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Slow gallbladder emptying reverts to normal but small intestinal transit of a physiological meal remains slow in celiac patients during gluten-free diet

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Abstract

Background *Alterations of small intestinal transit and gallbladder (GB) motility have been reported in celiac disease (CD) in studies involving, in most cases, non-physiological experimental conditions and artificial stimuli to motility. Our aims were to quantitate non-invasively small intestinal transit time and GB emptying during administration of a physiological and palatable solid meal, and to assess the effect of gluten-free diet (GFD).* ***Methods*** *We simultaneously measured mouth-to-cecum transit time (MCTT) using a validated H_2 breath test, and GB motility using ultrasonography. We studied CD patients before (n = 19) and during (n = 14) GFD, and healthy volunteers (n = 24) following administration of a physiological solid meal (Kcal 539).* ***Key Results*** *Mouth-to-cecum transit time was more prolonged in CD (mean ± SEM: 235 ± 96 min) than in controls (169 ± 65 min, P = 0.0039). The GB fasting volume and postprandial residual volume were significantly higher in CD than in controls, and GB emptying constant was slower in CD than in controls. During GFD, GB emptying reverted to normal, but MCTT remained unchanged (229 ± 69 min) and more prolonged in CD than in controls (P = 0.0139). During GFD, duodenal infiltration with lymphocytes and mast cells persisted higher than that in controls, and the number of mast cells lying in proximity of nervous endings did not change.* ***Conclusions & Inferences*** *Slow postprandial MCTT in response to a physiological meal does not revert to normal during GFD, an effect mirroring incomplete histopathologic recovery.*

Keywords *emptying, gallbladder intraepithelial lymphocytes, mast cells.*

INTRODUCTION

Celiac disease (CD) is an autoimmune enteropathy that develops in genetically predisposed patients triggered by the ingestion of gluten of wheat and of related prolamines contained in barley and rye.[1] Functional alterations of the gastrointestinal tract have been reported in CD, and these alterations include intestinal and gallbladder (GB) dismotility.[2] Information on small intestine dismotility has been mainly obtained by manometric studies[3–5] involving invasive studies, but little information is available on how this dismotility may affect the transit of a normal meal along the small intestine in these patients. Attempts have been made to measure mouth-to-cecum transit time (MCTT) non-invasively using hydrogen breath testing following lactulose administration.[6–8] Using this technique, MCTT has been reported to be slower in CD patients as compared to normal controls,[9–13] a defect reversed during GFD.[11,13] Results based on lactulose are, however, hampered by the fact that lactulose markedly accelerates the transit of the meal along the small intestine.[7]

Due to these technical limitations, no valid information is available to what extent small intestinal dismotility affects the transit of a palatable physiological meal, and on whether this phenomenon reverts to normal during GFD. This latter point is of

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particular interest because although reconstitution of villous architecture occur in most patients, duodenal lymphocytic infiltration persists in a substantial proportion of CD patients during prolonged adherence to GFD.[14] Persistent low-grade inflammation is thought to be an important factor affecting neuromuscular function of the gastrointestinal tract.[15,16] In particular, the mast cells placed in proximity of nerve endings[17] may play an important role. Previous studies showed significantly higher number of mucosal mast cells in untreated celiac disease as compared with controls.[18] These observations raise the possibility that persistent infiltration of lymphocytes or other immune cells in CD patients on a GFD may be accompanied by persistent alteration of small intestinal transit.

The GB motility has been extensively studied[2] non-invasively in CD patients more commonly using ultrasonography[19–21] to measure changes in GB volume, but these studies also have been carried out in response to a variety of non-physiological stimuli.

The aim of our study was to measure simultaneously MCTT by hydrogen breath testing and GB motility by ultrasonography in response to a physiological and palatable solid meal in CD patients and in normal controls, and to assess the effect of GFD. A further aim was to assess the characteristics of the lymphocytic infiltration of the duodenum before and during GFD to assess mucosal immune activation and neuro-immune interactions as a possible basis of long term motor dysfunction.

MATERIALS AND METHODS

Patients

As part of a preliminary validation study, eight healthy subjects participated to a reproducibility study involving measurement of MCTT on 2 consecutive days, and nine subjects participated to a further validation study comparing MCTT measured after administration of the meal and after lactulose.

Nineteen CD patients participated in the study for measurement of postprandial MCTT (n = 17) and GB emptying (n = 19). In addition to MCTT, 15 patients had colonic transit time (CoTT) measured radiologically. Diagnosis of CD was based on positive antiendomysial and/or antitransglutaminase antibodies and on typical duodenal histopathology classified according to Marsh-Oberhuber.[22] Fourteen CD patients have been studied twice, before and during GFD diet given for at least 1 year. Adherence to GFD was assessed by interview and scored according to a 4-point Likert scale that includes no dietary indiscretions (score 1), one serving with gluten per month (score 2), <4 serving per month (score 3), or ≥4 serving per month (score 4).

