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[^{13}C]Octanoic acid breath test for non-invasive assessment of gastric emptying in diabetic patients: validation and relationship to gastric symptoms and cardiovascular autonomic function

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Summary Since there is a need for a widely applicable non-invasive test to assess gastric emptying in diabetic patients, we evaluated the sensitivity, specificity, and reproducibility of the [^{13}C]octanoic acid breath test as compared with scintigraphy. Moreover, we examined the relationship between the breath test indices and gastric symptoms, cardiovascular autonomic function, and metabolic parameters. Forty healthy control subjects and 34 diabetic patients were studied. Three indices of gastric emptying, assessed by the breath test, were computed: half-emptying time ($t_{1/2breath}$), gastric emptying coefficient (GEC), and lag phase. Furthermore, the half-emptying time, measured by scintigraphy ($t_{1/2scint}$), was calculated and gastric symptoms and cardiovascular autonomic neuropathy (CAN) were scored. The coefficients of variation of day-to-day reproducibility in 10 healthy subjects were 29.6 % for $t_{1/2breath}$, 7.4 % for GEC, and 46.5 % for lag phase. An abnormal delay for $t_{1/2scint}$ (> 100 min) or $t_{1/2breath}$ (> 200 min) was noted in 12 patients. Based on the results for $t_{1/2scint}$, the sensitivity of $t_{1/2breath}$ and GEC was 75 % and the specificity was 86 %. Both $t_{1/2breath}$ ($r_s = 0.523$; $p < 0.05$) and GEC ($r_2 = -0.594$; $p < 0.05$) were significantly associated with the gastric symptom score. A significant relationship to the CAN score was demonstrated for $t_{1/2breath}$ ($r_s = 0.448$; $p < 0.05$), GEC ($r_s = -0.467$; $p < 0.05$), and $t_{1/2scint}$ ($r_s = 0.602$; $p < 0.05$). There were no significant associations of the breath test indices with the blood glucose levels during the test, HbA_{1c}, age, and duration of diabetes. In patients with abnormal $t_{1/2scint}$ ($n = 12$) not only was $t_{1/2breath}$ significantly prolonged and GEC reduced, but also the scores of CAN and gastric symptoms were significantly increased as compared with those who had a normal $t_{1/2scint}$ ($n = 22$). We conclude that the [^{13}C]octanoic acid breath test represents a suitable measure of delayed gastric emptying in diabetic patients which is associated with the severity of gastric symptoms and CAN but not affected by the blood glucose level. [Diabetologia (1996) 39: 823–830]

Keywords Gastric emptying, diabetic gastroparesis, scintigraphy, breath test, cardiovascular autonomic function.

Severe diabetic gastroparesis is associated with considerable morbidity from disabling symptoms that may be difficult to manage, metabolic instability, and poor quality of life [1, 2]. Although a relatively high prevalence of gastrointestinal symptoms has been shown in both diabetic and non-diabetic subjects [3], these symptoms may be absent in diabetic patients with gastroparesis, possibly due to an involvement of visceral afferent nerve fibres [4]. The scintigraphic technique is generally accepted as the 'gold standard' for the evaluation of disordered gastric emptying in diabetic patients. However, it is associated with exposure to radiation and requires expensive equipment with limited availability [1]. Hence, an alternative non-invasive approach is needed particularly to allow

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Abbreviations: CAN, Cardiovascular autonomic neuropathy; CV, coefficient of R-R interval variation; GEC, gastric emptying coefficient; LF, low-frequency; MCR, mean circular resultant; MF, mid-frequency; ROI, region of interest; $t_{1/2breath}$, breath test half-emptying time; $t_{1/2scint}$, scintigraphic half-emptying time.

for clinical application in metabolically unstable asymptomatic patients as well as assessment in epidemiological and prospective studies. A suitable candidate for this purpose could be the newly developed [^{13}C]octanoic acid breath test that allows measurement of the gastric emptying rate of solids [5]. The indices of this test have been shown to correlate closely with the scintigraphic half-emptying time in healthy subjects [5] and to discriminate between different pharmacological effects on gastric emptying of solids [6]. However, it remains to be established whether the [^{13}C]octanoic acid breath test provides a reliable method for measuring gastric emptying of solids in diabetic patients [7]. Recently, a correlation has also been demonstrated between the scintigraphic half-emptying time of liquids and the [^{13}C]acetate breath test [8].

Several factors including hyperglycaemia [9, 10], hyperinsulinaemia [11], obesity [12, 13], and cardiovascular autonomic neuropathy (CAN) [14–17] have been found to be associated with delayed gastric emptying in both non-diabetic and diabetic subjects. In contrast, several studies have found either no relationship to CAN [7, 9, 18], an association of obesity with more rapid gastric emptying [19], or no correlation between gastrointestinal motility and hyperglycaemia [15, 20]. Furthermore, it has been emphasized that the association of delayed gastric emptying with gastrointestinal symptoms is relatively poor or lacking and therefore a causal relationship between them appears unlikely [1, 21]; however, a significant correlation has also been described [22].

