Electrolyte composition of endoscopically collected duodenal drainage fluid after synthetic porcine secretin stimulation in healthy subjects

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Background: **Traditional pancreatic function tests are sensitive for the diagnosis of pancreatic exocrine insufficiency but are cumbersome and difficult to perform. A sedationless endoscopic pancreatic function test that has the potential for wide clinical application was developed by us, but data on the results of this method in healthy subjects are lacking. This study analyzed endoscopically collected duodenal fluid from healthy subjects after synthetic porcine secretin stimulation.**

Methods: **Healthy subjects underwent the sedationless endoscopic pancreatic function test. After secretin stimulation, duodenal aspirates were obtained every 5 minutes for 1 hour. The collected fluid was analyzed for electrolyte concentrations.**

Results: **Sixteen healthy subjects (8 women, 8 men; median age 34.5 years) underwent the endoscopic pancreatic function test. The concentrations of the sodium ($[Na^+]$) and potassium ($[K^+]$) cations remained constant, similar to normal concentrations in plasma (median $[Na^+]$, 155 mEq/L; median $[K^+]$, 4.3 mEq/L). The concentrations of the bicarbonate ($[HCO_3^-]$) and chloride anions increased and decreased, respectively, in an inverse and reciprocal manner, similar to the previously characterized "secretory curve." The median peak $[HCO_3^-]$ was 108 mEq/L IQR: 99-110). By the 20-minute collection, the $[HCO_3^-]$ was greater than 80 mEq/L for 94% (15/16) of subjects, the historic cut point for $[HCO_3^-]$ in studies based on traditional methods of pancreatic function testing.**

Conclusions: **Endoscopic collection of pancreatic fluid reproduces the anion-cation secretory curve described by prior studies of pancreatic secretory physiology based on traditional collection methods. (Gastrointest Endosc 2004;60:351-5.)**

Direct tests of pancreatic exocrine function, first described by Lagerloef[1] in 1942, are used to evaluate steatorrhea and malabsorption. Hormonally stimulated function tests are considered the most sensitive for detection of early chronic pancreatitis.[2] The traditional method of direct function testing involves collection of duodenal juice by using a double-lumen gastroduodenal tube. The pancreatic secretory curve in healthy subjects was characterized in seminal studies by Dreiling and Hollander[3] and others[4,5] conducted during the 1950s to 1960s, which used this type of collection method. These early experiments defined the well-known normal patterns of basal and stimulated pancreatic electrolyte secretion: (1) concentrations of sodium ($[Na^+]$) and potassium ($[K^+]$)remain constant at all rates of secretion and are approximately isotonic to plasma; (2) as secretory flow increases, the concentrations of bicarbonate ions ($[HCO_3^-]$) and chloride ions ($[Cl^-]$) exhibit a reciprocal relationship—as $[HCO_3^-]$ progressively increases, $[Cl^-]$ decreases.

Although the traditional secretin-stimulated test of pancreatic function is a sensitive indicator of pancreatic insufficiency and may allow early detection of chronic pancreatitis, its use has been largely restricted to a few academic centers. Placement of a gastroduodenal tube is difficult and time consuming for the practicing physician. Moreover, the test is uncomfortable for patients, because it is performed without sedating the patient. Fluoroscopy also is required to confirm proper positioning of the

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tube. Moreover, the fluid is collected over 60 to 80 minutes and requires significant resources. Because of these formidable obstacles, a simpler and more practical methodology is needed if pancreatic function testing is to become clinically useful.

A purely endoscopic method for pancreatic function testing that involves collection of timed duodenal aspirates after stimulation of the pancreas by administration of cholecystokinin (CCK) or secretin was previously described by us.[6,7] This secretin-stimulated endoscopic pancreatic function test (ePFT) has been shown to differentiate patients with chronic abdominal pain from those with moderate to advanced chronic pancreatitis.[7] Another group has similarly found that an endoscopic secretin test differentiates patients with normal and with abnormal pancreatograms.[8] In addition, the ePFT minimizes costs compared with the traditional test of pancreatic function.[6] However, a major criticism of the ePFT has been the lack of data for healthy, normal subjects.

A synthetic porcine secretin now is approved by the Food and Drug Administration for clinical use in the United States and has been found to be equivalent to biologic secretin,[9,10] thereby making it possible to evaluate the results of the ePFT in healthy subjects. The aim of this study was to assess the pancreatic secretory curve and to characterize electrolyte concentrations in the duodenal juice of healthy subjects by using our endoscopic collection method. It was hypothesized that the electrolyte composition of timed duodenal aspirates obtained by using this endoscopic method replicates the pattern observed with the traditional collection method, thereby further validating the use of this test in clinical practice.