Twenty-four asymptomatic medical students and health professionals participated in the study as controls for simultaneous measurement of MCTT by hydrogen breath testing (n = 20) and GB emptying (n = 24) by ultrasonography in response to a solid meal. Beside lack of symptoms, all control subjects reported a negative family history for gastrointestinal disorders, and tested negative to antiendomysial and antitransglutaminase antibodies.

Duodenal biopsies of six asymptomatic subjects investigated for mild anemia with no gastrointestinal complaints have been retrospectively identified to act as controls for characteristics of duodenal lymphocytic infiltration.

Clinical methods

The timetable of the studies was as follows: patients and controls were admitted in the morning to a day-case Unit after an overnight fasting. Thirty minutes after mouthwash with clorexidine, hydrogen breath test and measurement of GB volume by ultrasonography was carried out at time −10 min and 0 to provide fasting values.[23] At time 0, the solid meal was administered within 15 min, and starting at time 0 min, postprandial breath testing and GB ultrasonography were carried out at 10 min intervals up to a maximum 510 min for MCTT and up to 90 min for GB volume. The solid meal consisted of gluten-free bread 45 g (Rustico®, Dr. Schär. GmbH, Bolzano, Italy), butter 15 g, one chicken egg (medium size, 53–63 g), baked beans 100 g (white kidney beans as source of non-absorbable carbohydrates), olive oil 10 g^{-1}, sugar 10 g^{-1}, and tea 200 mL^{-1}. The composition of the meal (539 Kcal) was 37% carbohydrates, 13% protein, and 50% lipids, the latter to ensure strong stimulus to GB contraction.

Mouth-to-cecum transit time was measured using hydrogen breath testing with the patients in the sitting position immediately after each measurement of GB volume. Samples of alveolar air were obtained by asking the patient to collect mid-expiratory breath in 20 mL^{-1} plastic gas-tight syringes. Samples were immediately analyzed in duplicate using a gas-chromatograph (Mycrolizer SC; Quintron, Milwaukee, WI, USA). At the beginning and at the end of each individual study, the gas-chromatograph was calibrated with reference gas (hydrogen 10 and 50 ppm). Use of antibiotic during the month preceding testing was ruled out in all CD patients and healthy controls.

The GB diameters were measured by a single operator (FB) by ultrasonography (Aloka Flexus SSD-1100; Aloka Holding Europe AG, Zurich, CH, Switzerland) using a convex 3.5 MHz probe. Longitudinal, anterior-posterior, and lateral-lateral GB diameters have been measured in duplicate by positioning the caliper on the mucosal side of the GB wall with the patient lying on his back.

The CoTT was measured within 7 days of other measurements using a modification[12] of the method of Metcalf et al.[24] involving oral administration of radiopaque markers of different shapes on successive days followed by plain abdominal X-ray taken on day 3.

Duodenal histology

A minimum of four biopsies were taken in CD patients in proximal and distal duodenum and oriented mucosa-up using acetate cellulose filters. The severity of duodenal histopathologic alteration was classified according to Marsh-Oberhuber.[22] Tissue sections were stained with haematoxilin and eosin and with immunohistochemical reagents to identify and quantitate intraepithelial T cells (CD3), T cells with antigen binding capacity (CD4), cytotoxic T cells (CD8), macrophages (CD68 and S100), and mast cells (tryptase positive and CD117). The number of cells positive to different antibodies was counted at microscopy in 10 high power fields. One of us (GB) quantitated mast cells in

close vicinity (<5 μm) of nerve fibers (% mast cell-nerve fiber/area) using previously validated methods described in detail elsewhere.[17] For this purpose, we used specific monoclonal antibodies directed against tryptase for mast cells (1 : 2000 dilution; Dakopatts, Glostrup, Denmark) and a rabbit polyclonal neuron-specific enolase as a general neuronal marker (1 : 500 dilution; Dakopatts).

Calculations and expression of results

Mouth-to-cecum transit time was calculated as the time interval between administration of the meal or lactulose (time 0) and the beginning of a progressive and uninterrupted rise of hydrogen concentration with 10 ppm taken as a minimum value of increment above baseline values.[8]

The CoTT was calculated by differential counting of the residual markers on day 3 after ingestion of radiopaque markers as visualized on a plane X-ray.[12,24]

The GB volume has been calculated by the following formula[23]:

$$GB\ volume = \pi * longitudinal\ diameter * lateral - lateral\ diameter * anterior - posterior\ diameter$$

The following measurements have been carried out:

