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Influence of age, gender, and body mass index

Nandhra, Gursharan Kaur; Mark, Esben Bolvig; Di Tanna, Gian Luca; Haase, Anne-Mette; Poulsen, Jakob; Christodoulides, Stephanos; Kung, Victor; Klinge, Mette W; Knudsen, Karoline; Borghammer, Per; Andersen, Katrine O; Fynne, Lotte; Sutter, Nanna; Schlageter, Vincent; Krogh, Klaus; Drewes, Asbjørn Mohr; Birch, Malcolm; Scott, S Mark

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MAIN TITLE

Normative values for region-specific colonic and gastrointestinal transit times in 111 healthy volunteers using the 3D-Transit electromagnet tracking system: influence of age, gender and body-mass index

RUNNING TITLE

Normative values for regional colonic transit

AUTHORSHIP

Gursharan Kaur Nandhra[1,2], Esben Bolvig Mark[3,4], Gian Luca Di Tanna[5], Anne-Mette Haase[6], Jakob Poulsen[3], Stephanos Christodoulides[1], Victor Kung[1], Mette W. Klinge[6], Karoline Knudsen[7], Per Borghammer[7], Katrine O. Andersen[6], Lotte Fynne[6], Nanna Sutter[6], Vincent Schlageter[8], Klaus Krogh[6], Asbjørn Mohr Drewes[3,4], Malcolm Birch[1,2] & S. Mark Scott[1]

AFFILIATIONS

[1]GI Physiology Unit, The Blizard Institute, Barts and the London School of Medicine and Dentistry, Queen Mary University of London, UK

[2]Clinical Physics, Barts Health NHS Trust, The Royal London Hospital, UK

[3]Mech-Sense, Department of Gastroenterology and Hepatology, Aalborg University Hospital, Denmark

[4]Department of Clinical Medicine, Aalborg University, Denmark

[5]Statistics Department, The George Institute for Global Health, Sydney, Australia

[6]Neurogastroenterology Unit, Department of Hepatology and Gastroenterology, Aarhus University Hospital, Denmark

[7]Department of Nuclear Medicine and PET Centre, Aarhus University Hospital, Denmark

[8]Motilis Medica SA, Lausanne, Switzerland

CORRESPONDING AUTHOR

Gursharan Kaur Nandhra,

Clinical Physics,

Barts Health NHS Trust,

The Royal London Hospital,

56-76 Ashfield Street,

Whitechapel,

London.

E1 1BB

Email: g.k.kalsi@qmul.ac.uk

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ABSTRACT

Background

The 3D-Transit electromagnet tracking system (Motilis Medica, SA, Lausanne, Switzerland) is an emerging tool for the ambulatory assessment of gastrointestinal (GI) transit and motility. Using this tool, we aimed to derive normative values for region-specific colonic and GI transit times and to assess the influence of age, gender and body-mass index (BMI).

Methods

Regional and total colonic transit times (CTT), gastric emptying (GET), small intestinal (SITT), and whole gut (WGTT) transit times were extracted from 111 healthy volunteers from the

United Kingdom and Denmark (58 female; median age: 40 years [range:21–88]). The effects of age, gender and BMI were assessed using standard statistical methods.

Key Results

The ascending, transverse, descending and rectosigmoid colon transit times accounted for 32%, 34%, 17% and 17% of total CTT in females, and 33%, 25%, 14% and 28% of total CTT in males. CTT and WGTT values were seen to cluster at intervals separated by approximately 24 hours, providing further evidence of the non-continuous nature of these measurements. Increasing age was associated with longer CTT (*p=0.021*), WGTT (*p=0.000*) ascending (*p=0.004*), transverse (*p=0.000*) and total right (*p=0.000*) colon transit times, but shorter rectosigmoid (*p=0.004*) transit time. Female gender was significantly associated with longer transverse (*p=0.049*) and descending (*p=0.000*) colon transit times, but shorter rectosigmoid (*p=0.000*) transit time. Time of entry into the rectosigmoid was significantly different between females and males (*p=0.015*). Increasing BMI was significantly associated with shorter WGTT (*p=0.012*).

Conclusions & Inferences

For the first time, normative reference values for region-specific colonic transit have been presented. Age, gender and BMI were seen to have an effect on transit times.

Abstract word count: [259] (max 250)

KEYWORDS:

Gastrointestinal, ingestible capsule, colon, motility, transit time

KEY POINTS:

- Localization of region-specific transit abnormalities within the colon may impact management of gastrointestinal (GI) motility disorders.
- Normative reference ranges for methods that assess GI transit and motility, such as the 3D-Transit electromagnet tracking system, are therefore essential to distinguish between normal and pathological physiology.
- For the first time, normative reference values for regional colonic transit have been presented using a minimally-invasive ambulatory method. Age, gender and body-mass index appear to have an effect on region-specific colonic and GI transit times.

Word count: [80] (max: 80)

ABBREVIATIONS:

GI: gastrointestinal; BMI: body-mass index; CTT: colonic transit times; GET: gastric emptying; SITT: small intestinal transit time; WGTT: whole gut transit time; ROM: radio-opaque markers; WMC: wireless motility capsule; TPC: time percent change; CI: confidence intervals; ICC: intraclass correlation coefficient

INTRODUCTION

Regional assessment of gastrointestinal (GI) motility can provide further insights into normal and pathological GI physiology. This may aid in advancing our understanding of GI motility disorders, particularly those of the lower GI tract such as the irritable bowel syndrome and chronic constipation, which present a substantial healthcare and socioeconomic burden.[1-3] Localization of region-specific transit abnormalities may complement recognized tests of colonic and anorectal function[4] and facilitate an effective diagnosis and management of such conditions.[5]

Recommended methods[6] to clinically assess colonic motility include the use of radio-opaque markers (ROM)[7] and colonic scintigraphy.[8] Although such techniques are well established, there are some inherent disadvantages which limit their use.[5] For instance, lack of standardization is a common issue, particularly with the ROM method, which has over ten published protocols.[9] Alternatively, colonic scintigraphy is a more quantitative and physiological method[10] however, it is expensive, time consuming and restricted to specialist centres.[9,11] More importantly, these methods only provide snapshots of the transit of ingested markers, rather than a single continuous measurement, due to limited scanning time (scintigraphy) or restrictions on number of X-rays taken (ROM) in an effort to minimize radiation exposures.

