Non-invasive assessment of gastrointestinal motility disorders in diabetic patients with and without cardiovascular signs of autonomic neuropathy

B Werth, B Meyer-Wyss, G A Spinas, J Drewe, C Beglinger

Abstract

Twenty six patients with insulin dependant diabetes mellitus underwent a gastric emptying test, a gall bladder contraction test, an orocaecal transit study, and a colon transit test. Eleven patients had signs of cardiovascular autonomic neuropathy, 15 patients were without signs of cardiovascular autonomic neuropathy. Mean gastric clearance of radioopaque markers ingested with a meal averaged 29.5 (2.3) markers per six hours in subjects without cardiovascular autonomic neuropathy compared with 17.8 (2.3) markers per six hours in patients with cardiovascular autonomic neuropathy (p<0.02). Gall bladder emptying in response to graded CCK8 stimulation was impaired in five of 11 patients with cardiovascular autonomic neuropathy, whereas it was normal in the patients without cardiovascular autonomic neuropathy (p<0.01). Oral caecal transit times were not significantly different in the two patient groups, whereas colonic transit was slower in the patients with cardiovascular autonomic neuropathy compared with the group without cardiovascular autonomic neuropathy (p<0.02). There was no correlation between disturbed gastric clearance, impaired gall bladder contraction, and prolonged colonic transit time in the patients with cardiovascular autonomic neuropathy nor was there a correlation between any disturbed motor function and age or duration of diabetes. It is concluded that autonomic neuropathy can affect motor functions throughout the gastrointestinal tract. Any disturbed motor function in the gut could therefore be one of the numerous expressions of diabetic neuropathy affecting the cardiovascular, the endocrine or the gastrointestinal system.

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Autonomic neuropathy is a frequent complication of long standing diabetes mellitus. Clinically autonomic neuropathy is well known to produce symptoms caused by cardiovascular dysfunction and cardiovascular reflexes are used to diagnose autonomic neuropathy. The gastrointestinal tract, however, is another area that may become severely affected by autonomic neuropathy. The prevalence of symptoms caused by gastrointestinal dysfunction may reach 76% in an unselected population of diabetic outpatients. Delayed gastric emptying as a result of gastroparesis is probably the best known and most extensively evaluated motor disturbance of the gastrointestinal tract in diabetic patients. Other parts of the digestive system may, however, also be affected and in a number of different studies constipation and diarrhea as well as impaired gall bladder contraction in diabetic patients have been attributed to autonomic neuropathy. Despite these many reports, a comprehensive evaluation of the motor function at different levels of the gastrointestinal tract in diabetic patients has never been carried out. Thus, in the present study we have evaluated gastric clearance of indigestible markers, orocaecal transit time, colonic transit time, and gall bladder contraction in two groups of diabetic patients who differed as to whether they had or did not have signs of autonomic neuropathy affecting the cardiovascular system.

Methods

PATIENTS

Diabetic patients attending our outpatient clinic were evaluated for participation in this study. Patients were selected on a random, consecutive basis. They were asked to participate only if they had no history of gastrointestinal diseases or abdominal surgery other than appendectomy, and if they had no symptoms that could be attributed to gastrointestinal motility disorders. Twenty six patients in whom the presence or absence of autonomic neuropathy affecting the cardiovascular system was established participated in this study. The study protocol was approved by the Human Ethical Committee of the University of Basel and written informed consent was obtained from each subject. Clinical data of the patients studied are given in Table I.

ASSESSMENT OF CARDIOVASCULAR AUTONOMIC NEUROPATHY

Patients were considered to have cardiovascular autonomic neuropathy if at least two of four cardiovascular tests were found to be pathological. None of the patients was taking any