GB basal volume (mL^{-1}) = fasting volume

GB residual volume (mL^{-1}) = minimal postprandial GB volume

GB emptying t ½ (min^{-1}) = time to postprandial reduction of GB volume to 50% of basal volume

GB t max (min^{-1}) = time to maximum postprandial GB volume

GB emptying constant (K) = relationship between time and log of postprandial GB volume at 10 min intervals

Results are expressed as mean values ± SEM. Normality of distribution of results was assessed using the D'Agostino & Pearson omnibus normality test. Statistical comparison was carried out using parametric (*t*-test for paired and for unpaired observations) and non-parametric tests (Wilcoxon test for paired observations and Mann–Whitney test for unpaired observations) as appropriate. The correlation and regression analysis was carried out using the Graph-Pad Prisma Statistical Package (La Jolla, CA, USA). A *P*-value <0.05 was used to indicate statistical significance. Meal composition was characterized using the MycroDiet software® (Downlee Systems, Chapel-en-le-Fith, UK).

The study has been approved by our Institutional Ethic Committee, and all patients and controls involved have given written informed consent to the study.

RESULTS

Validation studies

Coefficient of variation of duplicate MCTT measurements was 5%, 8%, 4%,0%, 12%, 0%, 39%, and 9% in eight individual subjects, and mean value was 9 ± 12%. The MCTT was significantly faster during lactulose administration than after administration of the meal in each individual subject, and mean value was 57 ± 20 min *vs* 176 ± 75 min for the two stimulants, respectively ($P = 0.0008$). There was no correlation ($P = 0.2163$) between results for MCTT obtained with the physiological meal and with lactulose.

Celiac patients *vs* healthy controls

Twenty-four healthy controls and 19 CD patients entered the study, and characteristics for each individual are reported in Table 1. Mean age was higher in CD patients (34 ± 10 years) than in controls (27 ± 8 years, $P = 0.0073$), but there was no difference in female to male ratio (16/3 *vs* 16/8, respectively) and in BMI in the two groups (20.9 ± 4.0 *vs* 21.4 ± 2.6 respectively).

Intestinal transit The MCTT was significantly greater in CD patients than in healthy controls (248 ± 98 min *vs* 169 ± 65 min, $P = 0.0059$; Table 2 and Fig. 1). There was no difference in CoTT in the two groups (30.6 ± 17.6 h *vs* 47.5 ± 15.0 h, in CD and in healthy controls, respectively, NS: Fig. 1). The MCTT was inversely related to BMI in healthy controls ($y = 424.6 - 23.43x$, $P = 0.0334$; Fig. 2) and there was a trend for a similar relationship in CD patients. There was no relationship between age and MCTT both in healthy controls ($r = -0162$) and in CD patients ($r = -0240$).

Gallbladder motor function Mean fasting GB volume was significantly larger in CD patients than in healthy controls (32.2 ± 19.1 mL^{-1} *vs* 17.6 ± 5.1 mL^{-1}, respectively, $P = 0.0041$), as was residual postprandial GB volume (8.5 ± 8.8 mL^{-1} *vs* 2.2 ± 1.7 mL^{-1}, respectively, $P = 0.0038$; Fig. 1). The GB emptying constant was significantly lower in CD patients than in healthy controls (−0.0331 ± 0.0212 *vs* −0.0582 ± 0.0379, respectively, $P = 0.0071$). There was no relationship between age or BMI and GB fasting GB volume, postprandial residual volume, and emptying constant in healthy controls ($r = -0.0292$, 0.0227, and −0.1963 for age and 0.2308, 0.0558, and −0.0127 for BMI, respectively) and in CD patients ($r = 0.03841$, 0.1622, and −0.1272 for age and 0.2553, 0.4211, and 0.3305 for BMI, respectively).

Effect of gluten-free diet

Fourteen of the 19 CD patients enrolled in the study have been studied twice, before and during GFD. Results for each individual patient are reported in Table 3. Mean age at baseline study was 33 ± 10 years as compared to 35 ± 10 years at repeat study indicating a mean length of GFD of 2 years prior to repeat study. Length of GFD was 1 year or more in individual patients, and all patients scored 1 at Likert scale for adherence to GFD. The BMI remained virtually unchanged during GFD (21.3 ± 3.3) as compared to baseline (20.9 ± 4.2). The T-TG antibody titer decreased in each individual patient and mean value

Table 1 Anthropometric characteristics of healthy controls (HC) and celiac patients (CD). Histologic (Marsh classification), serologic [tissue-transglutaminases antibodies level (tTG), and upper limit of normal (u.l.n.) of tTG] and clinical characteristics (GI = gastrointestinal) are shown for CD patients