The aim of the present study was to evaluate the sensitivity, specificity, and reproducibility of the [^{13}C]octanoic acid breath test in the assessment of gastric emptying as compared with scintigraphy in healthy subjects and diabetic patients and to examine the possible associations of the breath test parameters with the severity of upper gastrointestinal symptoms, cardiovascular autonomic neuropathy, and blood glucose levels.

Subjects and methods

Subjects. After obtaining informed consent, 34 insulin-treated diabetic patients were recruited from the in patient clinic of the Diabetes Research Institute. Criteria for entry into the study included the presence of either insulin-dependent or non-insulin-dependent diabetes mellitus classified according to the criteria of the National Diabetes Data Group [23], duration of diabetes 3 years or more, and fasting blood glucose levels prior to the assessment of gastric emptying between 4.5 and 15.5 mmol/l. Patients were excluded if they had a history of gastrointestinal surgery, peptic ulcer, cirrhosis of the liver, were taking medication that may influence gastric emptying and autonomic nerve function as well as evidence of neuropathy other than of diabetic origin, unstable angina pectoris, heart failure, arrhythmias, and peripheral arterial disease with intermittent claudication. Measurement of gastric emptying and cardiovascular autonomic and peripheral nerve function was performed in the absence of ketonuria for at least 1 week.

[^{13}C]Octanoic acid breath test. The ^{13}C-labelled octanoic acid breath test was performed as previously described by Ghoos et al. [5]. In brief, after an overnight fast the subject was given a test meal consisting of a scrambled egg with the yolk doped with 100 µl [^{13}C]octanoic acid (1–^{13}C, 99 %; Promochem, Wesel, Germany). Yolk and egg white were baked separately but ingested together with two slices of white bread and 5 g of margarine, followed immediately by 150 ml of water. The test meal comprised approximately 250 kcal (42 % carbohydrates, 18 % protein, 40 % fat) and was consumed within 10 min between 08.00 and 08.30 hours, 30 min after s.c. injection of the usual insulin. The insulin dose was adjusted according to the fasting blood glucose and the meal modification. The principle of the test is based on a prompt solubilization of octanoic acid in egg yolk and disintegration of the labelled solid phase in the duodenum, followed by a rapid absorption by the intestinal cells and preferential oxidation to $^{13}CO_2$ in the liver [5, 24–26]. The appearance of $^{13}CO_2$ in breath is primarily determined by the rate of delivery of the test meal from the stomach into the duodenum [5]. During the 4-h test period the subjects remained in the sitting position in a quiet room. Breath samples were collected using aluminium-coated bags with a volume of 1.5 litres (Tecobag; Tesseraux Container, Bürstadt, Germany), then taken from the bags and filled in 10-ml vacuum tubes (Vacutainer; Becton Dickinson, Heidelberg, Germany) at baseline (0 min) and thereafter at 10-min intervals during the first hour and every 15 min for the remaining 3 h. $^{13}CO_2$ in the breath samples was analysed using a Finnigan MAT Model 251 isotope ratio mass spectrometer (IRMS) (Finnigan, Bremen, Germany). The Pee-Dee-Belemnite (PDB) standard was used for calibration. Endogenous CO_2 production was assumed to be 5 mmol per m^2 of body surface per min [27]. Body surface was calculated using the weight-height formula suggested by Haycock et al. [28]. The exhalation of $^{13}CO_2$ in breath, i.e. the change of the $^{13}C/^{12}C$ ratio, was determined as the difference above baseline and the results were expressed as a percentage of the ^{13}C recovery per min and cumulatively over 4 h. Mathematical curve fitting using non-linear regression analysis was performed according to Ghoos et al. [5]. The following parameters were computed as suggested by Ghoos et al. [5]: half emptying time: $t_{1/2breath} = (-1/k) \cdot \ln(1-2^{-(1/\beta)})$,-66; gastric emptying coefficient (GEC): given as ln (a) in the formula $y(t) = a \cdot t^b \cdot e^{-ct}$; and lag phase: $t_{lagb} = (1/k) \cdot \ln(b)$-66.

Normal ranges for the half-emptying time and GEC of the [^{13}C]octanoic acid breath test were established in 40 healthy control subjects. Their clinical characteristics are given in Table 1. The day-to-day reproducibility of $t_{1/2breath}$, GEC, and lag phase was assessed in 10 of these healthy subjects with test-retest intervals of up to 1 week.

