

COLONIC CONTENT IN HEALTH AND ITS RELATION TO FUNCTIONAL GUT SYMPTOMS

Short title: colonic content

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Abbreviations: CT: computed tomography
IBS: irritable bowel syndrome

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ABSTRACT

Background. Gut content may be determinant in the generation of digestive symptoms, particularly in patients with impaired gut function and hypersensitivity. Since the relation of intraluminal gas to symptoms is only partial, we hypothesized that non-gaseous component may play a decisive role.

Methods. Abdominal CT scans were evaluated in healthy subjects during fasting and after a meal (n=15) and in patients with functional gut disorders during basal conditions (when they were feeling well) and during an episode of abdominal distension (n=15). Colonic content and distribution were measured by an original analysis program.

Key results. In healthy subjects both gaseous (87 ± 24 mL) and non-gaseous colonic content (714 ± 34 mL) were uniformly distributed along the colon. In the early postprandial period gas volume increased (by 46 ± 23 mL), but non-gaseous content did not, although a partial caudad displacement from the descending to the pelvic colon was observed. No differences in colonic content were detected between patients and healthy subjects. Symptoms were associated with discrete increments in gas volume. However, no consistent differences in non-gaseous content were detected in patients between asymptomatic periods and during episodes of abdominal distension.

Conclusions & Inferences. In patients with functional gut disorders, abdominal distension is not related to changes in non-gaseous colonic content. Hence, other factors, such as intestinal hypersensitivity and poor tolerance of small increases in luminal gas may be involved.

Key words: colonic content; intestinal gas; abdominal distension; functional gut disorders; food ingestion.

Key messages

- Using an original program for abdominal CT image analysis our study describes the distribution of non-gaseous content in the undisturbed colon.
- Meal ingestion induced a caudad displacement of non-gaseous content in the distal colon.
- Patients with functional gut disorders complaining of episodic abdominal distension did not show differences in non-gaseous colonic content versus healthy subjects, either when asymptomatic or during distension episodes.

For Peer Review

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INTRODUCTION

The mechanism of symptoms in functional gut disorders has not been fully elucidated. Two major pathophysiological mechanisms are currently accepted: abnormal motility and increased gut sensitivity (1;2). We hypothesized that in patients with functional bowel disorders even small variations in intraluminal content could trigger abnormal sensory-reflex responses and elicit symptoms. Among gut contents, and particularly in the colon, increased gas volume has been proposed as a symptom triggering culprit. During the past 15 years our laboratory has conducted a systematic research program quantitating normal intestinal gas handling, content and distribution, uncovering abnormalities in patients with functional gut disorders, particularly in patients complaining of abdominal bloating and distension, symptoms frequently attributed to intestinal gas.

Indeed, intestinal gas is easily visualized in abdominal CT scans. We developed and validated an original CT analysis program to measure the volume of gas in different regions of the gut (3-6). Using this program we described the pattern of intestinal gas in healthy subjects and in patients with episodic abdominal distension (7). During asymptomatic periods no differences were observed between both groups. During episodes of abdominal distension some patients exhibited an intraluminal gas pattern outside the normal range, but the abnormalities were relatively mild. Furthermore, in most patients no abnormalities in intraluminal gas volume could be detected during the symptomatic episodes.

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3 Expanding our initial hypothesis, we further postulated that gas may not
4 be the sole offending component, and that the solid-liquid component of gut
5 contents may play a role distending certain segments of the lower gut. At this
6 stage, we developed a program for analysis of the non-gaseous (solid-liquid)
7 content of the gut and applied it to measure colonic content. With this new
8 method we revised the CT scans previously acquired (7) with two aims, first, to
9 describe the basal pattern of colonic content in healthy subjects and the
10 physiological variations related to eating, and second, to describe the pattern of
11 content in patients with functional gut symptoms and to determine the temporal
12 relation of contents to symptoms. To these goals we analyzed a series of paired
13 studies in healthy subjects during fasting and after a meal, and in a group of
14 patients with episodic abdominal distension during symptomatic episodes and
15 during asymptomatic intervals between distension episodes.
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34 MATERIAL AND METHODS