	Age		Gender		BMI		Marsh	tTG U/mL^{-1}	tTG u.l.n	GI symptoms	Extra-GI manifestations
n	HC	CD	HC	CD	HC	CD	CD	CD	CD	CD	CD
1	54	26	F	F	22.1	16.8	3b	10.9	7	IBS-D	/
2	18	47	F	F	19.7	21	3a	15.1	7	/	/
3	21	24	F	F	18.9	17	3c	24.4	7	IBS-D	anemia, thyroditis
4	24	27	M	F	24.2	16.3	3c	21.0	7	abdominal discomfort	/
5	43	56	F	F	19.6	24.2	3b	20.9	8	dyspepsia	anemia, thyroditis
6	22	23	F	F	17.7	22.6	3c	18.0	7	diarrhea	anemia
7	20	30	F	F	19.8	18.1	3c	100.0	8	/	anemia, thyroditis
8	23	41	F	F	18.4	29.6	3c	100.0	8	IBS-D	anemia, folate deficiency
9	26	22	F	F	19.4	20	3c	19.8	8	/	anemia, vit D deficiency
10	26	40	M	M	22.8	28.4	2	19.0	8	IBS-D	/
11	26	34	M	F	23.3	22.5	3c	20.0	8	IBS-D	hyposideremia
12	26	30	M	F	21.0	20.7	3c	93.9	8	IBS-D	hyposideremia
13	26	25	F	F	22.9	18	3a	57.4	8	/	anemia
14	25	34	F	M	26.8	17.9	3c	11.8	8	chronic diarrhea	anemia
15	26	54	F	F	27.4	20.8	3b	39.9	30	chronic diarrhea	/
16	29	33	F	F	18.0	22.9	3a	23.1	8	IBS-D	folate deficiency
17	26	38	M	F	20.9	19.2	3c	106.	8	/	thyroiditis
18	25	33	M	F	23.7	15.1	3c	54.9	8	/	anemia
19	19	35	M	M	24.2	25.3	3c	100.0	8	IBS-D	/
20	26		F		19.2						
21	27		M		22.1						
22	26		F		19.3						
23	28		F		21.5						
24	32		F		20.1						
Mean	27	34			21.4	20.8		45.1			
SD+	8	10			2.6	4.0		36.0			
P	0.073										

fell from 31 ± 27 U mL^{-1} at baseline to 2 ± 9 U mL^{-1}, (P = 0.005) during GFD.

Intestinal transit The MCTT decreased during GFD in six individual CD patients, remained unchanged in two, and increased in six patients. Mean value of MCTT did not change during GFD (229 ± 69 min^{-1}) as compared to baseline (235 ± 96 min^{-1}) and remained significantly higher than that in healthy controls (169 ± 65 min^{-1}, P = 0.0139; Fig. 3). Mean value for CoTT remained unchanged during GFD (30.6 ± 11.6 h) as at baseline (32.1 ± 17.1 h, NS), and was similar to that observed in healthy controls (47.5 ± 15.0 h).

Gallbladder motor function Fasting GB volume decreased in each individual patient and mean value decreased from 32.1 ± 21.4 mL^{-1} at baseline to 20.6 ± 9.6 mL^{-1} (P = 0.0549; Fig. 3) during GFD, a value similar to that observed in healthy controls (17.6 ± 5.1 mL^{-1}). Postprandial residual volume also decreased in each individual patient and mean value volume decreased from 8.3 ± 8.9 mL^{-1} at baseline to 2.4 ± 1.7 mL^{-1} during GFD (P = 0.0134), a value similar to that observed in healthy controls (2.2 ± 1.7 mL^{-1}). The GB emptying constant increased during GFD from −0.0325 ± 0.0159 at baseline to −0.0835 ± 0.0449 (P = 0.0006), a value similar to that observed in healthy controls (−0.0582 ± 0.0379).

Duodenal histopathology At baseline, 13 patients had villous atrophy of variable severity and 1 patient had lymphocytic duodenal infiltration and no villous atrophy. During GFD, villous structure was reconstituted in 10 patients and persisted in 4 patients.

All subtypes of intraepithelial lymphocytes were more abundant in CD patients than in controls. The CD3 count was 42 ± 6 *vs* 18 ± 3 (P = 0.0001), CD8 was 36 ± 6 *vs* 14 ± 2 (P = 0.0001), CD4 was 18 ± 10 *vs* 4 ± 1 (P = 0.0044), CD 68 was 14 ± 7 *vs* 5 ± 1 (P = 0.0058), CD 117 was 14 ± 5 *vs* 6 ± 2 (P = 0.0021), respectively in CD patients and in controls. The density of S100 protein positive cells (8 ± 4 *vs* 2 ± 1, P = 0.0011) and of the tryptase secreting mast cells (12 ± 5 *vs* 6 ± 2, P = 0.0092, respectively) was also higher in CD patients than in control. There was a significant decrease of CD3 cells (36 ± 9, P = 0.0261) during GFD as compared to baseline: CD4 (18 ± 10), CD8 (32 ± 6),