Scintigraphy. Scintigraphic studies of gastric emptying were performed as previously reported [29]. Patients were studied in the fasting state at 08.00 hours. They injected their usual insulin dose 30 min prior to the test meal which consisted of a pancake (Diamant Backmischung für Eier-Pfannkuchen; Plange, Hamburg, Germany), dietary marmalade, and 400 ml of mineral water. In total the test meal comprised approximately 330 kcal (54 % carbohydrates, 10 % protein, 36 % fat). The pancake was labelled with 100 Mbq ^{99m}Tc-Sn-colloid before it was fried. Scintigraphy started immediately after consumption of the pancake and was performed with a Picker Dynascan gamma camera system (Espelkamp, Germany). Patients were examined in the supine position from ventral and dorsal sides for 1 min, and thereafter they were studied dynamically for 60 min (120 images, 30 s each) in the sitting position from the ventral side. Additional ventral and dorsal supine scans were acquired 60 and 120 min after consumption

Table 1. Clinical characteristics of the non-diabetic and diabetic subjects studied

	Control subjects	Diabetic patients Normal $t_{1/2scint}$	Diabetic patients Delayed $t_{1/2scint}$
n	40	22	12
Sex (male/female)	21/19	7/15	6/6
Age (years)	43.0 ± 15.1	48.0 ± 15.4	49.1 ± 14.3
Weight (kg)	71.3 ± 12.8	67.8 ± 7.6	68.0 ± 13.8
Height (cm)	171 ± 9.2	168 ± 8.4	170 ± 10.8
Smokers *(n)*	14	6	5
IDDM/NIDDM	–	19/3	11/1
Duration of diabetes (years)	–	20.0 ± 12.9	22.5 ± 8.2
HbA_{1c} (%)	–	9.8 ± 1.9	9.7 ± 1.9
Insulin dose (IU/day)	–	38.8 ± 10.1	40.4 ± 11.3
Serum creatinine (mmol/l)	–	71.6 ± 12.4	96.4 ± 51.3
Micro/macroalbuminuria	–	1/1	3/4[a]
Retinopathy	–	12	11[a]
Polyneuropathy stage 1/stage 2	–	5/12	2/10
Cardiovascular autonomic neuropathy	–	3	7[a]
Blood glucose during breath test			
0 min (mmol/l)	–	10.8 ± 3.3	10.2 ± 4.2
60 min (mmol/l)	–	11.8 ± 4.1	11.5 ± 4.2
150 min (mmol/l)	–	9.7 ± 4.0	11.2 ± 3.9
240 min (mmol/l)	–	8.3 ± 4.1	10.0 ± 3.2

Values shown are mean ± SD or number of patients. $t_{1/2scint}$: half-emptying time as assessed by scintigraphy; [a] $p < 0.05$ vs diabetic patients with normal $t_{1/2scint}$

of the pancake. The geometric mean of the values obtained from the dorsal and ventral scans was calculated. Data analysis included a qualitative judgement of gastric function from the dynamic image sequence as well as the quantification of gastric content and disappearance rate by the region of interest (ROI) technique. The half-emptying time ($t_{1/2scint}$) was calculated by graphic interpolation of the data obtained at 30, 60, and 120 min after ingestion.

Normal ranges for the scintigraphic parameters, including the percentage retention of activity after 60 and 120 min and the half-emptying time, have been established previously in 10 (4 male, 6 female) healthy subjects aged 25.5 ± 3.2 (21–31) years. The mean ± SD (range) for the percentage radioactivity retained in the stomach was 81.9 ± 6.6 (72–96) % after 30 min, 59.4 ± 10.3 (43–77) % after 60 min, and 22.3 ± 10.7 (7–40) % after 120 min. The scintigraphic half-emptying time was 74 ± 16 (52–100) min. Thus, values for $t_{1/2scint}$ over 100 min were defined as abnormally delayed. These normal ranges are consistent with those recently reported by Rothstein and Alavi [30].

Our usual scintigraphic procedure was not changed in order to adhere to the standard approach as it is practised under routine clinical conditions. Therefore, the breath test and scintigraphy were performed in a randomised order on 2 separate days rather than simultaneously despite the possibility that this approach could have introduced an additional source of variability. Among the patients studied, 12 were identified as having delayed $t_{1/2scint}$, while 22 patients had normal $t_{1/2scint}$.

Assessment of upper gastrointestinal symptoms. Prior to the measurement of gastric emptying, upper gastrointestinal symptoms were assessed by a standard questionnaire proposed by Horowitz et al. [9]. The following complaints were defined as 'gastric' symptoms: anorexia, nausea, early satiety, upper abdominal discomfort or distention, vomiting, and abdominal pain. They were scored as: 0 = normal, 1 = mild (symptom could be ignored if the patient did not think about it), 2 = moderate (symptom could not be ignored, but did not influence daily activities), 3 = severe (symptom influenced daily activities). The maximum possible total score was 18 [9].