35 **Participants**

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38 Healthy individuals [11 women, 4 men; 39±4 years age (mean±SE); 19-
39 62 years range] with no gastrointestinal complaints and patients complaining of
40 episodic abdominal distension i.e. severe distension episodes in contrast to mild
41 or no distension in-between episodes (15 women; 53±3 years age; 37-74 years
42 age), were included in the study. Patients were recruited from the
43 Gastroenterology outpatient clinic and healthy subjects by public advertisement
44 (3;7). All participants were instructed to fill-out a digestive symptoms
45 questionnaire based on Rome criteria; this information was used to establish a
46 diagnosis in patients, and to verify the absence of symptoms in healthy
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3 subjects. All patients (n=15) were diagnosed as having functional disorders
4 based on Rome III criteria (8), 7 of them as functional bloating (8.3±2.2 bowel
5 movement/wk, 3.3±0.4 score on the Bristol stool form scale), 4 constipation-
6 predominant IBS (1.3±0.3 bowel movements/wk, 2.1±0.3 Bristol score), 3 with
7 alternating IBS (2.1±0.1 bowel movements/wk, 1.3±0.1 Bristol score), and 1
8 diarrhea-predominant IBS. The protocols of the specific studies had been
9 approved by the Institutional Review Board of the University Hospital Vall
10 d'Hebron and all subjects gave their written informed consent to participate in
11 the studies.
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24 25 **CT scanning**

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27 Abdominal CT scans were obtained with a helical multislice CT
28 scanner, exposure 120 kV and 50 mAs, using the available dose reduction
29 options (tube current modulation). Section thickness and image reconstruction
30 depended on the scanner model (2.5-mm thickness, 1.6-mm interval
31 reconstruction with Mx8000, Philips Medical Systems, Best, The Netherlands;
32 2-mm thickness, 1.5-mm interval reconstruction with Somatom Sensation 16 or
33 Somatom Definition AS, Siemens Medical Solutions, Forchheim, Germany).
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With these characteristics the total effective dose was 2.4 mSv, similar to the dose of a CT colography and approximately 1/4 of the dose of a standard CT scan. Images were obtained in the supine position during a single breath-hold. No oral or intravenous contrast medium was administered. At the time of each CT scan, the participant's subjective sensation of abdominal distension was measured on a graphic rating scale graded from 0 (no distension) to 6 (extremely severe distension).

Experimental design

All participants underwent two abdominal CT scans. Healthy subjects were studied: a) in the morning during fasting and b) in the postprandial period (109 ± 21 min after a meal). Patients with episodic abdominal distension were studied on two different symptomatic status: a) during a spontaneous episode of severe distension, and b) at baseline (in between episodes) when they considered their abdomen showed minimal or no distension. All healthy subjects included in a previous study with paired fasting and postprandial scans were selected for the current study (3); patients were randomly selected among those with paired basal and distension scans included in previous studies (7). CT scanning and analyses were performed blindly, with the investigator unaware of the condition (patient or healthy subject) and the symptom status (basal or symptomatic) of the participants.

Data analysis

Morphovolumetric analysis of CT images was performed using an original software program specifically developed in our laboratory and. Total colonic content was measured using a computer-assisted semiautomatic method: the operator selects in each image the intracolonic content, which is then measured by anisotropic contrast enhancement and region growing algorithms. Gaseous component of intracolonic content was automatically measured by applying a user-defined threshold (3;4). Using a three-dimensional reconstruction program with 360° rotation over the three dimensions, volumes in

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3 selected regions of the colon were also measured. Analysis of each scan
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5 involves about 60 min operator's time. The reproducibility of the measurements
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7 was originally validated *in vivo* by measuring intestinal gas before and after
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9 injection of known volumes of air within the gut (15). The software used in this
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11 study was built with two open-source toolkits: the Insight Segmentation and
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13 Registration toolkit (9) and the Visualization toolkit, both used for 3D computer
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15 graphics, image processing and visualization (10).
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20 21 **Statistical analysis**

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23 Statistical analysis was performed using the SPSS 18.0 statistical
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25 package. Mean \pm SE of the features measured were calculated in each group of
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27 participant. The Kolmogorov-Smirnov test was used to check the normality of
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29 data distribution. Parametric normally-distributed data were compared by
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31 Student's *t*-test for paired or unpaired data; otherwise, the Wilcoxon signed rank
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33 test was used for paired data and the Mann-Whitney *U* test for unpaired data.
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39 **RESULTS**

