Variability of gastrointestinal transit in healthy women and men

L P Degen, S F Phillips

Abstract

Background and Aims—Measurements of gastrointestinal transit are made in clinical and research gastroenterology, yet their intrinsic variability is not well characterised. In particular, an influence of hormones on transit has been proposed as the basis for gastrointestinal symptoms that vary with the menstrual cycle. Our aims were to quantify individual differences in transit during the menstrual cycle in healthy women and to compare these with the intrinsic variability in healthy men.

Methods—On two occasions, whole gut transit was assessed scintigraphically and colonic transit quantified by radio-opaque markers. Thirty-two healthy volunteers (12 women, 20 men) were studied, women during the follicular and luteal phases, men twice within a similar four week period. Diets and exercise were standardised prior to and during both studies.

Results—Colonic transit was significantly faster in men, and postprandial gastric emptying was also more rapid; other indices of regional transit were not different between the sexes. Total colonic transit time was equally well reflected by the scintigraphic and radio-opaque marker methods. Important intraindividual differences were noted in both sexes. The variances in our samples predicted an 80% chance of detecting (with 95% confidence) a mean effect of menstrual hormones on transit that was in the same range as the intrinsic variation in men.

Conclusions—Colonic transit was faster in men than in women. Although group means in the two studies were almost identical, single assessments of transit in subjects sometimes exhibited considerable variability, implying broad biological variations. Given this intrinsic variability, the influence of menstrual hormones on gastrointestinal transit must be small and of doubtful clinical significance.

Keywords: transit, reproducibility, sex differences.

Rates at which food and digestive products traverse the gastrointestinal tract reflect the integrated activity of intestinal smooth muscle, are thought to be relevant to certain symptoms,1-3 and have been quantified by several methodologies.1-7 Scintigraphic assessments of gastric emptying are perhaps best established, and they are now standard in many nuclear medicine facilities; reports of their reproducibility have been published.8-10 Scintigraphy has now been applied also to small bowel and colonic transit3 5 and the techniques have been simplified enough to make clinical tests for whole gut transit feasible.11-13 However, the day to day reproducibility of small bowel and colonic transit have not been reported, even though these indices are required for adequate interpretation of clinical and experimental studies. Moreover, bowel habits may vary daily, especially in patients with functional gastrointestinal disorders. It is important, therefore, that the normal biologi-

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because transit may be altered in clinically obese patients. In addition women must have had a regular menstrual cycle for the last six months, the length needed to be 28±4 days and there had to be no major symptoms during the menstrual cycle. Excluded also were lactating women and those with a pregnancy within the last year.

With standardised questions, the smoking habits as well as the consumption of alcohol and coffee were assessed. After discussion of the procedure in detail, written consent was obtained for a protocol approved previously by the Institutional Review Board and the Radiation Control Committee of Mayo Clinic. All female volunteers had to have had a negative plasma β human chorionic gonadotrophin pregnancy test no longer than 48 hours before each scintigraphic study.

Experimental protocol
Three days prior to and during the entire study, volunteers ingested a weight maintaining diet, based on the Harris-Benedict equation, with adjustment for daily physical activity. Volunteers were asked to avoid unusually intensive physical activity. Meals were provided by the Mayo General Clinical Research Center and their composition was normalised to 53% carbohydrate, 17% protein, and 30% fat. Fibre intake was standardised to 15 gram per day, consisting of 60% (9 gram) water insoluble and 40% (6 gram) water soluble fibre.

On each of days 2–4, volunteers ingested at 9 am a capsule containing 24 radio-opaque markers (SITZMARKS, Lafayette Pharmacal, 4200 South Hulen Street, Fort Worth, TX 76109). At 9 am on day four, an abdominal x-ray was obtained for assessment of the distribution of the radio-opaque markers.

On day four at 7 am, after fasting since midnight, the scintigraphic transit study begun and it finished 48 hours later.

At the beginning of each study period, all participants reported their physical activity within the last week, corresponding to the Harvard Alumni Activity Survey questionnaire and completed the self report inventory SCL-90-R to reflect their pattern of psychological symptoms within the past week. Immediately before the transit study, a venous blood sample was draw in women, to measure the concentrations of progesterone and oestradiol.