<table>
<thead>
<tr>
<th>TABLE I</th>
<th>Clinical data of 26 diabetic patients with and without cardiovascular signs of autonomic neuropathy (CAN)</th>
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<tbody>
<tr>
<td>Patients with CAN</td>
<td>Patients without CAN</td>
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<tr>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Patients (n)</td>
<td>11</td>
</tr>
<tr>
<td>Age (yr), mean and range</td>
<td>41 (21–64)</td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>7/4</td>
</tr>
<tr>
<td>Diabetes type (IDDM/NIDDM)</td>
<td>10/1</td>
</tr>
<tr>
<td>Diabetes duration (yr)</td>
<td>22 (10–53)</td>
</tr>
<tr>
<td>HbA1C (%)</td>
<td>7.5 (6.1–9.2)</td>
</tr>
<tr>
<td>Retinopathy</td>
<td>9 (82%)*</td>
</tr>
<tr>
<td>Peripheral neuropathy</td>
<td>6/5(55%)</td>
</tr>
<tr>
<td>Nephropathy</td>
<td>5 (42%)*</td>
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</table>

*p<0.01; †p<0.04; ‡p<0.068.
medication known to interfere with cardiovascular reflexes. The tests for cardiovascular autonomic neuropathy assessment included beat-to-beat variation at rest\(^1\) and during forced respiration,\(^2\) heart rate response to standing,\(^3\) and Valsalva manoeuvre.\(^4\) The tests were done using the ProSciCard Program (Pro Science GmbH, Linde, FRG) whereby a standard electrocardiograph (ECG) recording was amplified by an ECG preamplifier and fed to a Pro Science laboratory computer. All tests were performed after overnight fasting and before injecting the morning insulin dose. The test procedures were as follows:

**Beat-to-beat variation at rest**
After the patient had remained in a recumbent position for at least 10 minutes and the heart rate had reached a steady state, R-R intervals were recorded continuously for five minutes or over 170 beats. From the first 150 R-R intervals free of artefacts, the coefficient of variation as well as the root mean square of successive differences were computed.

**Beat-to-beat variation during forced respiration**
The patient was asked to breathe deeply at a rate of six breaths per minute, the inspiration lasting for four seconds, the expiration for six seconds.\(^5\) Breathing rhythm was established by the movement of a bar graph displayed on the computer screen. Coefficient of variation and root mean square of successive differences were calculated for 100 R-R intervals free of artefacts. From the breathing cycle with the maximal heart rate variation, the longest (R-Rmax) and the shortest R-R interval (R-Rmin) were determined and the difference R-Rmax–R-Rmin as well as the quotient R-Rmax/R-Rmin (E/I ratio) calculated. In addition, the 'mean circular resultant'\(^6\) was computed. This parameter was particularly independent of the intrinsic heart rate.

**Heart response to standing (Ewing test)**
While the ECG was recorded, the patient was asked to change from supine to standing position. The ratio between the length of the R-R intervals at beats number 30 and 15 after standing (R-R30/R-R15) were calculated. In addition, a modified quotient was calculated based on the shortest R-R interval between beat number 5 and 25 and the longest interval between beat number 21 and 45.

**Heart rate response to Valsalva manoeuvre**
The sitting patient was asked to blow into a mouthpiece connected to a manometer held at 40 mm Hg pressure for 15 seconds and then to breathe normally. Timing was controlled for by optical and acoustical computer signals. The ratio between the longest R-R interval during the first 15 seconds after the manoeuvre and shortest R-R interval during the manoeuvre was calculated. Test results were compared with age related reference values derived from a study on 168 non-diabetic healthy subjects.\(^7\)

**Experimental procedures: assessment of gastrointestinal motor functions**

**Gall bladder contraction**
Gall bladder contraction was assessed by high resolution real time ultrasonography (Aloka SSD650) with a 3-5 MHz probe on a sector scanner. Details on the procedure have been published previously.\(^8\) Longitudinal sonograms of the gall bladder were recorded on film. Scans were obtained by manipulating the transducer so that it followed the appropriate long axis of the gall bladder and the largest gall bladder diameters at each time were recorded. No gall stones, gall bladder wall thickening, or other pathology were identified in any subject. Gall bladder volumes were calculated from the measured parameters with the software of the ultrasound unit, assuming that the shape of the gall bladder approximated a solid with elliptical sections, the eccentricity depending on that at the level of the greatest transverse section. These assumptions and the mathematical formula used to calculate the volume have been described and validated by Everson and colleagues.\(^9\)

All patients were studied after overnight fasting. After a basal period of 30 minutes, increasing doses of synthetic cholecystokinin octapeptide (CCK8) were infused intravenously, each dose for 30 minutes (2-4, 7-2, 21-6 pmol/kg/hour). Every 10 minutes, three images of the gall bladder were taken and the mean of the three measurements was taken as the response at this time point.