Laboratory methods. Glycated haemoglobin (HbA_{1c}) was determined by the HPLC technique using a Diamat analysing system (Bio-Rad, Munich, Germany). The normal range for this laboratory is under 6.3 % of total haemoglobin. Capillary blood glucose was taken at the start of the breath test (0 min) and during the test after 60, 150, and 240 min using heparinized capillaries (20 μl) and was measured by the hexokinase method on an ACP 5040 autoanalyser (Eppendorf, Hamburg, Germany). Fasting and stimulated (7 min after 1 mg glucagon i. v.) C-peptide was analysed by RIA (RIAmat C-peptide; Byk-Mallinckrodt, Dietzenbach, Germany). Urinary albumin excretion rate was determined from 24-h samples using the immuno-nephelometric technique (Array Protein System, Beckman, Fullerton, Calif., USA). Serum creatinine was measured by the p-aminophenazone (PAP) method (Boehringer, Mannheim, Germany).

Cardiovascular autonomic function. Autonomic reflex tests based on heart rate variability were performed using a ProSciCard computer system (MediSyst, Linden, Germany) as previously described [31]. The systolic blood pressure response to standing was performed using a Dinamap 1846 SX monitoring system (Critikon, Norderstedt, Germany). Normal ranges were established in 120 healthy subjects aged 32 (15–67) years [31]. We have recently suggested the following seven indices to be included in the test battery: 1) coefficient of R-R interval variation (CV) at rest, 2) spectral power in the low-frequency (LF) band and 3) mid-frequency (MF) band, 4) mean circular resultant (MCR) of vector analysis during deep breathing, 5) maximum/minimum 30:15 ratio, 6) Valsalva ratio, and 7) postural change in systolic blood pressure. Definite CAN was defined as three or more abnormalities among these seven indices [32]. The CAN score was defined as the ratio between the number of abnormal parameters divided by the total number of parameters tested in an individual patient (maximum score = 1).

Peripheral nerve function. Electrophysiological tests, thermal discrimination and vibration perception thresholds and neurological examination were performed as previously described

Table 2. Parameters of the [^{13}C]octanoic acid breath test, gastric symptom scores, and cardiovascular autonomic neuropathy (CAN) scores in healthy control subjects and diabetic patients with normal and delayed half-emptying time as assessed by scintigraphy ($t_{1/2scint}$)

	Control subjects	Diabetic patients Normal $t_{1/2scint}$	Diabetic patients Delayed $t_{1/2scint}$
n	40	22	12
Half-emptying time ($t_{1/2breath}$) (min)	95.8 ± 36.7[a]	140.3 ± 69.3[b]	228.8 ± 85.9[c]
Gastric emptying coefficient	3.19 ± 0.38[a]	2.83 ± 0.44[b]	2.21 ± 0.52[c]
Lag phase (min)	35.3 ± 22.5[a]	72.1 ± 60.8[b]	106.8 ± 54.7
Cumulative $^{13}CO_2$ excretion			
60 min (%)	6.27 ± 2.06[a]	4.64 ± 1.47[b]	3.65 ± 1.61
120 min (%)	18.53 ± 3.84[a]	15.8 ± 3.4[b]	12.3 ± 4.8[c]
240 min(%)	37.52 ± 5.42[a]	35.9 ± 7.0	30.2 ± 5.9[c]
Gastric symptom score	–	1.77 ± 2.11	4.17 ± 2.89[c]
CAN score	–	0.18 ± 0.21	0.76 ± 0.33[c]

Values shown are mean ± SD. [a] $p < 0.05$ vs diabetic patients with delayed $t_{1/2scint}$; [b] $p < 0.05$ vs control subjects; [c] $p < 0.05$ vs diabetic patients with normal $t_{1/2scint}$

[33]. Peripheral neuropathy was defined using a staging approach [32].

Retinopathy assessment. Colour retinal photographs were taken after pupillary dilatation using a CR3–45 NM non-mydriatic retinal camera (Canon, Tokyo, Japan) and were judged by an experienced examiner.

Statistical analysis

Results are expressed by the arithmetic mean ± SD. Differences between groups were analysed using the *t*-test for two independent samples. Qualitative data were given as absolute or relative frequencies which were analysed by the Fisher's exact test. Pearson correlation coefficient *(r)* and non-parametric Spearman rank correlation coefficient (r_s) were used to study associations between variables. Day-to-day reproducibility was computed from the standard error (SE) around the regression of the paired values and expressed by the coefficient of variation (CV = SE · 100 %/mean). The sensitivity and specificity of the [^{13}C]octanoic acid breath test in the assessment of gastric emptying as compared with scintigraphy were determined according to the approach proposed by Bailar and Mosteller [34]. A two-tailed test was used in all analyses. The level of significance was set at $\alpha = 0.05$. Significance levels were corrected for repeated testing using the Bonferroni method.