Women had two studies based on the menstrual cycle. Day 1 of the cycle was defined as the first day of menstrual flow. One transit study was performed on day 7–10 (= follicular phase) and one on day 21–24 (=luteal phase). At random, seven women had the first study during the follicular and five during the luteal phase. Men had the study repeated at equivalent times; an initial assessment was followed by the second within 14–17 days.

Study procedure
Gastric, small bowel, and colonic transit was measured by the non-invasive scintigraphic method developed in our laboratory. Briefly, polystyrene Amberlite 120-IR-Plus resin pellets (average diameter 1 mm; range 0.5–1.8 mm) were labelled in an acid medium with 100 μCi of 111InCl3. The efficiency of the labelling was >98%, as judged by thin layer chromatography. A capsule filled with approximately 0.5 g pellets and coated with one layer of methacrylate was given to fasting volunteers. As expected, the capsule dissolved in the ileocecal region and thereafter marked ileocecal transfer and colonic transit of contents.

External radioactive markers were placed over both anterior superior iliac spines to estimate the location of the capsule. As soon as the radiolabelled capsule passed into the small bowel, a breakfast was ingested within five minutes. It consisted of two scrambled eggs, one slice of whole wheat bread, and skimmed milk (35% protein, 52% carbohydrate, 13% fat, 219 kcal). The scrambled eggs were mixed and cooked with 1 mCi of 99mTc labelled Amberlite 410 resin pellets (average diameter 1 mm) to a firm consistency to provide a solid medium. These pellets had been labelled with 99mTc sodium pertechnetate in a neutral medium.

Four hours after breakfast, a standardised non-radiolabelled lunch (chicken, potato, butter, tapioca pudding, and water; 535 kcal) and, eight hours after breakfast, a dinner (steak, salad, dessert; 21% protein, 49% carbohydrate, 30% fat; 561 kcal) was consumed. During the study volunteers were permitted normal physical activity.

Gammacamera imaging
Gammacamera imaging started immediately after completion of ingestion of the radio-labelled breakfast with a large field of view gamma camera with a medium energy parallel hole collimator (GE Starcam, General Electric, Milwaukee, WI). Anterior and posterior images were acquired with the subject erect. For the 99mTc counts a 140 keV, and for the 111In counts a 245 keV, energy window (each with ±20% window) was utilised. The estimated whole body dose equivalent was 130 m Rem.

Images were obtained at the following times (hours) after ingestion of the labelled meal, 0, 0.5, 1, 2, 3–5, 8–10, 12, 24, and 48. For each image, two minutes of acquisition were selected. Using variable regions of interest, the radioactivity was quantified in the stomach and ascending colon for 99mTc and in four regions of the colon (ascending, transverse, descending, rectosigmoid) for 111In. The geometric means of the counts obtained from anterior and posterior images were calculated for each region and then corrected for radionuclide decay. The downscatter of 111In into the 99mTc window was adjusted. For two days, stools were collected and the radioactivity for 111In counts was assessed and corrected for decay.
TABLE 1. Gastric emptying and small bowel transit. Reproducibility studies in healthy women (n=12) and men (n=20)

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Sex</th>
<th>Mean</th>
<th>SEM</th>
<th>Median</th>
</tr>
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<tbody>
<tr>
<td>Gastric lag phase (minutes)</td>
<td>F1</td>
<td>56</td>
<td>7</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>F2</td>
<td>56</td>
<td>6</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>M1</td>
<td>55</td>
<td>5</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>M2</td>
<td>58</td>
<td>3</td>
<td>58</td>
</tr>
<tr>
<td>Gastric half emptying (T1/2) minutes</td>
<td>F1</td>
<td>202</td>
<td>26</td>
<td>184</td>
</tr>
<tr>
<td></td>
<td>F2</td>
<td>198</td>
<td>22</td>
<td>201</td>
</tr>
<tr>
<td></td>
<td>M1</td>
<td>153</td>
<td>9</td>
<td>157</td>
</tr>
<tr>
<td></td>
<td>M2</td>
<td>163</td>
<td>13</td>
<td>148</td>
</tr>
<tr>
<td>Small bowel transit (minutes)</td>
<td>F1</td>
<td>196</td>
<td>22</td>
<td>179</td>
</tr>
<tr>
<td></td>
<td>F2</td>
<td>210</td>
<td>23</td>
<td>184</td>
</tr>
<tr>
<td></td>
<td>M1</td>
<td>181</td>
<td>19</td>
<td>186</td>
</tr>
<tr>
<td></td>
<td>M2</td>
<td>196</td>
<td>13</td>
<td>190</td>
</tr>
</tbody>
</table>

F1 is the study performed during the follicular and F2 during the luteal phase of the menstrual cycle in women.