PATIENTS AND METHODS

Study population

Healthy, adult subjects capable of giving informed consent were recruited. A focused medical history and examination were obtained for every subject. All women subjects underwent a urine pregnancy test before the ePFT. Our institutional review board approved the research protocol. An equal number of men and women were recruited. Inclusion criteria were the following: age 18 to 65 years, weight 40 to 100 kg, and the ability to give informed consent. Exclusion criteria were the following: pregnancy; allergy or known sensitivity to secretin; a history of alcoholism, illicit drug use, acute or chronic pancreatitis, vagotomy, gastrectomy, inflammatory bowel disease, or liver disease; and recent use of narcotic analgesic or anticholinergic medication.

Endoscopic collection method

Study subjects underwent upper endoscopy without sedation by using an endoscope with a 6-mm-diameter insertion tube (GIF 160-XP; Olympus America Corp., Melville, N.Y.). Before endoscopy, oropharyngeal anesthesia was induced with a topical spray of a local anesthetic (Cetacaine, Cetylite Industries, Pennsauken, N.Y.). Fluid within the stomach was aspirated as completely as possible and was discarded. A test dose (0.2 mcg) of synthetic porcine secretin (ChiRhoClin, Inc., Burtonsville, Md.) then was administered intravenously. If, after 5 minutes, no adverse reaction was observed, a full dose (0.2 mcg/kg) was given. At time "0", a baseline duodenal aspirate was obtained (bottle A). Duodenal aspirates then were obtained at 5-minute intervals for 1 hour (bottles B-M). All duodenal fluid samples were immediately placed on ice and transferred to the laboratory for analysis.

Bicarbonate analysis

Bicarbonate concentrations were determined as total carbon dioxide by a rate pH measurement when using reagents and an auto-analyzer (CX3 Delta; Beckman-Coulter, Brea, Calif.). After acidification of the specimen, bicarbonate forms carbon dioxide gas, which passes through a silicone membrane and results in a rate of pH change in a bicarbonate solution between the membrane and a pH electrode. The rate of pH change is related to the initial [HCO_3^-]. When necessary, fluid specimens were diluted with normal saline solution to bring [HCO_3^-] within the range of measurement for this method.

Statistical methods

Descriptive statistics, such as mean, median, and standard deviation, are reported. Empirical 95% confidence intervals for mean concentrations at each time point were achieved by generating 1000 bootstrapped samples. The average trend with 95% confidence intervals for each electrolyte concentration was plotted over time. To estimate the effects of time, age, and gender on each of the outcome variables, mixed regression models were used to account for repeated measures for each patient. A p value of 0.05 was considered significant for all analyses.

RESULTS

Seventeen healthy subjects were recruited, of which 16 (8 women, 8 men; median age 34.5 years, range 19-51 years) were able to tolerate the sedationless ePFT. One patient could not tolerate intubation, and the procedure was discontinued before secretin administration. No adverse event or reaction to the synthetic porcine secretin was encountered.

A longitudinal analysis was performed to determine the effects of age and gender on electrolyte secretion, adjusted for collection time. None of the electrolyte concentrations were significantly related to age. For men, the median peak [HCO_3^-] was significantly higher compared with women (112 vs.

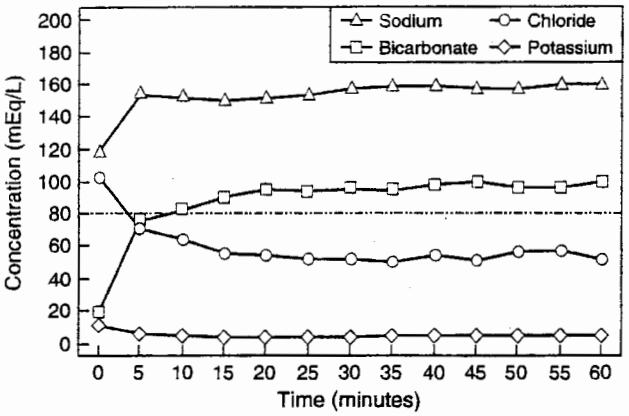

Figure 1. Median electrolyte concentrations vs. time for healthy subjects. Sodium and potassium concentrations remain relatively constant; bicarbonate and chloride concentrations increase and decrease, respectively, in an inverse, reciprocal manner.