Ingestible capsule-based technologies provide a continuous means of assessing GI motility within a minimally invasive, radiation-free and ambulatory setting.[11] One such commercially

available system is the wireless motility capsule (WMC; SmartPill, Medtronic, USA), which measures whole gut and regional transit times using stereotypical changes in pH to identify the capsule's progression from one GI region to the next.[12] However, the WMC can only assess *total* colonic transit as no robust pH or pressure landmarks have been identified to evaluate specific colonic regions.[6,11] Such information can be obtained using the Motilis 3D-Transit system (Motilis Medica, SA, Lausanne, Switzerland), an emerging research tool that tracks the location and orientation of up to three ingestible electromagnetic capsules.[13] The system has already been used in several clinical studies assessing whole gut and regional GI transit in healthy and patient populations.[13-16] However, the novelty of the system lies in providing a detailed analysis of colonic motility in terms of region-specific colonic transit times,[17,18] anatomical lengths of colonic segments[19] and colonic motility patterns.[20]

Most diagnostic methods of GI motility have normative reference ranges for the whole gut or specific GI regions.[5,6,21] Such information is fundamental to the diagnostic capabilities of the investigation, allowing a patient to be diagnosed as having a normal or abnormal result. As the 3D-Transit system is a relatively new tool, it is therefore vital to define normal ranges of transit measurements using this system and to demonstrate its reliability in doing so. Therefore, our primary aim was to derive normative values and their measurement uncertainties for region-specific colonic and GI transit from a cohort of healthy volunteers using the 3D-Transit system. Our secondary aim was to evaluate the effects of age, gender and body-mass index (BMI) on region-specific colonic and GI transit times.

MATERIALS AND METHODS

Study population

The study population consisted of 128 healthy volunteers who participated in 3D-Transit studies conducted at three research facilities in the United Kingdom and Denmark between March 2012 and November 2017 as follows: 73 from Aarhus, Denmark as part of various clinical studies trialing the 3D-Transit system in healthy subjects and in patients with functional GI disorders;[13-16] 30 from London, United Kingdom as part of an observational study of colonic motility in constipation and ageing;[22] 25 from Aalborg, Denmark as part of clinical studies assessing the impact of opioid treatment on regional GI transit[17,18] and in studies demonstrating the novel capabilities of the 3D-Transit system for the assessment of colonic motility.[19,20] Despite being pooled from separate studies, consistency of test protocols was maintained.

All subjects were screened for eligibility against the following general inclusion criteria: healthy volunteers aged between 21 – 85 years; Barthel index ≥ 11[23] for elderly subjects; no co-existing acute or chronic diseases at the time of recruitment (except hypertension and hypercholesterolemia for elderly subjects), Cleveland Clinic Constipation Score < 8[24], no history of chronic GI symptoms, surgery or use of prescribed medication affecting GI motility; possessing capacity to understand the study information sheet and giving informed consent. The exclusion criteria were: vulnerable subject groups (e.g. elderly with dementia); pregnancy, intention to become pregnant, or breastfeeding during the study period; recent childbirth in the last 6 months; previous history of recreational drug abuse; daily alcohol or nicotine consumption; participation in any other studies within 14 days of enrolment;

planned medical/surgical treatments during the study period; operation of heavy machinery or motor vehicles during the study; non-removable piercings or metal implants; the use of prescribed medicine and/or herbal medicine; abdominal diameter (defined as circumference at the level of the umbilicus) of >140 cm to avoid signal loss between detector and electromagnetic capsule. All participating sites and studies received approval from the respective local research Ethics Committees (see Supporting Information – Appendix A) and written informed consent was obtained from all subjects prior to enrolment.

3D-Transit electromagnet tracking system

As described previously,[11,13] the 3D-Transit system (Motilis Medica SA, Lausanne, Switzerland) consists of ingestible electromagnetic capsules (Ø 8.3 mm; length 21.5 mm), which when activated and swallowed, emit an electromagnetic tracking signal that is detected by an external detector plate (160 mm x 160 mm x 11 mm; weight: 145 g) positioned over the abdomen. Each capsule contains an electromagnet, an electronic module and a battery which lasts between 60 – 120 hours, depending on the frequency of the emitted electromagnetic tracking signal (5 Hz or 10 Hz). During a recording, the electromagnetic tracking signal is saved onto a microSD memory card (Swissbit AG, Switzerland). Once a recording is complete, the data is downloaded to a computer and converted into 3D-space-time coordinates using dedicated software (Version 0.4, Motilis Medica, SA, Lausanne, Switzerland), which enables visualization of a capsule's 3D-position in the gut and changes in its 3D-orientation that reflects gut contractile activity.[13]

177 **Study protocol**

178 As the studies were performed at three different research facilities, there were some
179 variations in capsule ingestion time, meal composition and timing and the number of
180 capsules ingested during the study period.[13,14,16,17,22] For this reason, only the first capsule
181 recordings were selected for analysis as these were collected under a similar protocol across
182 all study sites. All subjects fasted for at least 6 – 8 hours before ingesting a meal followed by
183 the capsule with a glass of water. Depending on the study protocol, the total caloric intake
184 of the capsule ingestion meals ranged between 250 kcal (e.g. muesli breakfast bars) and 602
185 kcal (e.g. breakfast meal consisting of oats/cornflakes, 1 tablespoon raisins/2 teaspoons
186 sugar, skimmed milk, 1 slice wholegrain bread with plant-based margarine and 1 portion
187 jam or ham).

188 After capsule ingestion, subjects were instructed not to eat again for six hours to avoid
189 prolonging gastric emptying.[25] Once the capsule had been ingested, subjects were allowed
190 to leave the research facilities and proceed with their normal daily activities, including using
191 public transportation and going to work. Any strenuous physical activities in relation to work
192 and all sporting activities were prohibited. Subjects were asked to wear the external
193 detector plate at all times throughout the study period except during showering. Once all
194 capsules had been expelled, subjects were asked to return to the research facility where the
195 data from the external detector plates were downloaded and capsule expulsion confirmed
196 using the dedicated 3D-Transit software.

197

198

3D-Transit system data analysis

Gastrointestinal transit times

Gastric emptying (GET), small intestinal (SITT), colonic (CTT) and whole gut (WGTT) transit times were extracted from each recording by the primary investigator (GKN) as described by Haase et al.[13] In brief, this involved identifying the following four time points: (i) **Ingestion:** start of the recording, (ii) **Duodenum:** the time when the capsule progresses from the stomach into the duodenum. This is characterized by a change in contractile frequency from a regular and cyclical 3 contractions per minute (cpm) to an irregular 9 – 12 cpm, as reflected in the capsule's orientation angles (iii) **Right Colon:** identified as the time when the capsule progresses from the ileum to the caecum, identified as a drop in the contractile frequency to approximately 3 cpm, (iv) **Expulsion:** characterized by a large shift in the capsule's trajectories indicating a bowel movement, followed by a loss of signal which denotes the end of the recording. From these time points, the following GI transit times were determined:

- GET: duration between capsule ingestion and passage into the duodenum
- SITT: duration between the duodenum and the right colon time points
- CTT: duration between the right colon and capsule expulsion time points
- WGTT: duration between capsule ingestion and expulsion