Table 2 Mouth–cecum transit time (MCTT), colonic transit time (CoTT), gallbladder (GB) volume, and emptying constant (K) in healthy controls (HC) and in celiac patients (CD)

	MCTT min^{-1}		CoTT h^{-1}		GB fasting volume mL^{-1}		GB residual volume mL^{-1}		GB emptying K	
n	HC	CD	HC	CD	HC	CD	HC	CD	HC	CD
1	140	180	24.0	24.0	17.3	8.7	1.7	1.4	−0.0356	−0.0389
2	290	260	49.2	31.2	17.1	8.3	1.1	1.4	−0.0283	−0.0191
3	140	480	49.2	/	14.4	26.2	1.1	0.8	−0.0267	−0.0434
4	80	210	66	67.2	21.6	13.0	7.1	0.7	−0.0545	−0.0321
5	220	190	49.2	31.2	16.2	31.7	2.3	26	−0.1300	−0.0203
6	180	270	/	45.6	18.7	64.8	2.4	30	−0.0910	−0.048
7	120	180	/	42.0	17.2	31.8	2.9	5.5	−0.0285	−0.0375
8	290	260	/	33.6	12.9	30.3	3.5	9.7	−0.0229	−0.0147
9	190	210	/	9.6	17.1	30.0	0.3	8.7	−0.0580	−0.0141
10	160	130	/	25.2	10.2	56.9	4.6	5.9	−0.0282	−0.0383
11	/	70	/	38.4	14.8	23.5	0.6	4.6	−0.0327	−0.0148
12	140	320	/	/	16	25.6	1.5	8.3	−0.0438	−0.0235
13	/	230	/	/	19	16.9	3	2.4	−0.1060	−0.0397
14	/	300	/	4.8	11.5	81.4	1.6	11	−0.0880	−0.07
15	110	200	/	4.8	23.7	33.3	1.8	3.3	−0.0960	−0.0925
16	110	/	/	/	14	15.3	1.4	3	−0.1690	−0.0022
17	130	/	/	43.2	18.1	43.2	4.1	16	−0.0810	−0.0233
18	90	380	/	12.0	14.2	26.4	1.5	1.6	−0.0410	−0.0395
19	190	340	/	45.6	23.2	44.6	0.4	22	−0.0418	−0.018
20	300	/	/	/	15.9	/	0.1	/	−0.0397	/
21	150	/	/	/	32.4	/	1.9	/	−0.0525	/
22	200	/	/	/	13.4	/	3.3	/	−0.0425	/
23	/	/	/	/	27.8	/	0.6	/	−0.0412	/
24	150	/	/	/	15.5	/	4.8	/	−0.0170	/
Mean	169	248	47.5	30.6	17.6	32.2	2.2	8.5	−0.0582	−0.0331
SD+	65	98	15.0	17.6	5.1	19.1	1.7	8.8	0.0379	0.0212
P	0.0059				0.0041*		0.0038*		0.0071*	

*Non-parametric tests.

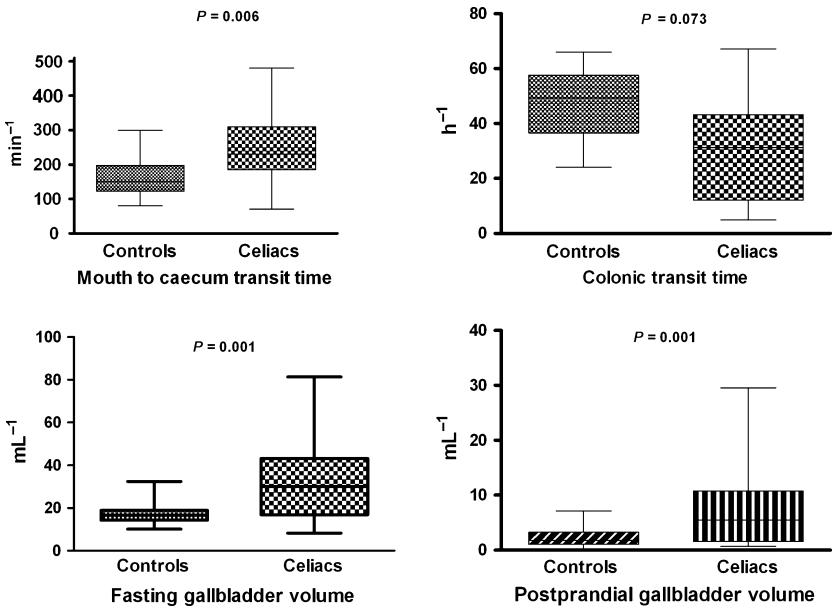

Figure 1 intestinal transit times and gallbladder volumes in healthy controls and in celiac patients.