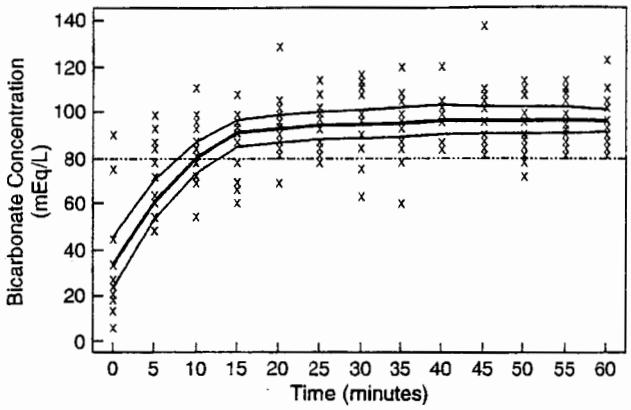

Figure 2. Bicarbonate concentration in duodenal aspirates. Duodenal fluid bicarbonate concentrations are shown for each of the 16 subjects. The average trend *(central line)* is shown, along with 95% "boot-strapped" confidence intervals.

98 mEq/L; $p = 0.003$). Gender did not affect $[Na^+]$ or $[K^+]$.

Electrolyte concentrations

The median $[HCO_3^-]$, $[Cl^-]$, $[Na^+]$, and $[K^+]$ were calculated for each time point (Fig. 1). The $[Na^+]$ and $[K^+]$ remained relatively constant (approximately isotonic to plasma). The median $[Na^+]$ for all time points was 155 mEq/L (interquartile range [IQR] 150-160). The median $[K^+]$ for the entire collection period was 4.3 mEq/L (IQR 4.0-4.9).

The anion concentrations exhibited the expected inverse relationship: as $[HCO_3^-]$ increased across the collection period, $[Cl^-]$ decreased reciprocally. The median $[HCO_3^-]$ was 90 mEq/L (IQR 83-102); mean $[Cl^-]$ was 60 mEq/L (IQR 47-72). The peak $[HCO_3^-]$ and nadir $[Cl^-]$ were calculated for each patient. The median peak $[HCO_3^-]$ for all patients was 108 mEq/L (IQR 99-110). All patients had a peak $[HCO_3^-]$ greater than 80 mEq/L. The median nadir $[Cl^-]$ was 44 mEq/L (IQR 37-51).

For all patients, the $[HCO_3^-]$ plotted for each time point is shown in Figure 2. The proportion of patients at each time point who had a $[HCO_3^-]$ greater than the traditional cut point, 80 mEq/L,[11] are shown in Table 1. By the 20-minute collection, 94% (15/16) of the healthy subjects had a $[HCO_3^-]$ greater than 80 mEq/L. In a mixed regression model, no significant differences were found between mean $[HCO_3^-]$ at 20 minutes and thereafter.

Table 1. Proportion of healthy subjects with duodenal fluid $[HCO_3^-] > 80$ mEq/L

Time (min)	0	5	10	15	20	25	30
No. with $[HCO_3]$ > 80 mEq/L	1	7	11	12	15	15	14
Percentage $[HCO_3]$ > 80 mEq/L	0	44	69	75	94	94	88
Time (min)	35	40	45	50	55	60	
No. with $[HCO_3]$ > 80 mEq/L	14	16	16	15	16	16	
Percentage $[HCO_3]$ > 80 mEq/L	88	100	100	94	100	100	

DISCUSSION

The present study demonstrates that the ePFT reproduces the electrolyte secretory pattern of previous studies of pancreatic physiology that used the traditional method for collection of duodenal pancreatic juice. This suggests that the measurement of $[HCO_3^-]$ in timed endoscopic aspirates is comparable with measurement in continuous collections obtained via a gastroduodenal tube. These results further support the use of the ePFT in clinical practice.