Region-specific colonic transit times

Data for each subject were exported from the 3D-Transit software to a graphical user interface written in Matlab (version R2016b; MathWorks Inc., Massachusetts, USA) for the

extraction of region-specific colonic transit times using the method described by Mark et al.[19] Briefly, this involved first cleaning the recordings (performed by two investigators – GKN and EBM) to identify movements of the capsule that reflect real GI activity and filter out artifacts e.g. those caused by detector movement that distort a capsule's 3D-trajectory (Figure 1(a)). The following six distinct anatomical landmarks of the colon were then identified from the 'cleaned' recordings by the primary investigator (GKN): (i) start of the colon, (ii) hepatic flexure, (iii) mid-point of the transverse segment, (iv) splenic flexure, (v) end of the descending colon (vi) end of the colon (Figure 1(b)). Where retrograde motion of a capsule occurred between segments of the colon i.e. movement of the capsule back into the preceding segment, the landmark was identified as the point in time when the capsule last moved into the distal segment without further retrograde motion. These landmarks were then used to determine the following regional colonic transit times (Figure 1c)):

- ascending transit time: defined as the time taken for the capsule to traverse from the start of the colon to the hepatic flexure;
- transverse transit time: defined as the time taken for the capsule to traverse from the hepatic flexure to the splenic flexure;
- descending transit time: defined as the time taken for the capsule to traverse from the splenic flexure to the end of the descending segment;
- recto-sigmoid transit time: defined as the time taken for the capsule to traverse from the descending end to the end of the colon;
- total right colon transit time: defined as the time taken for the capsule to traverse from the start of the colon to the mid-point of the transverse segment;

- total left colon transit time: defined as the time taken for the capsule to traverse from the mid-point of the transverse segment to the end of the colon.

Study endpoints

The primary study endpoints were GET, SITT, CTT, WGTT and the region-specific colonic transit times (ascending, transverse, descending, rectosigmoid, total left and total right colon). The effects of age, gender and BMI on the primary study points were evaluated as exploratory endpoints. The inter- and intra-rater reliability of the identification of the colonic landmarks and hence, the regional colonic transit times was assessed by two independent experienced raters (GKN and EBM) who analyzed data from 32 subjects, with the first rater (GKN) re-analyzing the data after a two-week gap for intra-rater reliability. The reliability of regional GI transit times has already been published by our group.[26]

Statistical analysis

The primary study endpoints were summarized using number of observations, median, 95% confidence intervals and 5^{th} and 95^{th} percentiles. The associations between the primary endpoints (GET, SITT, CTT, WGTT and the regional colonic transit times) and a covariate set which included age, gender and BMI were assessed using mixed-effects Poisson regression models with study site as a random effect. The coefficients of the Poisson regression models were reported as a time percent change (TPC), whereby TPC > 1 indicates an increase in the predictor variable while TPC < 0 indicates a decrease. Statistical significance was set at $p < 0.05$.

To determine the inter- and intra-rater reliability of the colonic landmark identification and the regional colonic transit times, the intraclass correlation coefficients (ICC) and their 95% confidence intervals (CIs) were calculated based on a single rating, absolute agreement, 2-way random-effects model. The colonic landmark time points were subtracted from the time point when the capsule enters the caecum to convert the data into hours for the ICCs to be determined. ICC values range between 0 and 1 with a higher value indicating better reliability (<0.5, poor; 0.5-0.75, fair; 0.75-0.9, good; >0.9, excellent).[27] All analyses were performed using proprietary software (Stata Release 15, College Station, StataCorp LLC, TX, USA and SPSS Statistics Version 25, IBM, NY, USA).

RESULTS

Participant characteristics

In total, 128 3D-Transit recordings were available for analysis. Of these, 17 (13.3% – 6 from Aalborg, 6 from Aarhus and 5 from London) were excluded due to technical issues with the capsule, external battery, or the recording, which resulted in major signal loss or incomplete recordings. Regional GI and colonic transit times were extracted from the remaining 111 recordings (Aarhus: 67, London: 25, and Aalborg: 19). 85% of capsules were ingested in the morning, 10% in the afternoon (between 12noon and 2pm) and in 5% of recordings, the capsule ingestion time was not available. Demographic data was missing in 6 subjects, although gender was known. A summary of the subject demographics is presented in Table 1.

Region-specific colonic and gastrointestinal transit times

Region-specific colonic and GI transit times are presented in Table 2, as a whole group and also by gender (see also Figure 2). In 7 recordings, it was noted that subjects ingested a second meal while the capsule was still in the stomach. This has the potential to prolong gastric emptying[21,25] so these 7 recordings were excluded from the GET analysis but included in all other analyses. Of particular note, WGTT and CTT values were seen to cluster at intervals separated by approximately 24 hours (Figure 3(a) and 3(b)). 35% of capsules were expelled at approximately 24 hours after capsule ingestion, with a second peak (15%) observed at around 48 hours (Figure 3(a)). For CTT, 30% of capsules were expelled from the colon approximately 16 hours after capsule entry into the caecum, with a second peak in expulsions (12%) occurring at approximately 42 hours (Figure 3(b)).

Figure 3(c) shows the position of a capsule in the colon at 24 hours since ingestion for each 24-hour cluster shown in Figure 3(a), where the first 24-hour cluster includes capsules with WGTT < 36 hours and the second 24-hour cluster includes capsules with WGTT > 36 hours. As shown, 70% of capsules with WGTT > 36 hours were observed to reside in the right side of the colon, with 6% still in the small intestine. In contrast, 44% of capsules with WGTT < 36 hours had already been expelled within 24 hours of capsule ingestion, with the remaining bulk of capsules (55%) residing in the left side of the colon. Capsule location at 60 minutes and 30 minutes prior to expulsion is presented in Figure 3(d). At 60 minutes, 92% of capsules were seen to be located in the left side of the colon. This increased to 97% at 30 minutes prior to defecation.

The timing of capsule expulsions by hour of day are presented in the Supporting Information, Appendix B, Figure S1, which shows that most capsule expulsions (38%) occurred in the morning, between 0600 and 0800 (Figure S1(a)), irrespective of whether the WGTT was less than or greater than 36 hours (Figure S1(b)). Two small peaks in capsule expulsions were observed at 1300 (7%) and 1800 (5%) corresponding with lunch and evening meal times. Minimal capsule expulsions occurred in the early hours of the morning.

Influence of age, gender and body-mass index

The results of the Poisson regression analyses assessing the influence of age, gender and BMI on the GI and region-specific colonic transit times are presented in Table 3 in the form of time percent change (TPC) and their 95% confidence intervals (CI). Increasing age was associated with longer CTT (TPC= 1.003, 0.26% increase per year, *p = 0.021*), WGTT (TPC = 1.003, 0.35% increase per year, *p = 0.000*) and transit times in the ascending colon (TPC = 1.006, 0.56% increase per year, *p = 0.004*), transverse colon (TPC = 1.008, 0.85% increase per year, *p = 0.000*) and total right colon (TPC = 1.007, 0.73% increase per year, *p = 0.000*). Rectosigmoid transit time was seen to decrease with increasing age (TPC = 0.992, 0.76% decrease per year, *p = 0.004*).