Results

[^{13}C]Octanoic acid breath test in non-diabetic subjects. No significant associations were noted between $t_{1/2breath}$ and age ($r = -0.05$), weight ($r = -0.21$), and height ($r = -0.21$; NS) nor between the GEC and age ($r = 0.13$), weight ($r = 0.19$), and height ($r = 0.29$). However, $t_{1/2breath}$ was significantly associated with the GEC ($r = -0.82$; $p < 0.05$) and the lag phase ($r = 0.91$; $p < 0.05$). The GEC was also significantly correlated with the lag phase ($r = -0.74$; $p < 0.05$) in the control group. The coefficients of variation of intrasubject day-to-day reproducibility were 29.6 % for $t_{1/2breath}$, 7.4 % for GEC, and 46.5 % for lag phase. The mean values for the breath test indices in the control group are given in Table 2.

Sensitivity and specificity of [^{13}C]octanoic acid breath test. A delayed $t_{1/2scint}$ (> 100 min) was noted in 12 patients, while in 22 patients $t_{1/2scint}$ was normal (≤ 100 min). The clinical characteristics of the two diabetic groups and the control subjects are given in Table 1. There were no significant differences between the groups regarding any of the parameters listed, except for nephropathy, retinopathy, and CAN which were significantly more frequent in the patients with delayed $t_{1/2scint}$ than in those with normal $t_{1/2scint}$ ($p < 0.05$).

The mean $^{13}CO_2$ excretion curves, expressed as the percentage dose of $^{13}CO_2$ excreted per min in the healthy subjects and diabetic patients are shown in Figure 1. There was a significant delay in $^{13}CO_2$ excretion in the diabetic group as compared with the control group at 30–120 min ($p < 0.05$). The $^{13}CO_2$ excretion curves in the diabetic groups with normal or delayed $t_{1/2scint}$ and the control group for comparison are illustrated in Figure 2. A significant delay in the $^{13}CO_2$ excretion curve in the patients with abnormal $t_{1/2scint}$ was noted between 75 and 135 min when compared with those showing normal $t_{1/2scint}$ ($p < 0.05$) and between 60 and 150 min when compared with the control group ($p < 0.05$). In addition, there was a significant delay in the $^{13}CO_2$ excretion curve at 60–90 min in the patients with normal $t_{1/2scint}$ compared with the control group ($p < 0.05$).

The results of the parameters of the [^{13}C]octanoic acid breath test in the diabetic groups with normal and delayed $t_{1/2scint}$ and the control subjects are presented in Table 2. Half-emptying time ($t_{1/2breath}$), GEC, and cumulative $^{13}CO_2$ excretion at 120 min were significantly impaired in the group with delayed $t_{1/2scint}$ compared with both the normal $t_{1/2scint}$ group and the control subjects ($p < 0.05$). Each of the breath test parameters, except for cumulative

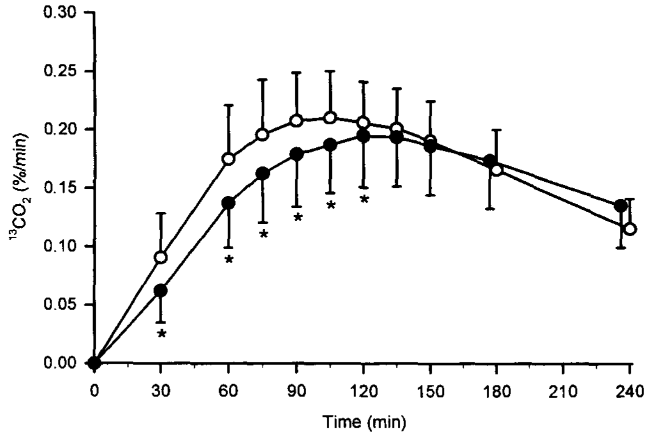

Fig. 1. The $^{13}CO_2$ exhalation curves (mean ± SD) in the healthy non-diabetic subjects (○) and diabetic patients (●). * $p < 0.05$ vs non-diabetic subjects