**Gastric clearance of indigestible markers**
Gastric emptying was assessed on a different day after overnight fasting by means of 10 radio-opaque markers ingested with a standard test meal.\(^10\) These markers were custom made polyurethane particles impregnated with barium sulphate, of density 1-7 g/ml, measuring 2\(\times\)2\(\times\)10 mm. Radiographs of the upper abdomen (24\(\times\)30 cm) were taken 1, 5, 3, 4-5, and 6 hours after a meal and marker ingestion by high voltage technique (100 kV; 20 mAs) to minimise radiation exposure. Two observers independently assessed the films to count the number of markers retained in the stomach. If all markers had passed from the stomach in less than six hours, subsequent radiographs were not taken. The test meal consisted of 10 radioopaque markers, orange juice, crunchy nut cornflakes, 100 ml milk, and biscuits with cheese amounting to a total of 69-5 g carbohydrates, 10-4 g fat, and 7-8 g protein (413 kcal). Twenty minutes before ingestion of the meal, the usual insulin dose was administered by the patients. The meal was eaten within five minutes.

**Orocaecal transit time**
Orocaecal transit time was measured by hydrogen breath analysis.\(^11\) The test meal contained 12 ml lactulose and was eaten after overnight fasting by each subject within five minutes, again 20 minutes after insulin injection. End expiratory breath samples were collected at baseline before intake of the meal and then every 15 minutes for

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11. End expiratory breath samples were collected at baseline before intake of the meal and then every 15 minutes for
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up to five hours using a commercially available double bag designed to collect alveolar air. Hydrogen concentration (ppm) in the breath samples was measured on a hydrogen analyser (GMI Medical, Renfrew, Strathclyde, Scotland). Orocaecal transit time was defined as the time between meal intake and initial rise in hydrogen concentration of at least 10 ppm above fasting levels. The tests were done in a quiet room with patients sitting at a table.

**Colonic transit time**

Colonic transit time was assessed by means of radioopaque markers. The subjects ingested three sets of 20 markers at 24 hour intervals and a single abdominal radiograph was taken of the subject supine 24 hours after ingestion of the last set of markers. Two investigators independently evaluated the films to count the number of markers within the colon. Colonic transit time was calculated as 1-2× the sum of markers in the colon.

**STATISTICAL ANALYSIS**

Results are expressed as mean (SEM) if not specified otherwise. Statistical evaluation was performed by analysis of variance, Student's t test or χ² tests where appropriate.

**Results**

Twenty six diabetic patients, of whom 22 were insulin dependent and four non-insulin-dependent (Table 1), were studied. Eleven of these patients had signs of autonomic neuropathy affecting the cardiovascular system and in 15 patients there were no signs of autonomic cardiovascular dysfunction. The two patient groups did not differ with respect to age and gender, but there were significant differences as to mean Hb A₁C levels, in duration of diabetes and the presence of retinopathy (Table 1).

**GALL BLADDER CONTRACTION**

Mean fasting gall bladder volumes were similar in the two patient groups (17.2 (2.8) ml in patients with cardiovascular autonomic neuropathy and 17.9 (1.6) ml in patients without). Intravenous infusion of graded doses of cholecystokinin 8 induced a dose dependent and significant decrease in gall bladder volume in both groups of patients. Gall bladder contraction was significantly (p<0.05) weaker, however, in patients with cardiovascular autonomic neuropathy as compared with those without (Fig 1) as in the former patients only the highest dose of cholecystokinin 8 induced a significant decrease of gall bladder volumes. In patients without cardiovascular autonomic neuropathy infusion of dose – the highest of cholecystokinin 8 induced after 30 minutes a reduction of the gall bladder volume to 19 (5)% of fasting volume. In contrast, in patients with cardiovascular autonomic neuropathy the gall bladder volume decreased in response to the same dose of cholecystokinin 8 to only 51 (6)%, the difference being highly significant (p<0.01). Gall bladder contraction during cholecystokinin 8 infusion at doses reproducing physiological blood levels – that is, up to 7.2 pmol/kg/hour) was impaired in five of 11 patients with cardiovascular autonomic neuropathy whereas it was normal in the patient group without cardiovascular autonomic neuropathy according to the criteria of Pomeranz and Shaffer.