With the exception of GET and rectosigmoid transit times, females were generally seen to have longer GI and regional colonic transit times (Figure 4). The ascending, transverse, descending and rectosigmoid colon transit times on average accounted for 32%, 34%, 17% and 17% of total CTT in females, and 33%, 25%, 14% and 28% of total CTT in males. In

females, total right and left colon transit times accounted for 47% and 53% of total CTT, whereas in males, they accounted for 45% and 55% of total CTT. The regression analyses indicated that the female gender was significantly associated with longer transverse (TPC = 1.242, *p = 0.049*) and descending (TPC= 1.513, *p = 0.000*) transit times, which equates to a difference of 24.2% and 51.3% respectively, when compared to males. Rectosigmoid transit on the other hand, was significantly shorter in females (TPC = 0.672 *p = 0.000*) by approximately 32.8%. Notably, the median time of entry of a capsule into the rectosigmoid (normalized to overall CTT) was found to be significantly different between the male and female subjects (*p = 0.015*) i.e. capsules generally took longer to reach the rectosigmoid in female subjects, at a point in time that was closer to the expulsion time than in male subjects (Figure 4 (c)).

Increasing BMI was associated with significantly shorter WGTT (TPC = 0.988 *p = 0.012*), which equates to a reduction of 1.22% per unit increase in BMI. The result for the influence of BMI on GET was close to significance (IRR = 0.962 *p = 0.051*), equating to a reduction of 3.84% per unit increase in BMI.

Inter- and intra-rater reliability of colonic landmarks

The degree of inter-rater agreement in the identification of the hepatic flexure, splenic flexure and descending end was good to excellent, with the ICCs ranging between 0.86 and 0.93 (95% CI = 0.73–0.97), while the reliability of the transverse mid-point was fair (ICC=0.66, 95% CI = 0.41–0.82). The intra-rater reliability generally ranged between good to

353 excellent (ICC=0.84–0.99, 95% CI = 0.71–1.00). For the full set of results, see Supporting
354 Information – Appendix C, Table S1.

355

356 **Inter- and intra-rater reliability of regional colonic transit times**

357 The inter-rater reliability of the regional colonic transit times was fair to good with the ICCs
358 ranging between 0.63 and 0.86 (95% CI = 0.39–0.93). With the exception of the descending
359 colon transit time, the intra-rater reliability of the regional colonic transit times was good to
360 excellent with the ICC ranging between 0.84 and 0.95 (95% CI = 0.71–0.97). The descending
361 colon transit time intra-rater reliability was fair (ICC=0.63, 95% CI = 0.39–0.79). For the full
362 set of results, see Supporting Information – Appendix B, Table S1.

363

364 **DISCUSSION**

365 Normative reference values are essential for an investigation to be meaningful and aid in
366 the diagnostic assessment of GI motility disorders. We have presented normative reference
367 ranges for the 3D-Transit system and in doing so, demonstrated its ability to perform an in-
368 depth, continuous assessment of gut transit. The main findings of the study are discussed in
369 separate sub-headed sections below.

370 **Normative reference ranges for region-specific colonic and gastrointestinal transit times**

371 This is the first study that presents normative reference ranges for not only GI transit, but
372 also for region-specific colonic transit using a reliable and minimally-invasive method that
373 continually measures gut transit within an ambulatory setting. A comparison of our
374 normative reference values against published data obtained using conventional techniques

such as the ROM method and scintigraphy is difficult to perform due to differences in reporting formats. Nonetheless, a comparison against data published by Wang et al.[21] for the WMC, a similar capsule-based technique can be performed. However, this can only be performed for GI transit times as the WMC cannot reliably provide regional colonic transit times. To make the comparison, normative cut-offs for accelerated and delayed GI transit were extracted from the 3D-Transit system data in a similar fashion to those reported by Wang et al[21] – see Table 4. Small variations in the cut-offs can be attributed to differences in protocols, study populations and methodology; overall however, the GI transit time cut-offs obtained from the two methods are comparable e.g. upper limit of normal for CTT to the nearest 15 min: 47 h 45 min by 3D-Transit vs. 50 h 30 min by WMC.[21]

The accelerated and delayed transit cut-offs for the region-specific colonic transit times have also been included (Table 4) to demonstrate the system's potential to provide further insights into normal and pathological colonic function. However, there is currently no means of comparing this to published data due to variations in measurement methods and reporting.

24-hour clustering of whole gut and total colonic transit times

Our normative reference data showed that WGTT values cluster at intervals separated by approximately 24 hours, as per normal bowel habits.[21,28] By association, CTT values were also found to cluster in a similar manner, which further reinforces the point that these measurements should be described as non-continuous measurements in increments of 24 hours, rather than as a continuous measure, as done with ROM[7]. Interestingly, our data showed a phasic relationship between the location of a capsule in the colon and it's

expulsion within 24 hours of ingestion i.e. a capsule is more likely to get expelled within the first 24-hour cluster if it is located in the left side of the colon, particularly in the rectosigmoid segment.[29] If a capsule is still located in the right side of the colon at 24 hours since ingestion, there is a higher chance of it being retained in the colon until the next bowel movement, which is expected to occur within the next 24-hour cluster.

As for the timing of capsule expulsions, the majority were observed to occur in the morning, in response to waking and ingestion of a meal as previously reported by others.[28,30,31] A small percentage of capsules expulsions were also observed to occur at lunch and evening meal times, consistent with the stimulatory effect of meal ingestion on colonic motility.[30,31]

Effect of age on gastrointestinal and region-specific colonic transit times

The results from the Poisson regression analysis show that increasing age is associated with longer CTT, WGTT, ascending colon, transverse colon and total right colon transit times, but shorter rectosigmoid transit times. Existing literature on the influence of ageing on gut function is conflicting, whereby some studies report slower colonic transit in older individuals[32,33] whilst others report no effect of age on gut transit.[7,21,34] It is, however, known that certain risk factors emerge with advancing age such as the concomitant use of medications, reduced intake of dietary fiber and reduced levels of activity or impaired mobility which may have an impact on overall gut and colonic function.[35] Furthermore, some studies have reported intrinsic age-related changes in the colon such as the neurodegeneration of myenteric nerves, which may explain slower colonic transit in the elderly.[36,37] However, a recent large-scale study by Broad at al.[38] showed no changes in the number of myenteric and nitrergic neurons or intramuscular nerve densities in ascending

421 and descending colon tissue samples of elderly subjects. They did however, observe
422 significant functional changes in the ascending colon with increasing age such as an
423 increased likelihood of muscle relaxation, rather than contraction when electrically
424 stimulated, an increase in choline acetyltransferase immunoreactive neurons and a decline
425 in cholinergic function.[38] These changes may well manifest as longer transit times that we
426 have observed in the right side of the colon of elderly subjects.