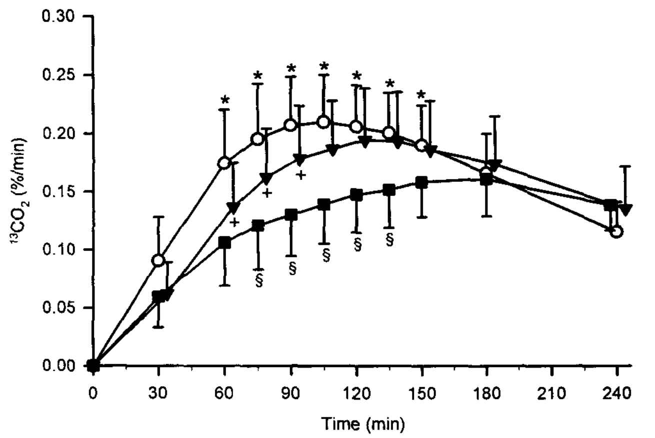

Fig. 2. The $^{13}CO_2$ exhalation curves (mean ± SD) in the diabetic groups with normal (▼) and delayed (■) scintigraphic half-emptying time ($t_{1/2scint}$) and the non-diabetic control group (○). * $p < 0.05$ for patients with abnormal $t_{1/2scint}$ vs control subjects; $^{+}p < 0.05$ for patients with normal $t_{1/2scint}$ vs control subjects; § $p < 0.05$ for patients with abnormal $t_{1/2scint}$ vs those with normal $t_{1/2scint}$

$^{13}CO_2$ excretion at 240 min, were also significantly impaired in the group with normal $t_{1/2scint}$ when compared with the control subjects ($p < 0.05$). Both the gastric symptom score and CAN score were significantly higher among the patients with delayed $t_{1/2scint}$ as compared with those showing normal $t_{1/2scint}$ ($p < 0.05$). However, in the patients with delayed $t_{1/2scint}$ the mean gastric symptom score indicated only a mild degree of symptoms, whereas the mean CAN score underlined moderate to severe cardiac involvement.

The specificity and sensitivity of $t_{1/2breath}$ and the GEC were calculated on the basis of normal ($t_{1/2scint} \leq 100$ min) or delayed ($t_{1/2scint} > 100$ min) scintigraphic half-emptying time. Values for $t_{1/2breath} > 200$ min and those for GEC less than 2.4 were defined as abnormal. The contingency table for this approach is shown in Table 3. Table 4 depicts the

Table 3. Contingency table to assess the sensitivity and specificity of the [^{13}C]octanoic acid breath test indices (half-emptying time: $t_{1/2breath}$ or gastric emptying coefficient: GEC) as compared with the half-emptying time as assessed by scintigraphy ($t_{1/2scint}$)

		Scintigraphic half-emptying time ($t_{1/2scint}$)		Total
		Delayed	Normal	
$t_{1/2breath}$/GEC	Delayed	9	3	12
	Normal	3	19	22
Total		12	22	34

Values are numbers of patients

Table 4. Sensitivity and specificity of the parameters of the [^{13}C]octanoic acid breath test

	Half-emptying time ($t_{1/2breath}$)	Gastric emptying coefficient
Cut-off value	200 min	2.4
Sensitivity (%)	75	75
Specificity (%)	86	86

resulting sensitivity and specificity of $t_{1/2breath}$ and GEC, respectively. Using the cut-off values given in Table 4 both the sensitivity and specificity were identical for $t_{1/2breath}$ and GEC.

Clinical correlates of gastric emptying. Both $t_{1/2breath}$ ($r_s = 0.52$; $p < 0.05$) and GEC ($r_s = -0.59$; $p < 0.05$) as well as $t_{1/2scint}$ to a lesser degree ($r_s = 0.33$; $p < 0.05$) were significantly associated with the gastric symptom score. A significant relationship to the CAN score was demonstrated for $t_{1/2breath}$ ($r_s = 0.45$; $p < 0.05$), GEC ($r_s = -0.47$; $p < 0.05$), and $t_{1/2scint}$ ($r_s = 0.60$; $p < 0.05$). Furthermore, scintigraphic gastric emptying after 60 and 120 min was significantly correlated with the CAN score ($r_s = 0.42$; $p < 0.05$ and $r_s = 0.60$; $p < 0.05$, respectively). The associations

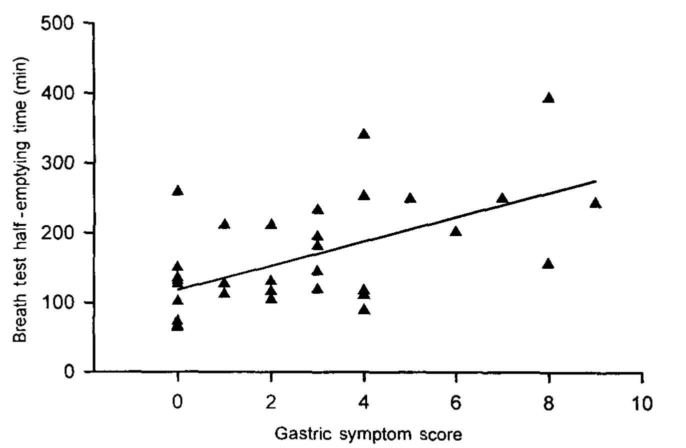

Fig. 3. Association between the breath test half-emptying time ($t_{1/2breath}$) and the gastric symptom score ($r_s = 0.523$; $p < 0.05$; $n = 33$)