The $[HCO_3^-]$ and $[Cl^-]$ in endoscopically collected duodenal aspirates were shown in the present study to increase and decrease, respectively, after augmentation by secretin. This observation is consistent with hypotheses for the reciprocal relationship between bicarbonate and chloride secretion. First, the exchange-diffusion hypothesis states that bicarbonate secreted by the acinar cells is exchanged for chloride as it moves through the pancreatic ducts. After stimulation by secretin, increased secretion causes an increased flow through the ducts, allowing less time for bicarbonate-chloride exchange and, thus, a higher concentration of bicarbonate in the resulting fluid.[12] Second, the two-component hypothesis states that acinar cells secrete sodium and

chloride-rich fluid at a low but constant rate. After stimulation of the pancreas by secretin, the chloride concentration is progressively diluted by large volumes of bicarbonate-rich fluid, leading to the characteristic reciprocal bicarbonate and chloride curves.[13]

The constancy and isotonicity of [Na^+] and [K^+] likewise were reproduced with the endoscopic method of collection. Interestingly, the first collection (time "0") tended to have lower [Na^+] and higher [K^+]. The most plausible explanation for this is contamination of the duodenal fluid aspirate with gastric juice. The time "0" fluid is collected after aspiration and discard of gastric fluid. It is likely that gastric juice (typically lower in [Na^+]) remains within the accessory channel of the endoscope and thereby contaminates the first sample of fluid. This could be precluded by aspirating and discarding more duodenal fluid (>5 mL) before the first collection of fluid for analysis, effectively "priming" the collection channel.

The ePFT has several potential advantages: (1) compared with the traditional method for testing pancreatic function, it is easy to perform, requires standard endoscopic equipment and technique, and is thus suitable for use in clinical practice; (2) less time is required compared with the standard gastroduodenal tube test, even if timed collections are obtained for a full hour; (3) endoscopic examination in conjunction with the ePFT rules out other causes of abdominal pain, such as peptic disease and malignancy; (4) fluoroscopy is not required, as with the traditional method, thereby eliminating exposure of patients and personnel to ionizing radiation; and (5) a technical charge has been established for the endoscopic collection of biliary and pancreatic fluid (CPT 89105).

Although evidence to support its use in clinical practice is growing, several criticisms of endoscopic pancreatic function testing have been raised.[14] One potential drawback is the need to administer sedative and analgesic drugs before and during endoscopy. Because of the possible effect of such agents on pancreatic secretion, traditional pancreatic function testing has been performed without sedation, which makes the test less practical. Although the concern that narcotic medications affect pancreatic secretion may be valid,[15] Saunders et al.[16] found that benzodiazepines do not alter pancreatic electrolyte secretion. In the present study, the need for conscious sedation was avoided by using an "ultrathin" endoscope. Almost all patients tolerated the endoscopic test for 1 hour, confirming the feasibility of a sedationless test. Unsedated endoscopy with ultrathin endoscopes generally has been found to be tolerable and, therefore, acceptable to patients.[17] However, further study of the effect of standard sedative and analgesic drugs on ePFT are needed.

Another criticism is that a full hour is required for performance of the ePFT. However, this actually represents a significant improvement over the traditional method. From 40 to 80 minutes are required to place a Dreiling tube (with or without endoscopic assistance) or two shingle-lumen tubes (CCK test) before testing can begin. When all necessary steps are considered, a traditional secretin or CCK test can require 1.5 to 2 hours. Thus, the ePFT requires less time overall, although it may still be too time consuming for use in clinical practice. Attempts have been made to develop a shortened test, either through a single timed collection of duodenal fluid[14] or by continuous collection of pure pancreatic fluid for a short time.[18,19] In most studies, the results obtained with these techniques have been suboptimal.[20]

To our knowledge, no study has analyzed the effects of gender on pancreatic secretion. The results of the present study suggest that secretin-stimulated [HCO_3^-] may be higher in men. This difference is intriguing and may relate to hormonal influences. Despite the observed differences, peak [HCO_3^-] greater than 80 mEq/L, the traditional cut point, were obtained in all patients. Further studies in larger numbers of subjects are needed to determine whether there are gender-related differences in pancreatic secretion.

In conclusion, the present study provides further evidence to support the validity of the secretin-stimulated ePFT. The data indicate that the ePFT replicates the pancreatic secretory curve and accurately describes pancreatic electrolyte outputs. Furthermore, the peak [HCO_3^-] cut point (80 mEq/L) used in traditional tests of pancreatic function also may be applicable for endoscopic timed aspirates. Furthermore, it may be possible to shorten the duration of the ePFT, because the optimal timing of duodenal fluid collection can be inferred based on the secretory curve. For definitive validation, rigorous comparison studies of the secretin-stimulated ePFT vs. the traditional gastroduodenal tube method in normal subjects and in patients with chronic pancreatitis are needed.

DISCLOSURE

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