<td>Thirst</td>
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<tr>
<td>Baseline_Saline</td>
<td>5.3 (2.7)</td>
<td>5.0 (2.6)</td>
<td>0.7359</td>
</tr>
<tr>
<td>Baseline GLP-1</td>
<td>4.9 (2.6)</td>
<td>5.5 (2.4)</td>
<td>0.5412</td>
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<tr>
<td>P, saline vs. GLP-1</td>
<td>0.5263</td>
<td>0.5578</td>
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<tr>
<td>tAUC_Saline cm/min</td>
<td>1,247 (670)</td>
<td>1,336 (500)</td>
<td>0.7137</td>
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<tr>
<td>tAUC GLP-1 cm/min</td>
<td>1,251 (605)</td>
<td>1,286 (549)</td>
<td>0.8779</td>
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<tr>
<td>P, saline vs. GLP-1</td>
<td>0.9397</td>
<td>0.5497</td>
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<tr>
<td>Well-being</td>
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<tr>
<td>Baseline_Saline</td>
<td>7.5 (2.5)</td>
<td>8.1 (1.4)</td>
<td>0.8882</td>
</tr>
<tr>
<td>Baseline GLP-1</td>
<td>8.4 (1.2)</td>
<td>8.1 (1.4)</td>
<td>0.4404</td>
</tr>
<tr>
<td>P, saline vs. GLP-1</td>
<td>0.8888</td>
<td>0.7500</td>
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<tr>
<td>tAUC_Saline cm/min</td>
<td>1,843 (523)</td>
<td>1,942 (329)</td>
<td>0.6031</td>
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<tr>
<td>tAUC GLP-1 cm/min</td>
<td>1,871 (474)</td>
<td>1,940 (289)</td>
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<tr>
<td>P, saline vs. GLP-1</td>
<td>0.6463</td>
<td>0.7695</td>
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<td>Nausea</td>
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<tr>
<td>Baseline_Saline</td>
<td>0.9 (1.3)</td>
<td>0.4 (0.4)</td>
<td>0.8503</td>
</tr>
<tr>
<td>Baseline GLP-1</td>
<td>0.9 (1.1)</td>
<td>0.9 (1.6)</td>
<td>0.9199</td>
</tr>
<tr>
<td>P, saline vs. GLP-1</td>
<td>0.9669</td>
<td>0.3135</td>
<td></td>
</tr>
<tr>
<td>tAUC_Saline cm/min</td>
<td>271 (344)</td>
<td>183 (150)</td>
<td>0.8883</td>
</tr>
<tr>
<td>tAUC GLP-1 cm/min</td>
<td>344 (348)</td>
<td>249 (307)</td>
<td>0.4621</td>
</tr>
<tr>
<td>P, saline vs. GLP-1</td>
<td>0.0663</td>
<td>0.8457</td>
<td></td>
</tr>
</tbody>
</table>

Data are mean values with SD in parentheses from 20 vagotomized subjects with pyloroplasty and 10 control subjects. tAUC, total area under the curve.
The lack of effect of GLP-1 on gastric volume in diabetic patients with vagal neuropathy (14), and suggests that GLP-1 inhibits gastric tone through a mechanism involving the vagus nerve. However, the lack of effect of GLP-1 on gastric emptying observed in the vagotomized subjects could also be a consequence of the pyloroplasty. The mechanism whereby GLP-1 inhibits gastric emptying may involve both reduced antral motility (70) and closure of the pylorus (60). Although the reduction of antral motility probably involves inhibition of vagal outflow (70), the closure of the pylorus is likely to be impeded by the pyloroplasty, and it is therefore plausible that latter is responsible for part of the loss of the GLP-1 effect in our patients. The lack of effect after vagotomy is consistent with the concept that the effect of GLP-1 is vagally mediated.

The accelerated gastric emptying observed in the vagotomized subjects leads to rapid delivery of nutrients to the small intestine, a pattern also seen in gastric bypass-operated subjects (36). This undoubtedly influences many of the parameters in this study e.g., GLP-1 secretion, PG levels, and insulin responses, and must be taken into account when evaluating the results. Nevertheless, the participants serve as their own control, so, although it may be difficult to compare vagotomized to control subjects (because of the difference in gastric emptying and therefore delivery of nutrients to the small intestine), comparison of the effect of saline and GLP-1 infusions within the groups is still justified.