**GASTRIC CLEARANCE OF INDIGESTIBLE MARKERS**

Mean gastric clearance of radioopaque markers ingested with the test meal was significantly (p<0.02) faster in patients without cardiovascular autonomic neuropathy as compared to patients with cardiovascular autonomic neuropathy (Fig 2). The area under the emptying curve (number of markers×h) averaged 29·5 (2·3) in patients without cardiovascular autonomic neuropathy compared with 17·8 (4·3) in

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Figure 1: Gall bladder contraction in response to graded intravenous doses of CCK8 in 11 diabetic patients with cardiovascular autonomic neuropathy (CAN) and 15 diabetic patients without CAN (mean (SEM)).

Figure 2: Cumulative gastric clearance of radioopaque markers in 11 diabetic patients with cardiovascular autonomic neuropathy (CAN) and 15 diabetic patients without CAN (mean (SEM)).
patients with cardiovascular autonomic neuropathy (p<0.02). Three of 11 patients with cardiovascular autonomic neuropathy (27%) had a gastric emptying test considered abnormal according to the criteria of Feldman and coworkers26 and our own normal values, although none of these patients had symptoms that could be attributed to the late gastric emptying.

**Oroaeval Transit Time**

Mean orocecal transit time of the test meal containing lactulose, assessed by hydrogen breath analysis was similar in the two patient groups: in patients without cardiovascular autonomic neuropathy it averaged 170 (19) minutes and in patients with cardiovascular autonomic neuropathy 138 (19) minutes. These results are not statistically different.

**Colonic Transit Time**

Mean colonic transit time was 35.4 (4.7) hours in patients without cardiovascular autonomic neuropathy, being much shorter (p<0.02) than in patients with cardiovascular autonomic neuropathy in whom it averaged 53.8 (5.5) hours. The results of segmental transit time are given in Table II. Transit time tended to be slower in all three segments of the colon (right, left, and rectosigmoid colon) in patients with cardiovascular autonomic neuropathy although the differences reached statistical significance in the right colon only.

**Correlations**

We have calculated potential correlations between any disturbed motor function and the presence or absence of cardiovascular autonomic neuropathy. Furthermore, we have analysed potential correlations between individual motor function disturbances and age or duration of diabetes using univariate histograms and bivariate scatter plots (generalised draftman’s display27). In no instance did we find any significant correlation (data not shown).

**Discussion**

There is good evidence that gastrointestinal motor functions become impaired as diabetic patients develop autonomic neuropathy. Interdigestive motor complexes are absent,25 postprandial antral motor activity is decreased,24 gastric emptying of radioopaque is delayed,26 and gall bladder contraction is reduced.26 A comprehensive evaluation of the motility at different levels of the gastrointestinal tract in diabetic patients, however, has not been so far reported. In the present study, we classified asymptomatic diabetic patients into two groups according to presence or absence of cardiovascular signs of autonomic neuropathy using standardised criteria.26-28 Motility was then assessed in each patient by non-invasive tests: gastric emptying was measured by means of solid radioopaque markers, gall bladder contraction in response to cholecystokinin 8 stimulation by ultrasonography, mouth-to-caecum transit time by using the lactulose hydrogen breath test, and colonic transit time again by means of indigestible radioopaque markers.

Gastric clearance of indigestible markers was significantly slower in patients with than in those without cardiovascular autonomic neuropathy. Gastric clearance had to be considered abnormal in three asymptomatic patients with cardiovascular autonomic neuropathy according to Feldman and coworkers as well as to our own criteria: in healthy subjects all 10 markers were emptied from the stomach by six hours. Thus, a radiograph six hours after meal intake showing incomplete gastric emptying of markers indicates delayed gastric emptying. Emptying of radioopaque markers does not reflect gastric emptying of food components, as it is known that indigestible particles larger than 1.5 mm are emptied from the stomach during the interdigestive period.28 It has, however, been suggested that clearance of radioopaque markers is a more sensitive test than standard radionuclide scintigraphy for documenting gastric motor dysfunction in diabetics.29 Here we extend these observations by showing that 25% of asymptomatic diabetic patients with cardiovascular autonomic neuropathy have abnormal gastric emptying of indigestible particles. This abnormality can be documented with a simple test that is readily available.