CD 68 (14 ± 8), CD117 (15 ± 6), S100 positive (7 ± 3), and tryptase positive cells (14 ± 10) remained unchanged during GFD.

Results for the number of mast cells identified at <5 μm from nerves are shown in Fig. 4 for six healthy controls and for nine CD patients studied before and

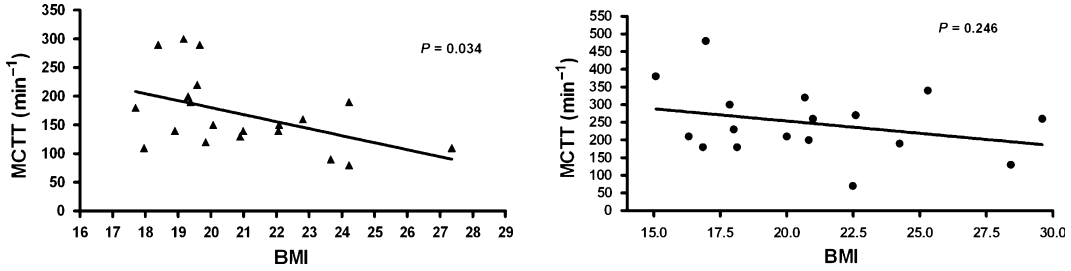

Figure 2 relationship between body mass index (BMI) and mouth-to-cecum transit time (MCTT) in healthy controls (left panel) and in celiac patients (right panel).

Table 3 Anthropometric and histological characteristics (Marsh classification), mouth-caecum transit time (MCTT), colonic transit time (CoTT), and gallbladder (GB) volume and emptying constant (K) in celiac patients studied before (B) and during (D) gluten free diet

							Transit time				GB volume					
			BMI		Marsh		MCTT min^{-1}		CoTT h^{-1}		Fasting mL^{-1}		Residual mL^{-1}		GB K	
Label	Age Years	Sex	B	D	B	D	B	D	B	D	B	D	B	D	B	D
1	26	F	16.8	16.6	3a	2	180	190	24.0	31.2	8.7	19.2	1.4	2.6	−0.0389	−0.0715
2	47	F	21.0	21.4	3b	2	260	130	31.2	62.4	8.3	23.3	1.4	3.8	−0.0191	−0.0341
3	24	F	17.0	19.0	3c	2	480	350	/	/	26.2	31.3	0.8	1.2	−0.0434	−0.0303
4	27	F	16.3	16.3	3c	2	210	210	67.2	38.4	13.0	2.0	0.7	1.8	−0.0321	−0.1200
5	56	F	24.2	24.2	3b	3a	190	210	31.2	34.8	31.7	21.6	26.1	3.1	−0.0203	−0.0650
6	23	F	22.6	20.7	3c	2	270	260	45.6	56.4	64.8	43.0	29.6	1.9	−0.0480	−0.0850
7	30	F	18.1	19.1	3c	3a	180	310	42.0	44.4	31.8	26.8	5.5	2.2	−0.0375	−0.1580
8	41	F	29.6	25.7	3c	3a	260	150	33.6	38.4	30.3	20.6	9.7	7.1	−0.0147	−0.0500
9	22	F	20.0	20.7	3c	2	210	290	9.6	28.8	30.0	16.7	8.7	0.7	−0.0141	−0.1360
10	40	M	28.4	27.4	2	2	130	140	25.2	/	56.9	25.1	5.9	3.5	−0.0383	−0.0950
11	34	F	22.5	24.4	3c	2	70	280	38.4	31.2	23.5	16.0	4.6	0.9	−0.0148	−0.0459
12	30	F	20.7	22.6	3c	3c	320	190	/	/	25.6	17.6	8.3	0.6	−0.0235	−0.0940
13	25	F	18.0	18.0	3a	2	230	200	/	/	16.9	12.7	2.4	1.5	−0.0397	−0.0294
14	34	M	17.9	21.7	3c	2	300	300	4.8	30.0	81.4	12.6	10.8	2.5	−0.0700	−0.1550
Mean	33		20.9	21.3			235	229	32.1	30.6	32.1	20.6	8.3	2.4	0.0325	−0.0835
+SD	10		4.2	3.3			96	69	17.1	11.6	21.4	9.6	8.9	1.7	0.0159	0.0449
P													0.0134*		0.0006	

*Non-parametric tests.

during GFD. Although the results showed increased mean values of mast cells <5 μm from nerves in CD patients (95 ± 79) compared with healthy controls (41 ± 40), these results did not reach statistical significance. There was virtually no change in results obtained in CD patients during GFD (67 ± 86) as compared to pre-GFD.