427

428 **Effect of gender on gastrointestinal and region-specific colonic transit times**

429 Several studies report longer regional transit times in females.[7,21,33,39,40] This is largely
430 attributed to the effects of the menstrual cycle and hormones.[33] For the first time, the
431 influence of gender has been studied on region-specific colonic transit times. Our results
432 show that the female gender is significantly associated with longer transverse colon (by
433 24.2%) and descending colon (by 51.3%) transit times. However, rectosigmoid transit was
434 significantly shorter in females (by 32.8%). The only comparable studies are those done by
435 Metcalf et al.[7] who reported longer right and left colon transit in females but did not see any
436 difference in rectosigmoid transit, and Abrahamsson et al.[40] who reported longer
437 descending transit in females. Interestingly, the shorter rectosigmoid transit times observed
438 in the female subjects was due to the fact that capsules generally took longer (than males)
439 to reach the rectosigmoid, as a consequence of longer transverse and descending colon
440 transit times, at which point the capsules were closer to the time when subjects would
441 empty their bowels. This effect may potentially explain the shorter rectosigmoid transit
442 times that we observed in elderly subjects.

443

Effect of BMI on gastrointestinal and region-specific colonic transit times

Our results show that increasing BMI is associated with shorter WGTT and GET, although the result for GET did not quite reach the significance level. Existing literature on the effects of increasing BMI on GI transit times is mostly focused on gastric motility. Most studies appear to associate obesity with accelerated gastric emptying, which has the potential to decrease the nutrient-induced satiety signal, the effect of which may stimulate over-eating. [41,42] A few studies have evaluated the effects of obesity on small intestinal transit times but the findings are conflicting.[41,42] There is even less evidence on the effects of obesity on colonic transit times. More conclusive studies are required to better understand the influence of BMI on, not only the upper but also lower GI function.

Comparison of 3D-Transit system with conventional methods

Unlike conventional radiological techniques which derive transit time measurements from mathematical assumptions, the 3D-Transit system provides a direct and continuous means of tracking the progression of an ingested marker as it traverses the GI tract. For instance, with the ROM technique, regional colonic transit time is determined by counting the number of markers in a given segment and multiplying it by a factor, which varies depending on the protocol.[40,43,44] This factor is based on the total number of markers ingested, hence the progression of markers within colonic segments is measured with respect to whole gut transit time rather than the total colonic transit time. This in turn has the effect of overestimating the overall colonic and segmental transit times, as gastric emptying and small bowel transit generally account for at least 6 – 10 hours of whole gut transit time.[45] Furthermore, the mathematical formulae used provide transit time values assumed to

467 follow a linear progression. It is clear from scintigraphy and previous 3D-Transit system
468 studies that major shifts in intra-luminal content occur only a few times per day, allied to
469 high amplitude propulsive contractions as seen on colonic manometry i.e. there is a staccato
470 progression.[20,46] In addition, colonic content is known to move both in the antegrade
471 (towards rectum) and retrograde directions.[47] Furthermore, most ROM studies provide only
472 three measurements of regional colonic transit,[48] namely the total right, total left and
473 rectosigmoid transit, as identified from bony structures on an X-ray film.[43] These segments
474 are not sufficient or accurate enough to describe regional colonic transit. Some ROM
475 protocols do exist, which divide the colon into 4 to 7 segments,[40,44,49] but these are not in
476 routine clinical use.

477

478 Colonic scintigraphy, by comparison, tracks the progression of a liquid-based radioisotopic
479 substance.[8] However, data interpretation can be difficult as the radioisotope tends to
480 spread out over a larger area of the colon. Transit measurements are therefore derived in
481 various reporting formats from estimates of radioactivity within specific regions.[48] Unlike
482 the ROM and colonic scintigraphy methods, the 3D-Transit system tracks the progression of
483 a single marker, which is the electromagnetic capsule, from the point of ingestion to
484 expulsion. This enables a path of capsule progression to be visualized, from which direct
485 colonic transit measurements can be made as illustrated in figures 1 and 2.

486

487 The previous, non-ambulatory version of the 3D-transit system, which used a stationary
488 detector plate has been compared against the ROM technique to demonstrate that the
489 position of the capsule correlates well with the progression of the markers through the

colon.[50] The non-ambulatory system was also validated against capsule endoscopy (PillCam, Medtronic, USA) for the measurement of gastric emptying and small intestinal transit time.[51] The current ambulatory version of the 3D-Transit system was validated against the ROM method for the measurement of WGTT.[13] The two methods were found to be comparable, though the 3D-Transit system's WGTT estimates were seen to be longer, a finding attributed to the size of the capsule in relation to ROMs.[13] The other comparative validation of the ambulatory version of the 3D-Transit system has been against magnetic resonance imaging, for the measurement of colorectal length.[19]

To date, the ambulatory 3D-Transit system has not been directly compared against similar, capsule-based systems such as the WMC. Nevertheless, although the modes of operation of both systems are different and the WMC is bigger due to the use of multiple sensors (3D-Transit capsule Ø 8.3 mm, length 21.5 mm; WMC Ø 11.7 mm; length 26.8 mm), both systems provide a similar set of normative values and cut-offs for accelerated and delayed GI transit as described previously. Additionally, both systems demonstrate the 24-hour clustering of WGTT and CTT values. This finding is, in itself, a validation of the two methods against each other.

Reliability of region-specific colonic transit times

The inter-and intra-rater reliability of the region-specific colonic transit times was generally good to excellent, with the exception of the descending colon transit time which was fair.

This could be due to its magnitude i.e. duration of the measurement is relatively small when compared to the other regional colonic measurements, making it sensitive to uncertainties in the placement of the colonic landmarks illustrated in Figure 1. However, the magnitude of the descending transit measurement is similar to that of the rectosigmoid measurement which is seen to have good reliability. This difference can be due to the use of a fixed landmark to determine the rectosigmoid transit times i.e. the colon segment end which makes it less variable than the descending colon transit measurement. The inter-rater reliability of the total right colon transit time was also seen to be fair. This can be attributed to the fair reliability result for the transverse mid-point landmark, which was used to determine the total right colon transit time and was subjectively more difficult to identify due to some retrograde motion of the capsule in this segment of the colon.

Limitations

The main limitation of the study is the loss of recordings. 17 of the 128 (13.3%) recordings were excluded from analysis due to technical issues with the system. This was consistent across all sites indicating a need to improve the system to avoid data loss. Another limitation is the manual analysis of recordings which may have an impact on the accuracy of the data if performed by inexperienced investigators.[26] Although the inter- and intra-rater reliability of the region-specific colonic transit times showed good agreement between experienced raters, there is a need to automate the method to ensure consistency and to improve the speed of processing as manual extraction of data is time-consuming.

Some variability in study protocols across the three research centers is a further limitation of the study whereby variations in capsule ingestion time, ingestion and study meal protocols and the study group populations may have influenced the measurements in a subtle manner that was difficult to detect. Further controlled studies are needed to refine these normative values.

In conclusion, we have for the first time presented normative reference values for region-specific colonic transit using a minimally-invasive ambulatory method. As a research tool, the 3D-Transit system has provided a dataset that expands upon current data provided by other clinically approved methods, thereby enhancing our understanding of normal and pathological physiology and the influence of factors such as age, gender and BMI.

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AUTHORSHIP STATEMENT

Guarantor of the article: Dr S Mark Scott PhD.