The frequency of gall bladder motor disorder in patients with diabetes mellitus is still controversial. Enlargement of fasting gall bladder volumes as well as impaired gall bladder contraction in response to sham feeding, to oral meal intake or to hormonal stimulation has been reported.26,27,28 In the present study we found that five asymptomatic diabetic patients with cardiovascular autonomic neuropathy had impaired gall bladder emptying in response to graded doses of cholecystokinin 8. These results closely agree with a study of Stone and coworkers30 who, using cholescintigraphy, observed a 21% reduction of gall bladder emptying in response to a maximal dose of cholecystokinin 8 in diabetic patients with cardiovascular autonomic neuropathy in comparison with patients without cardiovascular autonomic neuropathy. Discrepant results have been described by Fiorrucci and coworkers who were unable to confirm any difference in gall bladder emptying rates induced by hormonal stimulation.28 The discrepancy with this latter study and our present results cannot easily be explained. In both studies, gall bladder emptying was determined by ultrasonography and dose response curves were determined in both studies. Fiorrucci and coworkers, however, infused the synthetic cholecystokinin

<table>
<thead>
<tr>
<th>Table II: Total and segmental colonic transit times (hours) in 26 diabetic patients (mean (SEM))</th>
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<tbody>
<tr>
<td>Patients with CAN (n=11)</td>
</tr>
<tr>
<td>--------------------------</td>
</tr>
<tr>
<td>Total</td>
</tr>
<tr>
<td>Right colon</td>
</tr>
<tr>
<td>Left colon</td>
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<tr>
<td>Rectosigmoid</td>
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*p<0.02; CAN = cardiovascular autonomic neuropathy.
analogue caerulein, whereas we used human cholecystokinin 8. Because the relative molar potency of caerulein compared with that of the naturally occurring cholecystokinin for gall bladder contraction is not known, decreased gall bladder contraction in the study of Fiorrucci could have been masked by using caerulein.

Breath hydrogen analysis after oral lactulose containing meals is an easy, non-invasive, bedside test to measure mouth-to-caecum transit time. The test has several shortcomings, however: (i) it can only be accurately interpreted, if the small intestinal flora is normal; (ii) it requires a bacterial flora in the caecum which is capable of producing \( \text{H}_2 \); (iii) it only measures the transit of the head of the meal; and (iv) its reproducibility has been questioned. Its main advantage is the simplicity of the test. In the present study, we could not detect a significant difference in orocecal transit time between patients with and patients without cardiovascular autonomic neuropathy. We believe that methodological limitations of the lactulose hydrogen breath test may explain the present results and account for the reported controversies.

Colonic transit time was significantly longer in the patients with cardiovascular autonomic neuropathy. This resulted from slower regional transit through all three parts of the colon, although the difference was significant for the right colon only. These findings agree with a previous study of diabetic neuropathy where constipation was the most common gastrointestinal symptom. It has to be pointed out, however, that we selected asymptomatic patients and thus total colonic transit times have still to be considered in the high normal range.

The present study confirmed and extended previous observations that different gut motor functions can be impaired in diabetic patients with cardiovascular autonomic neuropathy. We did not find a correlation between disturbed gastric emptying, impaired gall bladder contraction, and prolonged colonic transit time in the patients with cardiovascular autonomic neuropathy; also, there was no correlation between any disturbed motor function and age or longstanding history of diabetes. These findings support the hypothesis that autonomic neuropathy affects motility throughout the gastrointestinal tract. Any disturbed motor function in the gut could therefore be one of the numerous expressions of diabetic neuropathy which can affect the cardiovascular, the endocrine or the gastrointestinal system. The lack of correlation between the four motility parameters further more implies that the disturbed motor functions develop independently and can affect any gut system. From our results one could speculate that impairment of gall bladder contraction is a sensitive parameter for the diagnosis of autonomic neuropathy in the gastrointestinal tract.

Such a hypothesis requires, however, confirmation in a large group of patients.

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