Gastric emptying was assessed by the gastric lag time, postlag emptying rate, and the half emptying time (T1/2). The gastric lag time (min) was the time taken for 10% of the radiolabel to empty from the stomach. The gastric postlag emptying rate (%/min) was characterised by the slope and estimated from linear regression analysis of data from the first point beyond the lag time until the time when 90% of the radiolabel had emptied from the stomach.

Small bowel transit time was assessed by subtracting the time for 10% of the radiolabelled breakfast to empty from the stomach from the time taken for 10% of the label to enter the colon.

Colonic transit was evaluated by the geometric centre of counts in the colonic regions of interest (ROI). The geometric centre was the weighted average of the proportions of counts in the four ROI of the colon.

Data analysis

Gastric emptying by the scintigraphic method was assessed by the gastric lag time, postlag emptying rate, and the half emptying time (T1/2). The gastric lag time (min) was the time taken for 10% of the radiolabel to empty from the stomach. The gastric postlag emptying rate (%/min) was characterised by the slope and estimated from linear regression analysis of data from the first point beyond the lag time until the time when 90% of the radiolabel had emptied from the stomach.

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Colonic transit was evaluated by the geometric centre of counts in the colonic regions of interest (ROI). The geometric centre was the weighted average of the proportions of counts in the four ROI of the colon. The regions, designated by numbers 1-4 as weighting factors were, respectively, the ascending, transverse, descending, and rectosigmoid colons. The stool was designated as region 5. The proportion in each region was multiplied by the weighting factor and the sum calculated. A low geometric centre indicated that most radiolabel was closer to the caecum, whereas a high value indicated that the major part of the radiolabel was closer to the stool.

Colonic transit time measured by radio-opaque marker method

The total number of all markers for each colonic segment was multiplied by a factor of 1.0 and designated as the mean colonic transit time for that segment. The mean total colonic transit time was the sum of the calculated mean segmental transit times.

Physical activity score, SCL-90-R score, smoking habits, alcohol, and coffee consumption

Assessments of total energy expenditure, expressed as kilocalories/week, and the psychological symptom scores for primary symptoms and global indices of distress were rated. Smoking habits were quantified by pack years of actual consumption, alcohol consumption by units a week (1 unit beer, wine or hard drink = 10 g alcohol), and coffee by cups a day.

Hormone concentrations

Serum concentrations of progesterone and oestradiol were assessed with enhanced luminoscence and radioimmunoassays, respectively. A concentration of progesterone above 2 ng/ml was regarded as consistent with the follicular phase, values below 0.7 ng/ml with the luteal phase.

Statistical analysis

Gastrointestinal transit data were expressed by box whisker plots showing the median, the interquartile, and the total ranges. Normally distributed values were analysed statistically by paired or unpaired t tests and non-parametric data by the Wilcoxon's signed rank test. When we compared the radio-opaque and scintigraphic methods, we assessed the correlation between both the methods for each study period. Correlations between the results were
Figure 2: Colonic transit in healthy men and women, expressed as the geometric centre of counts at 48 hours, shown as median values (bars), interquartile ranges (boxes), and range for men and women. Women were studied in the follicular (F) and luteal (L) phases of the menstrual cycle. Men were studied twice, 14 days apart. Colonic transit was significantly faster in men.

Figure 3: Total colonic transit as measured by the radio-opaque marker method, in healthy men and women, shown as median values (bars), interquartile ranges (boxes), and range for men and women. Women were studied in the follicular (F) and luteal (L) phases of the menstrual cycle. Men were studied twice, 14 days apart. Colonic transit was significantly faster in men at 24 hours.