Author contributions: Gursharan Kalsi: collation of data, data analysis, statistical analysis, interpretation of data, drafting of the manuscript; Esben Bolvig Mark: development of algorithms, data analysis, revised the manuscript for important intellectual content; Gian Luca Di Tanna: statistical analysis; Anne-Mette Haase, Jakob Poulsen, Stephanos Christodoulides, Victor Kung, Mette Klinge, Karoline Knudsen, Per Borghammer, Katrine Andersen, Lotte Fynne: data acquisition, revised the manuscript for important intellectual content; Nanna Sutter: collation of data, revised the manuscript for important intellectual content; Vincent Schlageter: technical support, performed a technical review of the article; Klaus Krogh, Asbjørn Mohr Drewes, Malcolm Birch & S. Mark Scott: study concept and

567 design, study supervision, interpretation of data, critical revision of the manuscript for

568 important intellectual content. All authors approved the final version of the manuscript.

REFERENCES

1. Canavan C, West J, Card T. The epidemiology of irritable bowel syndrome. *Clin Epidemiol.* 2014;6:71-80.
2. Sanchez MI, Bercik P. Epidemiology and burden of chronic constipation. *Can J Gastroenterol.* 2011;25 Suppl B:11B-15B.
3. Nellesen D, Yee K, Chawla A, Lewis BE, Carson RT. A systematic review of the economic and humanistic burden of illness in irritable bowel syndrome and chronic constipation. *J Manag Care Pharm.* 2013;19(9):755-764.
4. Carrington EV, Scott SM, Bharucha A, et al. Expert consensus document: Advances in the evaluation of anorectal function. *Nat Rev Gastroenterol Hepatol.* 2018;15(5):309-323.
5. Rao SS, Camilleri M, Hasler WL, et al. Evaluation of gastrointestinal transit in clinical practice: position paper of the American and European Neurogastroenterology and Motility Societies. *Neurogastroenterol Motil.* 2011;23(1):8-23.
6. Keller J, Bassotti G, Clarke J, et al. Expert consensus document: Advances in the diagnosis and classification of gastric and intestinal motility disorders. *Nat Rev Gastroenterol Hepatol.* 2018;15(5):291-308.
7. Metcalf AM, Phillips SF, Zinsmeister AR, MacCarty RL, Beart RW, Wolff BG. Simplified assessment of segmental colonic transit. In: *Gastroenterology.* Vol 92. United States1987:40-47.
8. Krevsky B, Malmud LS, D'Ercole F, Maurer AH, Fisher RS. Colonic transit scintigraphy. A physiologic approach to the quantitative measurement of colonic transit in humans. In: *Gastroenterology.* Vol 91. United States1986:1102-1112.
9. Dinning PG, Smith TK, Scott SM. Pathophysiology of colonic causes of chronic constipation. *Neurogastroenterol Motil.* 2009;21 Suppl 2:20-30.
10. Maurer AH. Gastrointestinal Motility, Part 2: Small-Bowel and Colon Transit. *J Nucl Med Technol.* 2016;44(1):12-18.
11. Gronlund D, Poulsen JL, Sandberg TH, et al. Established and emerging methods for assessment of small and large intestinal motility. *Neurogastroenterol Motil.* 2017;29(7).
12. Zarate N, Mohammed SD, O'Shaughnessy E, et al. Accurate localization of a fall in pH within the ileocecal region: validation using a dual-scintigraphic technique. *Am J Physiol Gastrointest Liver Physiol.* 2010;299(6):G1276-1286.
13. Haase AM, Gregersen T, Schlageter V, et al. Pilot study trialling a new ambulatory method for the clinical assessment of regional gastrointestinal transit using multiple electromagnetic capsules. *Neurogastroenterol Motil.* 2014;26(12):1783-1791.
14. Gregersen T, Haase AM, Schlageter V, Gronbaek H, Krogh K. Regional Gastrointestinal Transit Times in Patients With Carcinoid Diarrhea: Assessment With the Novel 3D-Transit System. *Journal of neurogastroenterology and motility.* 2015;21(3):423-432.
15. Haase AM, Gregersen T, Christensen LA, et al. Regional gastrointestinal transit times in severe ulcerative colitis. *Neurogastroenterol Motil.* 2016;28(2):217-224.
16. Knudsen K, Haase AM, Fedorova TD, et al. Gastrointestinal Transit Time in Parkinson's Disease Using a Magnetic Tracking System. *J Parkinsons Dis.* 2017;7(3):471-479.
17. Poulsen JL, Nilsson M, Brock C, Sandberg TH, Krogh K, Drewes AM. The Impact of Opioid Treatment on Regional Gastrointestinal Transit. *J Neurogastroenterol Motil.* 2016;22(2):282-291.
18. Poulsen JL, Mark EB, Brock C, Frokjaer JB, Krogh K, Drewes AM. Colorectal Transit and Volume During Treatment With Prolonged-release Oxycodone/Naloxone Versus Oxycodone Plus Macrogol 3350. *J Neurogastroenterol Motil.* 2018;24(1):119-127.