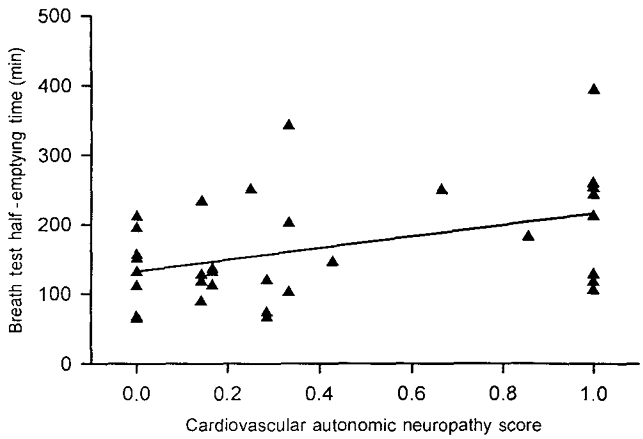

Fig. 4. Association between the breath test half-emptying time ($t_{1/2breath}$) and the cardiovascular autonomic neuropathy (CAN) score ($r_s = 0.448$; $p < 0.05$; $n = 32$)

between $t_{1/2breath}$ and both the gastric symptom score and CAN score are illustrated in Figures 3 and 4, respectively. There was one missing value for the gastric symptom score and two for the CAN score.

No significant associations with the blood glucose levels at 0, 60, 150, and 240 min were observed for $t_{1/2breath}$ ($r = -0.16$, $r = -0.15$, $r = 0.04$, $r = 0.08$) or for $t_{1/2scint}$ ($r = -0.06$, $r = -0.06$, $r = 0.12$, $r = 0.07$). Moreover, neither $t_{1/2breath}$ nor $t_{1/2scint}$ were associated with the HbA_{1c} levels ($r = 0.14$ and $r = -0.05$), age ($r = 0.15$ and $r = 0.03$), and duration of diabetes ($r = 0.01$ and $r = 0.24$).

Discussion

The results of this study demonstrate that the sensitivity, specificity, and reproducibility of the [^{13}C]octanoic acid breath test are acceptable for the assessment of gastric emptying rates when compared with a scintigraphic technique. Furthermore, we provide evidence for an association between delayed gastric emptying, assessed by the breath test, and the severity of gastric symptoms and cardiovascular autonomic neuropathy in diabetic patients, but no relationship to the blood glucose levels at the time the test was performed.

Scintigraphy is generally regarded as the reference method in the evaluation of disordered gastric emptying in diabetic patients, but this technique has several disadvantages in that it: 1) involves radiation exposure, albeit only a small amount, 2) requires expensive equipment and data analysis systems that are of limited availability, and 3) may not be readily accessible for routine clinical purposes due to the exclusive use of a gamma camera for several hours. Thus, there is a need for an alternative method that can be widely used in clinical and epidemiological studies. Whether the [^{13}C]octanoic acid breath test, which avoids radiation exposure, may represent a sensitive, specific, and reliable diagnostic tool for these purposes has not been previously established in diabetic patients.

Our approach in which the breath test and scintigraphy were performed on 2 separate days could have introduced a source of variability. However, we decided to adhere to the standard procedures as practised under routine clinical conditions rather than introducing modifications. Nevertheless, the sensitivity, specificity, and reliability of the [^{13}C]octanoic acid breath test were consistent with a recent study in which a [^{13}C]acetate breath test for gastric emptying of liquids and scintigraphy were performed simultaneously [8]. In that study, which included patients with different gastric emptying disorders, the sensitivity and specificity of the half-emptying time for liquids were 80 and 77.8 %, respectively [8]. These data are comparable to those found in our study, i.e. 75 and 86 % for both the half-emptying time ($t_{1/2breath}$) and gastric emptying coefficient (GEC), respectively. However, it has been suggested that measurement of gastric emptying of solids is more sensitive than that of liquids [35, 36], since the initial emptying rate of liquids may be more rapid in diabetic patients as compared with non-diabetic subjects [15]. Hence, we preferred to evaluate a breath test for assessment of solid gastric emptying rather than a test for measuring liquid emptying. Whether simultaneous labelling of the solid phase with [^{14}C]octanoic acid and the liquid phase with [^{13}C]glycine in a dual-carbon-labelled test [37] would increase the diagnostic information has not been examined in diabetic patients.