40 41 **Healthy subjects**

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43 Total colonic content during fasting was 801 \pm 45 mL of which 87 \pm 24 mL
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45 were gas and 714 \pm 34 mL non-gaseous (solid/liquid) content (Figure 1 and 2).
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47 Both gas and non-gaseous content were uniformly distributed along the colon
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49 (Figure 3). The reproducibility of the measurements during fasting and after the
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51 meal was good; in the early postprandial period, total colonic volume increased
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53 slightly, but even small changes in the distribution of both gaseous and non-
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55 gaseous components were consistently noted (Figures 2 and 3): the non-
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3 gaseous content decreased in the descending colon (by 15 ± 8 mL; $p=0.033$) and
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5 increased proportionally in the pelvic colon (by 19 ± 10 mL; $p=0.040$), whereas
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7 gas content increased selectively in the proximal part (ascending; $p=0.036$) and
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9 distal part of the colon (descending and pelvic; $p<0.034$ for both).
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14 Patients

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16 Patients studied during basal conditions, in-between distension episodes,
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18 reported mild symptoms (2.2 ± 0.3 distension score) and no consistent
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20 differences in colonic content gaseous and non-gaseous, were observed as
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22 compared to healthy subjects (Figures 2 and 3). The same patients during
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24 episodes of abdominal distension (4.6 ± 0.4 distension score) showed a mild
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26 increase in colonic gas content (up to 128 ± 32 mL from 94 ± 3 mL in the basal
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28 scan; $p=0.059$), but no significant differences in the non-gaseous content of the
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30 colon were observed); the reproducibility of the measurements during basal
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32 conditions and episodes of distension was good (Figure 2). The severity of
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34 symptoms during distension episodes (either absolute values or increase from
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36 basal) did not correlate with changes in intracolonic fecal mass.
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DISCUSSION

Our study describes and quantitates the normal pattern of content in the undisturbed colon, during fasting and the early postprandial period. The study further shows that patients with functional gut symptoms do not exhibit detectable abnormalities in non-gaseous colonic content.

The volume of colonic content measured in the present study by CT is remarkably similar to the volumes measured by MRI both by us and others (11-13). The volume of colonic content is much smaller than previously expected, and considering that the daily fecal output is around 150 mL, the turn-over of colonic biomass seems particularly rapid (14). Interestingly, intraindividual differences in non-gaseous colonic content are relatively small; the volume of gas is much more variable, but this variability involves relatively small quantities of intracolonic gas, because differences in absolute gas volumes among individuals are not large. **To note, the reproducibility of the measurements was good and the technique allowed detection of relatively small, but consistent and statistically significant intraindividual variations.**

The distribution of non-gaseous content within the undisturbed colon is quite uniform, indicating that the metabolic activity of colonic biomass takes place all along the entire length of the colon. Meal ingestion was not associated with changes in colonic content in the early postprandial period, probably because the postprandial scan was obtained before the bulk of unabsorbed meal residues arrived from the small bowel. However, meal ingestion induced a redistribution of non-gaseous content that decreased in the descending colon and proportionally increased in the pelvic segments. Conceivably, this caudad displacement of biomass is operated by the gastro-colonic reflex activated by

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3 ingestion of the meal (15;16). The situation may change at a later stage of the
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5 postprandial period when unabsorbed residues of small bowel chime enter the
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7 colon. Thus, in a recent study we observed a consistent increment in content all
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9 along the colon 6 h after ingestion of a test meal rich in residues (17).
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12 The gas component of intracolonic content after the meal changes
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14 according to a different pattern than the non-gaseous component. As
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16 previously described (3;7), the absolute volume of colonic gas increased during
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18 the early postprandial period. Gas increased in the ascending colon, which
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20 could be due to emptying of gas from the small bowel by a gastro-ileal reflex,
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22 because fermentable residues had probably not arrived at the time of scanning;
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24 at the same time an increase was observed in the distal colon, conceivably due
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26 to a caudad movement driven by the gastro-colonic reflex.
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30 In patients complaining of abdominal distension, changes in gas content
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32 and distribution have been previously reported (7). In that earlier study a
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34 relatively large population of patients was studied, allowing application of
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36 sophisticated analysis by machine learning techniques. This analysis showed
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38 that in-between symptomatic episodes, the intraluminal gas pattern in patients
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40 was undistinguishable from healthy subjects. During symptomatic episodes, a
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42 substantial proportion of patients were classified outside the normal range by a
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44 combination of abnormalities, although no single characteristic was found
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46 discriminant. The present data in a subpopulation of these patients corroborates
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48 the increase in gas content associated to abdominal distension, although the
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50 differences failed to reach statistical significance due to the smaller sample size.
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52 We acknowledge that healthy subjects and patients were not matched, but
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54 previous studies did not detect differences related to sex, age or BMI (3-7).
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3 The original contribution of the present study is that, contrary to our
4 hypothesis, no abnormalities in the non-gaseous component of colonic content
5 were observed in patients regardless of whether they were asymptomatic or
6 during one of their usual episodes of abdominal distension. Indeed, the range of
7 individual values in both populations overlap (see Figure 2), suggesting that the
8 lack of difference was not related to the sample size.
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17 These data indicate that abdominal distension in these patients may
18 develop with a normal amount of solid/liquid mass inside their colons.
19 Consequently, their symptoms are more likely related to luminal gas, which
20 makes sense, considering the temporal variations both of symptoms and gas
21 content, as opposed to the stability of non-gaseous colonic content.
22 Furthermore, increased colonic gas during symptomatic episodes is
23 quantitatively modest and only detected in a proportion of symptomatic patients;
24 hence, other factors, such as intestinal hypersensitivity, may be involved.
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34 Content in other segment of the gut, such as the stomach of small bowel, may
35 also play a role. Futures studies on gut content in relation to symptoms,
36 requiring repeated imaging over time, may benefit from less invasive
37 technologies that do not involve radiation, such as MRI (11-13).
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Author Contributions:

AB. Data analysis

EBarba. Study management and conduction of experiments.

EBurri. Study management and conduction of experiments.

DC. Conduction of experiments.

AA. Study design, supervision of studies, data analysis and manuscript revision.

SQ. Analysis of CT images.

EM. Adaptation of CT analysis program.

IN. Adaptation of CT analysis program.

J-RM. Data interpretation, manuscript revision.

FA. Study design, data interpretation, and manuscript preparation.

No competing interests declared

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FIGURE LEGENDS

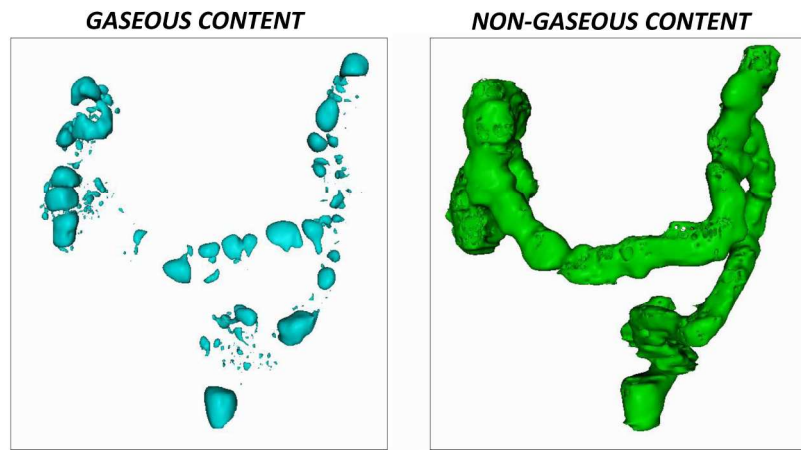
Figure 1. Independent representation of gaseous and non-gaseous components of colonic content from a CT scan in a healthy subject.

Figure 2. Colonic content. Total, non-gaseous and gaseous content are represented. Data are individual values (and means) in healthy subjects during fasting and in the early postprandial period, and in patients during basal conditions and during an episode of abdominal distension.

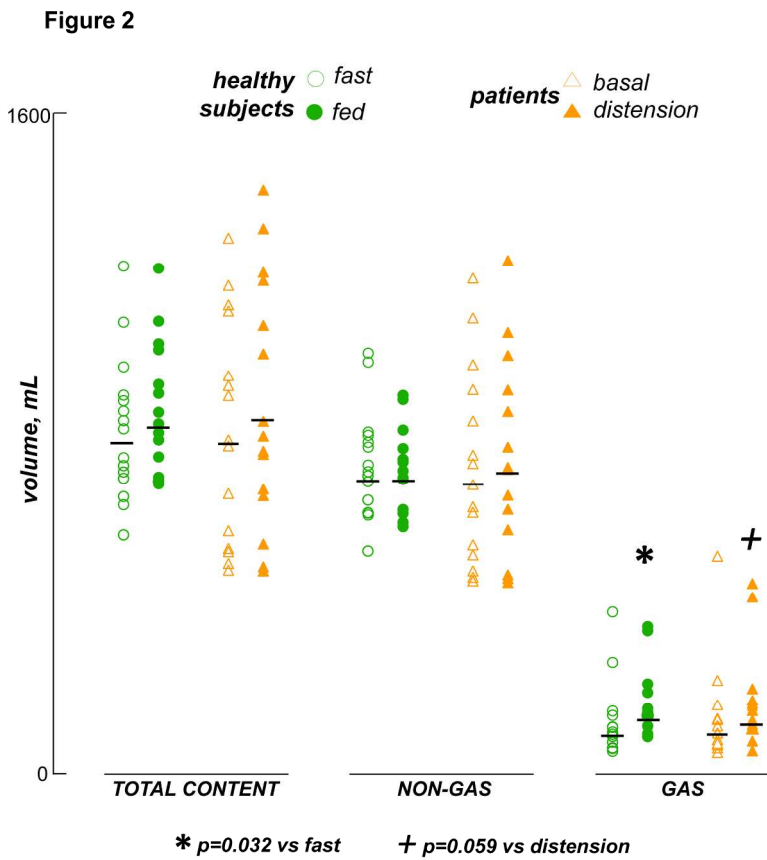
Figure 3. Distribution of colonic content. Figure shows total, non-gaseous and gaseous content in different colonic segments: ascending (**A**), transverse (**T**), descending (**D**) and pelvic (**P**). Data are mean values \pm SE in healthy subjects (fast vs early postprandial period) and in patients (basal conditions vs episodes of abdominal distension). No consistent difference between patients and healthy subjects were detected.