In agreement with the accelerated gastric emptying, PG increased rapidly after the standardized liquid mixed meal in the vagotomized subjects, with peak PG concentrations being much higher than in the control subjects. Despite this, the vagotomized subjects were effective at eliminating glucose from the plasma compartment, with levels returning to baseline after 2 h. Therefore, in accordance with previous observations (33, 34), vagotomy with pyloroplasty is not associated with impaired glucose tolerance, but rather an altered glucose homeostasis. However, despite the very high plasma levels of GLP-1 in the vagotomized subjects after infusion, the fractional lowering of postprandial PG was affected similarly in the two groups. It is unlikely that this is a consequence of changes in gastric emptying, insulin, or glucagon secretion, since GLP-1 had little effect on these parameters in the vagotomized subjects. A study by Corssmit et al. (9) indicated that truncal vagotomy does not affect the fasting (16–22 h) postabsorptive glucose metabolism or the levels of certain regulatory hor-
Vago- or parasympathetic nerves in the postprandial glucose metabolism. Our vagotomized subjects probably failed to secrete endogenous GLP-1 during the GLP-1 infusion, as also shown in rodents (2, 5, 35). On the other hand, the lower glucose levels during the GLP-1 infusion are likely to contribute to the missing response to the high GLP-1 levels under these conditions. Further studies are therefore needed to unravel these relationships. In contrast, in the control subjects, insulin secretion was suppressed during exogenous GLP-1 infusion, presumably because of the inhibition of gastric emptying by GLP-1, as previously shown (47).

Vagotomy with pyloroplasty was also associated with postprandial hyperglucagonemia during saline infusion as well as during GLP-1 infusion, with exogenous GLP-1 failing to suppress glucagon levels, particularly in the early phase of the meal. This is all the more surprising since the elevated GLP-1 levels in the vagotomized subjects would be expected to cause greater suppression (50). It is therefore possible that GLP-1’s glucagonostatic effects may also be impaired after vagotomy.

However, the mechanism, which could include an absent vagal signaling, reduced alpha cell and/or beta cell sensitivity, and/or gastrointestinal factors, remains to be clarified.

GLP-1 levels were higher in the vagotomized group than in the controls after the standardized liquid mixed meal on both days. Based on studies in rodents, it has been claimed that the vagus nerve plays an important role in GLP-1 secretion (58). However, vagotomy and vagus stimulation in pigs did not affect secretion of either GLP-1 or the other incretin hormone, glucose-dependent insulinotropic polypeptide (GIP) (6). Furthermore, in another detailed study in pigs, there were no changes in GLP-1 or GLP-2 during GLP-1 infusion, with exogenous GLP-1 failing to suppress glucagon levels, particularly in the early phase of the meal. This is all the more surprising since the elevated GLP-1 levels in the vagotomized subjects would be expected to cause greater suppression (50). It is therefore possible that GLP-1’s glucagonostatic effects may also be impaired after vagotomy.

Importance of the Vagus Nerve for GLP-1 Effects

GLP-1 after vagotomy, as also shown in rodents (2, 5, 35). On the other hand, the lower glucose levels during the GLP-1 infusion are likely to contribute to the missing response to the high GLP-1 levels under these conditions. Further studies are therefore needed to unravel these relationships. In contrast, in the control subjects, insulin secretion was suppressed during exogenous GLP-1 infusion, presumably because of the inhibition of gastric emptying by GLP-1, as previously shown (47).

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However, the mechanism, which could include an absent vagal signaling, reduced alpha cell and/or beta cell sensitivity, and/or gastrointestinal factors, remains to be clarified.
In conclusion, in vagotomized subjects with pyloroplasty the effect of exogenous GLP-1 on food intake and gastric emptying is diminished. Furthermore, vagotomized subjects with pyloroplasty exhibit altered glucose tolerance and approximately fivefold higher endogenous GLP-1 levels, presumably caused by accelerated gastric emptying. Additionally, vagotomy is associated with reduced insulinotropic and glucagonotropic static effects of exogenous GLP-1. Therefore, the vagus probably plays an important role in mediating the physiological and pharmacological effects of GLP-1. However, further studies aiming at eliminating the effects of the associated pyloroplasty are needed to define more clearly the contributions of vagal transmission to the effects of GLP-1.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