The intrasubject coefficients of variation for $t_{1/2breath}$, GEC, and lag phase in the non-diabetic group were 29.6, 7.4, and 46.5 %, respectively and are consistent with those recently reported by Ghoos et al. [5] in five healthy subjects. It is noteworthy that the intraindividual variability of the half-emptying time for liquids, as measured by the [^{13}C]acetate breath test, was 21.8 % [8]. Regarding the scintigraphic half-emptying time, an intrasubject variability of 30.2 % has been reported for solids and 34.4 % for liquids [38]. Since the expression of marker retention at single time points is associated with an even higher variability [38] and poorly represents the emptying process [39], the half-emptying time has been suggested as the parameter of choice [38]. However, our findings show a day-to-day reproducibility of the [^{13}C]octanoic acid breath test slightly superior to that of scintigraphy. For routine purposes either $t_{1/2breath}$ or GEC can be used as clinical parameters.

In this study we also examined the associations of the breath test parameters with the upper gastrointestinal symptom score, CAN score, and metabolic parameters. We found that in diabetic patients the increased half-emptying time, assessed by both the breath test and scintigraphy, was significantly correlated with the severity of CAN examined using a

scoring approach. Although the degree of these correlations was relatively modest, this finding is in line with expectations based on the biological variability of measures of gastric emptying and cardiovascular autonomic function as well as the fact that different organ manifestations are being evaluated by these methods. Moreover, the failure to demonstrate an association of delayed gastric emptying with CAN in previous studies [7, 9, 18, 40] could be due to the use of tests for the diagnosis of CAN that have received substantial criticism [31, 41]. In contrast, we have used a test battery that includes not only the standard indices but also those based on spectral and vector analysis of heart rate variability and provides an adequately sensitive and specific detection of CAN [31, 32]. Thus, although the correlations found in the present study were moderate, they support the concept that autonomic neuropathy constitutes one important underlying factor in the development of diabetic gastroparesis.

This study also showed an association between delayed $t_{1/2breath}$ and, to a lesser extent, $t_{1/2scint}$ and the severity of gastric symptoms, suggesting that the latter may at least in part be ascribed to impaired solid gastric emptying. The correlation between the gastric symptom score and gastric emptying was moderate, in line with a recent study that measured solid retention by scintigraphy [22]. However, it has been suggested that appropriate symptoms may be caused by disordered gastric motility when there is a marked emptying delay [35]. Thus, although visceral neuropathy may reduce symptom perception [4], it is possible that dyspeptic symptoms in patients with CAN are induced by small intestine dysregulation and hyperactivity [20].

It has been suggested that hyperglycaemia exerts a major influence on gastric motor function in that it slows gastric emptying in diabetic patients [1]. However, in other studies no association has been shown between hyperglycaemia and delayed gastric emptying as assessed by scintigraphy [15, 42]. In the present study no association between hyperglycaemia and delayed gastric emptying was noted, but the range of fasting blood glucose concentrations prior to the breath test was limited to 4.5–15.5 mmol/l. Thus, the [^{13}C]octanoic acid breath test is suitable for routine clinical measurements without a significant influence over this range of blood glucose levels. The possible effects of more pronounced blood glucose fluctuations on the test results should be assessed by glucose clamp studies.

The advantages of the [^{13}C]octanoic acid breath test include: 1) ease of performance for both the patient and examiner, 2) avoidance of radiation exposure, 3) the ability to be performed simultaneously in several patients, and 4) availability for widespread use, since the breath test samples can be collected away from the analytical unit to which they can be subsequently mailed. Until recently, sophisticated and expensive isotope-ratio mass-spectrometry (IRMS) was required for analysis of the breath samples. At present, the low-cost and easy-to-operate isotope-selective non-dispersive infrared spectrometry (NDIRS) is being increasingly employed to analyse the breath samples and should be available for clinical use in near future [43, 44]. However, limitations to the breath test including the lack of information on the possible influence of impaired absorption or liver disease should also be borne in mind. In addition, the breath test is limited to the assessment of gastric emptying, whereas scintigraphic techniques can be used to measure intestinal transit as well.

In conclusion, the [^{13}C]octanoic acid breath test represents a sufficiently sensitive, specific, reliable, and easily performed non-invasive method for the assessment of delayed gastric emptying in diabetic patients. Impairments revealed by this test are associated with gastric symptoms and cardiovascular autonomic neuropathy but not with elevated HbA_{1c} and blood glucose levels up to 15.5 mmol/l. It is conceivable that the test will become widely applicable in clinical routine and thereby could help to examine effects of treatment on the impaired gastric emptying [6] and to unravel the natural history of diabetic gastroparesis.

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