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Figure 1



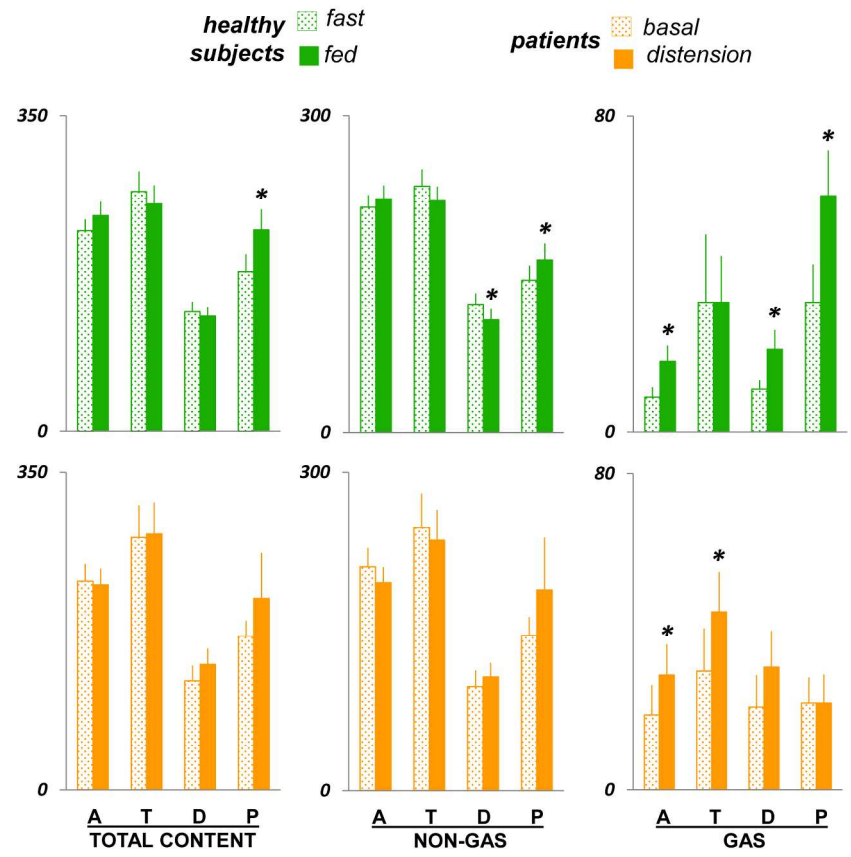
Independent representation of gaseous and non-gaseous components of colonic content from a CT scan in a healthy subject.
190x254mm (300 x 300 DPI)



Colonic content. Total, non-gaseous and gaseous content are represented. Data are individual values (and means) in healthy subjects during fasting and in the early postprandial period, and in patients during basal conditions and during an episode of abdominal distension.
 190x254mm (300 x 300 DPI)

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Figure 3



Distribution of colonic content. Figure shows total, non-gaseous and gaseous content in different colonic segments: ascending (A), transverse (T), descending (D) and pelvic (P). Data are mean values±SE in healthy subjects (fast vs early postprandial period) and in patients (basal conditions vs episodes of abdominal distension). No consistent difference between patients and healthy subjects were detected.

190x254mm (300 x 300 DPI)