DISCUSSION

Our study provides the first quantitative measurement of the transit time of a palatable solid test meal from mouth-to-cecum in CD. A particular aspect of our study is that our results have been obtained using a physiological stimulus to intestinal transit, a technical aspect of critical importance because previous studies have used lactulose as stimulant. Lactulose-based methodology is invalid as reported by Read et al.[7] and as confirmed by our own validation studies showing artificial increase of MCTT by a factor of three using lactulose as compared with the solid meal. The validity of our results is further confirmed by the reasonably good reproducibility of MCTT between duplicate measurements (CV 8%) observed in the present and in a previous study carried out in gallstone patients documenting accuracy of results by comparison with scintigraphic measurement of MCTT.[25] Mean age was slightly higher (7 years) in CD patients than in controls, and although this difference may theoretically influence results, the difference is in our opinion, too small to exert any effect as supported by our finding of lack of correlation of age with

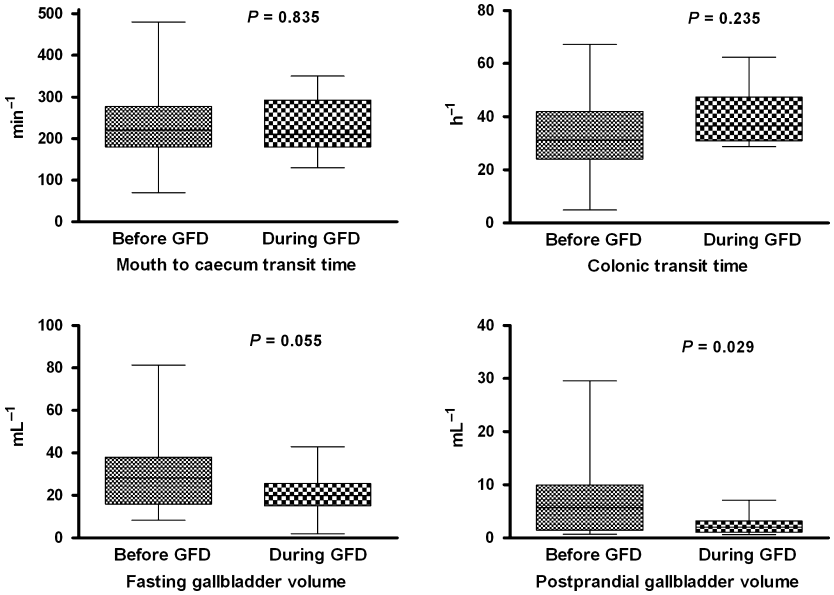

Figure 3 effect of gluten-free diet (GFD) on intestinal transit times and on gallbladder volumes in celiac patients.

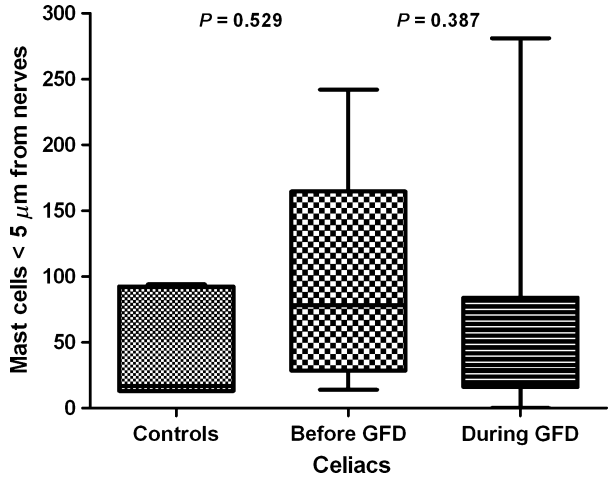

Figure 4 number of mast cells lying in proximity of nerve endings in healthy controls and in celiac patients studied before and during gluten-free diet (GFD).

MCTT and with parameters of GB motility in both groups.

In our study, MCTT was on average 60 min^{-1} slower in CD patients than in healthy controls, and most important, results indicate that MCTT in response to a physiological meal does not revert to normal during GFD. This observation contradicts previous observations of normalization of MCTT during GFD based on the invalid lactulose breath test.[9–11,13] In accordance with our results, studies measuring gastrointestinal motor activity have invariably reported an abnormal motility pattern with the characteristics of a neuropathic disorder in untreated CD patients,[3–5,26] a defect persisting in most adult CD patients during GFD. The mechanism involved in this persistent defect remains, however, unclear. Gastric emptying, that has been inconsistently reported to be slow in CD patients, is unlikely to play a role because it has been consistently reported to be normal during GFD.[2] Furthermore, there was no change during GFD of BMI, an anthropometric characteristic that may potentially affect gastric emptying (Table 2).[27]