19. Mark EB, Poulsen JL, Haase AM, et al. Assessment of colorectal length using the electromagnetic capsule tracking system: a comparative validation study in healthy subjects. *Colorectal Dis.* 2017;19(9):O350-o357.
20. Mark EB, Poulsen JL, Haase AM, et al. Ambulatory assessment of colonic motility using the electromagnetic capsule tracking system. *Neurogastroenterol Motil.* 2018:e13451.
21. Wang YT, Mohammed SD, Farmer AD, et al. Regional gastrointestinal transit and pH studied in 215 healthy volunteers using the wireless motility capsule: influence of age, gender, study country and testing protocol. *Aliment Pharmacol Ther.* 2015;42(6):761-772.
22. Christodoulides S. *Multidimensional risk factor assessment in chronic idiopathic constipation, with a focus on fibre*. London: St Bartholomew's and the Royal London School of Medicine and Dentistry, Queen Mary University of London; 2019.
23. Mahoney FI, Barthel DW. Functional Evaluation: The Barthel Index. *Md State Med J.* 1965;14:61-65.
24. Agachan F, Chen T, Pfeifer J, Reissman P, Wexner SD. A constipation scoring system to simplify evaluation and management of constipated patients. *Dis Colon Rectum.* 1996;39(6):681-685.
25. Kuo B, McCallum RW, Koch KL, et al. Comparison of gastric emptying of a nondigestible capsule to a radio-labelled meal in healthy and gastroparetic subjects. *Aliment Pharmacol Ther.* 2008;27(2):186-196.
26. Kalsi GK, Gronlund D, Martin J, Drewes AM, Scott SM, Birch MJ. Technical report: Inter- and intra-rater reliability of regional gastrointestinal transit times measured using the 3D-Transit electromagnet tracking system. *Neurogastroenterol Motil.* 2018:e13396.
27. Koo TK, Li MY. A Guideline of Selecting and Reporting Intraclass Correlation Coefficients for Reliability Research. *J Chiropr Med.* 2016;15(2):155-163.
28. Heaton KW, Radvan J, Cripps H, Mountford RA, Braddon FE, Hughes AO. Defecation frequency and timing, and stool form in the general population: a prospective study. *Gut.* 1992;33(6):818-824.
29. Lubowski DZ, Meagher AP, Smart RC, Butler SP. Scintigraphic assessment of colonic function during defaecation. *Int J Colorectal Dis.* 1995;10(2):91-93.
30. Duthie HL. Colonic response to eating. *Gastroenterology.* 1978;75(3):527-528.
31. Rao SS, Sadeghi P, Beaty J, Kavlock R, Ackerson K. Ambulatory 24-h colonic manometry in healthy humans. *Am J Physiol Gastrointest Liver Physiol.* 2001;280(4):G629-639.
32. Madsen JL, Graff J. Effects of ageing on gastrointestinal motor function. *Age Ageing.* 2004;33(2):154-159.
33. Graff J, Brinch K, Madsen JL. Gastrointestinal mean transit times in young and middle-aged healthy subjects. In: *Clin Physiol.* Vol 21.2001:253-259.
34. Saad RJ, Semler JR, Wilding GE, Chey WD. The Effect of Age on Regional and Whole Gut Transit Times in Healthy Adults. *Gastroenterology.* 2010;138(5).
35. Kim SE. Colonic Slow Transit Can Cause Changes in the Gut Environment Observed in the Elderly. *J Neurogastroenterol Motil.* 2017;23(1):3-4.
36. Gomes OA, de Souza RR, Liberti EA. A preliminary investigation of the effects of aging on the nerve cell number in the myenteric ganglia of the human colon. *Gerontology.* 1997;43(4):210-217.
37. Salles N. Basic mechanisms of the aging gastrointestinal tract. *Dig Dis.* 2007;25(2):112-117.
38. Broad J, Kung VWS, Palmer A, et al. Changes in neuromuscular structure and functions of human colon during ageing are region-dependent. *Gut.* 2019;68(7):1210-1223.
39. Sadik R, Abrahamsson H, Stotzer PO. Gender differences in gut transit shown with a newly developed radiological procedure. *Scand J Gastroenterol.* 2003;38(1):36-42.
40. Abrahamsson H, Antov S, Bosaeus I. Gastrointestinal and colonic segmental transit time evaluated by a single abdominal x-ray in healthy subjects and constipated patients. *Scand J Gastroenterol Suppl.* 1988;152:72-80.

667 41. Xing J, Chen JD. Alterations of gastrointestinal motility in obesity. *Obes Res.*
668 2004;12(11):1723-1732.
669 42. Mushref MA, Srinivasan S. Effect of high fat-diet and obesity on gastrointestinal motility. *Ann*
670 *Transl Med.* 2013;1(2):14.
671 43. Arhan P, Devroede G, Jehannin B, et al. Segmental colonic transit time. *Dis Colon Rectum.*
672 1981;24(8):625-629.
673 44. Bouchoucha M, Odinot JM, Devroede G, Landi B, Cugnenc PH, Barbier JP. Simple clinical
674 assessment of colonic response to food. *International Journal of Colorectal Disease.*
675 1998;13(5-6):217-222.
676 45. Camilleri M, Thorne NK, Ringel Y, et al. Wireless pH-motility capsule for colonic transit:
677 prospective comparison with radiopaque markers in chronic constipation.
678 *Neurogastroenterol Motil.* 2010;22(8):874-882, e233.
679 46. Cook IJ, Furukawa Y, Panagopoulos V, Collins PJ, Dent J. Relationships between spatial
680 patterns of colonic pressure and individual movements of content. *Am J Physiol Gastrointest*
681 *Liver Physiol.* 2000;278(2):G329-341.
682 47. Dinning PG, Szczesniak MM, Cook IJ. Proximal colonic propagating pressure waves
683 sequences and their relationship with movements of content in the proximal human colon.
684 *Neurogastroenterol Motil.* 2008;20(5):512-520.
685 48. Southwell BR, Clarke MC, Sutcliffe J, Hutson JM. Colonic transit studies: normal values for
686 adults and children with comparison of radiological and scintigraphic methods. *Pediatr Surg*
687 *Int.* 2009;25(7):559-572.
688 49. Lundin E, Graf W, Garske U, Nilsson S, Maripuu E, Karlbom U. Segmental colonic transit
689 studies: comparison of a radiological and a scintigraphic method. *Colorectal Dis.*
690 2007;9(4):344-351.
691 50. Hiroz P, Schlageter V, Givel JC, Kucera P. Colonic movements in healthy subjects as
692 monitored by a Magnet Tracking System. *Neurogastroenterol Motil.* 2009;21(8):838-e857.
693 51. Worsoe J, Fynne L, Gregersen T, et al. Gastric transit and small intestinal transit time and
694 motility assessed by a magnet tracking system. *BMC Gastroenterol.* 2011;11:145.
695

696 **TABLES**

Table 1: Subject Demographics

	Overall	Aalborg Denmark	Aarhus Denmark	London United Kingdom
N	111	19	67	25
Gender (female: male)	58:53	All male	33:34	All female
Median age [years]† (range)	40 (21 – 88)	26 (22 – 55)	46 (22 – 80)	72 (21 – 88)
Median BMI [kg/m²] † (range)*	24.0 (19.0 – 38.1)	23.0 (20.5 – 30.4)	24.4 (19.0 – 35.2)	24.0 (19.3 – 38.1)

N: number of recordings
BMI: body-mass index
†6 values missing

697

Table 2: Normative values for GI and region-specific colonic transit times (h:min)

Parameter	Group	N	Median	95% CI		5th percentile	95th percentile
				Lower Limit	Upper Limit		
Gastric emptying time†	All	104	2:41	2:29	3:06	0:32	6:01
	F	51	2:29	2:01	2:40	0:20	10:21
	M	53	3:06	2:41	3:35	0:31	5:56
Small intestinal transit time	All	111	4:47	4:20	5:06	1:50	9:29
	F	58	5:03	4:31	5:46	1:29	11:39
	M	53	4:23	3:54	5:05	2:08	9:12
Colonic transit time	All	111	21:06	18:39	23:54	5:55	47:44
	F	58	23:21	18:39	27:46	6:10	47:07
	M	53	19:36	16:11	22:04	4:44	55:22
Whole gut transit time	All	111	28:52	25:37	30:48	14:10	57:49
	F	58	30:28	25:20	44:01	19:11	56:38
	M	53	27:01	24:32	29:46	12:24	61:09
Ascending colon transit time	All	111	5:41	3:30	6:44	0:06	37:28
	F	58	5:57	3:30	6:31	0:02	38:25
	M	53	5:21	3:01	8:15	0:07	31:06
Transverse colon transit time	All	111	4:53	3:35	6:18	0:01	18:56
	F	58	6:49	4:13	9:10	0:05	20:47
	M	53	3:51	1:57	5:30	0:01	14:37
Descending colon transit time	All	111	1:54	1:01	2:58	0:00	13:59
	F	58	2:05	0:57	4:01	0:00	19:10
	M	53	1:30	0:34	3:06	0:00	11:10
Rectosigmoid transit time	All	111	2:18	0:55	5:11	0:00	20:37
	F	58	1:00	0:29	3:10	0:00	20:36
	M	53	4:41	1:23	7:39	0:00	23:44
Total right colon transit time	All	111	7:37	6:11	10:53	0:27	37:34
	F	58	8:19	6:15	11:42	0:25	38:25
	M	53	6:49	4:21	12:53	0:23	34:14
Total left colon transit time	All	111	11:01	9:54	13:52	0:43	34:07
	F	58	12:11	9:54	15:44	0:42	34:29
	M	53	10:55	6:44	15:14	0:35	31:39