Figure 4: Sex differences in colonic transit, expressed as the progression of the geometric centres of scintigraphic markers against time. High values for the GC, signifying faster colonic transit, became apparent in men after 12 hours (approximately). At that time the GC was approximately 2.6 indicating that the centre of counts was in the transverse colon.

based on linear regression analysis. The influence of covariates on gut transit was evaluated using multiple analyses of variance. Significance was declared at $p<0.05$.

In women, the indices were compared intra as well as interindividually for the follicular and luteal phase; in men, the first and second studies were compared. As the menstrual cycle had no measurable effect on gastrointestinal transit, for sex comparisons we used the two values for women and the two for men to obtain a single value for each individual.

Intraindividual variability between results at each level of the gut were not significantly different between sexes and consequently have been summarised together.

Results

CHARACTERISTICS OF THE GROUPS

The mean age in both groups was 29 years (range, women: 19–44; men: 21–45). Although the body mass index did not differ significantly between the sexes (mean (SEM) women: 23.97 (0.95); men: 25.48 (0.63), weight and height were significantly different ($p<0.001$). Men were heavier (84 (2) vs 68 (3) kg) and taller (182 (2) vs 168 (2) cm). Although men ingested slightly more calories, the values were not significantly different between sexes, as well as between the two studies (women: study 1: 2845 (420), study 2: 2562 (288) kcal/day, men: 3384 (570) and 3296 (479) kcal/day). Smoking, coffee, and alcohol consumption were not significantly different between paired studies.

Women had their menarche at a mean age of 13 (0–4) years, the mean cycle length was 28 (0–7) with a menstrual length of five (0–3) days. The number of pregnancies was one (0–5). The fluctuation of hormonal concentrations between the follicular and luteal phases was significant for progesterone and oestradiol ($p<0.001$ and $p=0.018$, respectively). Progesterone measured in the follicular phase was 0.57 (0–07), in the luteal phase 0.7 (1–11) ng/ml. Oestradiol concentrations were 79–67 (11–09) and 137–33 (16–43) pg/ml, respectively.

SEX DIFFERENCES

Gastric emptying

The lag phase for solid meals and the half times for gastric emptying were not significantly different between sexes (Table I). However, the postlag gastric emptying rate (Fig 1) was significantly faster in men than women ($p=0.005$). No significant change in any of the three indices of gastric emptying was noted during the menstrual cycle (Table I, Fig 1).

Small bowel transit

The index demonstrated no sex differences and no effect of the menstrual cycle (Table I). The variability in women was similar to that in men.
Variability of gastrointestinal transit in healthy women and men

Index of transit. The means and medians of these intrasubjective differences were not significantly different from 0 for the gastric lag phase, postlag emptying slope, and T\textsubscript{1/2} of gastric emptying. However, the ranges of these intrasubjective differences were broad. Similar conclusions pertain to small bowel transit (Table II).

Repeated measurements of colonic transit also showed mean results that were very reproducible. When differences between study 1 and study 2 for both sexes were combined, median differences of the GCs at six and 24 hours were very close to zero and the interquartile ranges were also quite narrow (Fig 6). However, outliers were noted and the total ranges of intrasubjective differences were wide (Fig 7). Radio-opaque marker transit yielded similar comparisons between replicate studies. Figure 7 shows the mean colonic transit times for both studies in all subjects.

Discussion
Epidemiological findings suggest that women are more constipated than are men and Heaton et al. suggested a hormonal cause, especially in women of childbearing age. It is noteworthy, therefore, that female hormones influence gastrointestinal muscle function in animals. Contractile activity was inhibited in vitro by progesterone and excited by oestradiol; moreover, gastrointestinal transit in rodents was influenced by both classes of hormones. To extend these findings and quantify the effects in humans, transit times through the gut were estimated with radio-opaque markers or lactulose H\textsubscript{2} breath excretion. The results are inconsistent. Some have indicated no significant difference between women and men, while the measurements were those of orocecal transit or mouth to anus transit. Others have suggested a significant prolongation of mean colonic transit in women but were unable to demonstrate changes of transit during the menstrual cycle.