Our results suggest a possible role of a persistent low-grade mucosal inflammation despite GFD. Indeed, these results are supported by the recent evidence indicating that low-grade mucosal inflammation plays an important role in persistent intestinal sensorimotor dysfunction. There has been particular interest in the potential role of low-grade intestinal inflammation in patients with irritable bowel syndrome.[28] In this condition, research has been focused on the role of mast cells. Firstly, the number of mast cells is increased in colonic[29] and in ileal[30] mucosa of patients with irritable bowel syndrome; secondly, these cells lie in close proximity with intrinsic and extrinsic innervation and are associated with the severity and frequency of perceived abdominal pain.[17] Finally, mucosal mediators released by mast cells from the intestinal mucosa of patients with irritable bowel syndrome have been reported to alter intestinal motor function[31] in animal models and activate submucosal enteric neurons in human colonic specimens.[32] In view of these considerations, it seems reasonable to speculate that the low-grade mucosal inflammation that

persists in the vast majority of CD patients despite prolonged adherence to GFD[14] could play a role in the described alteration of MCTT during GFD. For this reason, we have looked in detail into the changes of intraepithelial infiltration during GFD, and results revealed that despite significant decrease of inflammatory cells by comparison with prediet, lymphocitic and mast cells infiltration persisted significantly higher in CD patients during GFD than in controls. We have also determined in detail the number of mast cells in proximity to mucosal innervations as a possible marker of altered neuroimmune interactions. Although the results showed an increase in the number of mast cells located close to mucosal nerves in patients with CD in comparison with controls, the results did not reach statistical significance (Fig. 4). This is an area of interest that deserves further investigation.

Another important observation made in our study is that there was an inverse relationship between MCTT and BMI that was clearly evident in healthy controls (Fig. 2). This phenomenon is in keeping with similar observations by other authors, in healthy subjects,[32] in patients with advanced liver diseases,[33] in anorexia,[34] in CD,[13] and in men fed a high-fat diet.[35] Taken together, these studies support the hypothesis that MCTT may vary in different clinical conditions to maintain the nutritional status as suggested by Spiller et al.[9] and by Sadick et al.[13] by a mechanism involving prolonged absorption time for nutrients in otherwise diseased small intestine. Other mechanisms may potentially be involved in favouring nutrient absorption. Recent studies of Gass et al.[36] indicate that bile acid dramatically enhance the proteolysis of several dietary proteins, including some digestion-resistant protein with the notable exception of gluten. The enlarged bile acid pool and the slow enterohepatic circulation that characterize CD[37] imply more prolonged contact time of meal constituents with bile acid and with digestive enzymes, thus potentially favouring more complete digestion of dietary protein in addition to providing more absorption time. Whether these alterations may contribute to maintain the nutritional status in CD patients is, however, purely speculative.

Except for MCTT and BMI, we found no further relationship of anthropometric/clinical characteristics of CD patients and of healthy controls with MCTT or GB motility (results not shown), and in particular, there was no effect of age, presence of diarrhea, or adherence to GFD on the latter parameters.

In contrast with results of MCTT, CoTT was not altered in our CD patients, possibly because none of our patients (Table 1) had severe diarrhea which was the case for a subgroup of patients reported by Bai et al.[12] with rapid colonic transit and steathorrea. Results are, however, to be taken cautiously because of the limited number of measurements of CoTT in healthy controls.

Our study confirms a functional alteration of GB motility in CD patients, and a prompt return to normality during GFD. The mechanisms involved in this reversible alteration of GB motility is likely to be independent of reduced end-organ response to neurohormonal stimulation and the balance of evidence suggests a role for defective secretion of CCK by the damaged small intestinal mucosa, with return of CCK secretion to normality during GFD and reconstitution of villous structure.[2] Normalization of GB motility may have multiple potential beneficial effects by preventing biliary stasis in the GB, a risk factor for gallstone formation, and by speeding up the enterohepatic circulation of bile acid with more efficient cholesterol excretion with bile and greater utilization in the liver for de-novo bile acid synthesis.[37]

In conclusion, our study provides quantitative evidence of a functional alteration of intestinal motility, resulting in slow transit of a physiological meal along the small intestine, and normal transit in the colon. The slow MCTT may contribute to maintain the nutritional status in CD patients before GFD. The alteration of MCTT persists unchanged during GFD possibly in association with the persistence of mild mucosal inflammatory changes. Alteration of GB motility is also present in CD patients and is reversible during GFD.

AUTHOR CONTRIBUTION

FB contributed to planning the study, carried out and coordinated measurements involved, and drafted the paper; AM, SB, and CR contributed to measurements, recruitment, and follow-up of patients and controls; FL performed all endoscopies; VV examined all biopsies; VS and GB carried out the study on mast cells; AL contributed to planning and coordination, reviewed, and finalized the manuscript.

COMPETING INTERESTS

The authors have no competing interests. No financial support was received for this study.

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