N: No. of 3D-Transit recordings
CI: Confidence interval
F: female; M: male
†7 3D-transit recordings excluded as capsule was still in the stomach when subjects ingested second meal after capsule ingestion

Table 3: Poisson regression estimates of the time percent change (TPC) and their 95% confidence intervals (CI) for the effects of age, gender and BMI on the GI and regional colonic transit times. For gender comparison, the TPC was estimated by comparing females to males. Significant results highlighted in bold.

Parameter	Predictor Variable	TPC	95% CI for TPC		p-value
			Lower limit	Upper limit	
Gastric Emptying Time	Age	1.007	0.999	1.015	0.068
	Female	0.926	0.674	1.274	0.637
	BMI	0.962	0.924	1.000	0.051
	Constant	5.181	1.754	15.304	0.003
Small Intestinal Transit Time	Age	1.003	0.998	1.008	0.188
	Female	1.085	0.908	1.297	0.369
	BMI	0.977	0.953	1.002	0.073
	Constant	7.506	4.122	13.669	0.000
Colonic Transit Time	Age	1.003	1.000	1.005	**0.021**
	Female	1.013	0.907	1.130	0.822
	BMI	0.996	0.985	1.007	0.516
	Constant	24.621	18.101	33.489	0.000
Whole Gut Transit Time	Age	1.003	1.002	1.005	**0.000**
	Female	1.002	0.912	1.100	0.966
	BMI	0.988	0.978	0.997	**0.012**
	Constant	39.737	30.367	51.999	0.000
Ascending colon transit	Age	1.006	1.002	1.009	**0.004**
	Female	0.988	0.814	1.198	0.900
	BMI	0.983	0.965	1.002	0.077
	Constant	10.289	6.124	17.288	0.000
Transverse colon transit	Age	1.008	1.004	1.013	**0.000**
	Female	1.242	1.001	1.541	**0.049**
	BMI	0.995	0.975	1.015	0.605
	Constant	5.006	2.786	8.995	0.000
Descending colon transit	Age	0.999	0.994	1.004	0.678
	Female	1.513	1.236	1.852	**0.000**
	BMI	1.025	0.999	1.052	0.063
	Constant	1.809	0.961	3.404	0.066
Rectosigmoid colon transit	Age	0.992	0.987	0.998	**0.004**
	Female	0.672	0.558	0.809	**0.000**
	BMI	1.000	0.975	1.026	0.994
	Constant	9.016	4.902	16.584	0.000
Total Right Colon transit	Age	1.007	1.004	1.010	**0.000**
	Female	1.011	0.860	1.189	0.891
	BMI	0.988	0.972	1.003	0.120
	Constant	11.635	7.425	18.231	0.000
Total Left Colon transit	Age	0.998	0.995	1.001	0.297
	Female	1.060	0.950	1.183	0.295
	BMI	1.006	0.991	1.022	0.433
	Constant	11.913	8.283	17.135	0.000

Table 4: Normative reference values for accelerated and delayed GI and colonic transit. Accelerated transit defined as transit time values < 5th percentile and delayed transit defined as transit time values > 95th percentile of values indicated in Table 2.

Parameter	**Accelerated Transit (h:min)**	**Delayed Transit (h:min)**
GET	<0:30	>6:00
SITT	<2:00	>9:30
CTT	<6:00	>47:45
WGTT	<14:00	>58:00
Ascending colon transit time†	-	>37:30
Transverse colon transit time†	-	>19:00
Descending colon transit time†	-	>14:00
Rectosigmoid transit time†	-	>20:45
Total right colon transit time	<0:30	>37:30
Total left colon transit time	<0:45	>34:00

GET: Gastric emptying time; SITT: Small intestinal transit time; CTT: colonic transit time; WGTT: whole gut transit time
Transit times have been rounded-up to the nearest 15 min
†5th percentile values for these transit times are close to zero

701

702

703 **FIGURE LEGENDS**

704 **Figure 1:** Measurement of region-specific colonic transit times from a 3D-Transit recording.
705 (a) Raw "pre-cleaned" 3D-Transit recording. (b) After cleaning, colon segment start (CS),
706 hepatic flexure (HF), transverse midpoint (TM), splenic flexure (SF), end of descending colon
707 (DE) and colon segment end (CE) are identified. (c) Identified anatomical segments of the
708 colon are used to determine regional colonic transit times (TT)

709

710 **Figure 2:** Median region-specific colonic transit times (h: min) by gender. F: female, M: male;
711 figures in brackets: 95% CI for median; *$p < 0.05$ as per Figure 4

712

713 **Figure 3:** Clustering of whole gut (WGTT) and colonic (CTT) transit times at intervals
714 separated by 24 hours. (a) Frequency polygon for WGTT in hours (b) Frequency polygon for
715 CTT in hours (c) Location of capsule in the colon at 24 hours since ingestion for each 24-hour
716 cluster shown in Figure 3(a), where the first 24-hour cluster includes capsules with WGTT <
717 36 hours ($N = 75$) and the second 24-hour cluster includes capsules with WGTT > 36 hours (N
718 $= 36$) (d) Location of capsule at 60 minutes and 30 minutes prior to defecation

719

720 **Figure 4:** Effects of gender on (a) gastrointestinal transit times and (b) region-specific colonic
721 transit times (c) Boxplot of the time of entry of a capsule into the rectosigmoid segment of
722 the colon, normalised to overall CTT in female and male subjects. Median normalised time
723 of entry into the rectosigmoid was significantly different between the female (0.94) and
724 male (0.78) subjects ($p = 0.015$). Data points for the male (blue) and female (orange)

725 subjects have been superimposed over the boxplots to show that in females, capsules
726 generally take longer (by approximately 16%) to reach the rectosigmoid segment, at which
727 point the capsules are closer to expulsion (indicated by a value of 1 in the boxplot) than in
728 the male subjects. GET: gastric emptying time, SITT: small intestinal transit time, CTT:
729 colonic transit time, WGTT: whole gut transit time, ASC: ascending colon, TRA: transverse
730 colon, DESC: descending colon, RSIG: rectosigmoid, TOTAL R: total right colon, TOTAL L: total
731 left colon. Displayed transit times are median values; errors bars: 95% CI for median; *$*p <$
732 *0.05*