We studied healthy women and men twice under the same standardised conditions, in particular we controlled for diet, alcohol, and coffee consumption, physical activities, and psychological patterns. None of these factors exhibited consistent effects. Physical activity and the psychological patterns did not vary significantly during the menstrual cycle. By preselecting healthy, non-obese volunteers, weight and height, although significantly different between the sexes did not influence transit significantly. We confirmed that hormone concentrations fluctuated predictably during the menstrual cycle, but transit was not systematically different during the phases of the menstrual cycle, even if we excluded from the analysis three women who showed the least changes in circulating levels of progesterone during the luteal phase.

In both sexes, group median (or mean) values were essentially identical for replicate studies. Closer inspection of the results showed that, though group differences

**Intracellular Variability**

Individual differences between study 1 and study 2 for each person were evaluated for each

<table>
<thead>
<tr>
<th>TABLE II</th>
<th>Gastric emptying and small bowel transit. Interindividual variability</th>
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<td>Measurement</td>
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<tr>
<td>Gastric lag phase (minutes)</td>
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<tr>
<td>Gastric half emptying (T\textsubscript{1/2} minutes)</td>
<td>F</td>
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<td></td>
<td>M</td>
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<tr>
<td>Postlag slope (00/0 per minute)</td>
<td>F</td>
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<td>M</td>
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<td>Small bowel transit (minutes)</td>
<td>F</td>
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<td>M</td>
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</table>

*Delta refers to the difference between studies 1 and 2 for each person.*
between replicate tests (for example, median and interquartile ranges for delta values) were generally small, outliers were recorded. Moreover, the intrinsic variability of transit in men implied that any additional perturbation in women (for example, due to the menstrual cycle) would need to be quite large if it were to be statistically and clinically important. Our sample size provided an 80% statistical power, at a significance level of 0.05, to detect a difference of one standard deviation within a person. Based on this index, the menstrual cycle did not change gastrointestinal transit. Thus, though a minor effect of the menstrual cycle on transit cannot be excluded, any changes that might be found with larger samples would be small compared with intrinsic biological variations.

In both follicular and luteal phases, one of three indices of gastric emptying (postlag emptying slope) was significantly slower in women. However, two other indices of gastric emptying (T1/2 and lag phase), and small bowel transit, showed no sex differences. Thus, we feel any effects of sex on gastric emptying and small bowel transit are probably not of great biological significance. The differences between sexes for colonic transit were more impressive and consistent with previous studies. The scintigraphic method allowed us to compare GCs at various times, Figure 4 suggests that the sex difference was most obvious in the later scans, when the marker had reached the distal colon.

The reproducibility of group results was impressive. However, in a few persons, intra-individual variabilities were considerable, and these were seen at all levels of the gut. We feel these represent the biological variations within a person, which must be kept in mind when assessing the effects of any intervention, such as treatment with a drug. The magnitude of the intraindividual variabilities in colonic transit were similar for the scintigraphic and radiopaque techniques, suggesting that they reflect physiological changes in gut function, rather than being due to methodological artefacts. Factors that might contribute to this physiological variability include diet, physical activity, sex hormones, weight, height, alcohol, and coffee consumption, as well as psychological factors. We attempted to control for all of these, and none was significant.

In summary, we were unable to demonstrate in healthy women an effect of the menstrual cycle on gastric emptying, small bowel, and colonic transit. Thus, physiological effects of hormones can be disregarded during assessments of transit. Although gastrointestinal symptoms fluctuate during the menstrual cycle, our results imply that these variations should not be caused by changes in transit. One of these indices of gastric emptying showed a sex difference and slower colonic transit in women was again confirmed.

The study was supported in part by a grant from the Swiss National Science Foundation (LPO) and by grants DK32121 and RR00585 from the National Institutes of Health, Bethesda, MD, USA (SFP).

Figure 6: Intraindividual differences for colonic transit, expressed as geometric centres at six (A) and 24 (B) hours, for 12 women and 20 men, each of whom was studied on two occasions. The median value for delta (differences between studies 1 and 2) was close to zero, and half the subjects (interquartile range in the box) yielded very similar values for replicate studies. The entire range was wide, as influenced by several outliers.

Figure 7: Mean colonic transit time by the radio-opaque marker method; duplicate studies in 12 women and 20 men. Women were studied in the follicular (F) and luteal (L) phases of the menstrual cycle. Men were studied twice, 14 days apart.
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