Menstruation and not fiber intake may account for differences in bowel function between sexes: a secondary analysis of a recent randomized controlled trial

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Abstract

In a recent study, healthy males were found to have a higher stool frequency than healthy females. This secondary analysis investigates this difference between sexes by examining effects of fiber intake on stool frequency and menstruation on stool frequency and consistency [measured by Bristol Stool Score (BSS)]. Stool frequency data from males (n=58) and females (n=84) from the control group of this fiber intervention study were compared while accounting for dietary fiber intake. Questionnaire data addressing bowel function from women (n=125) was compared between three menstrual cycle phases (menstrual, days 1-3; mid-follicular, days 7-9; mid-luteal, days 19-21). Males trended toward higher total fiber intake than females [16.2 (11.4-20.2) vs. 14.1 (9.8-17.9) g/d, P=0.077], but fiber intake did not contribute to stool frequency when sex was accounted for in the same model. Females had higher stool frequency [1.33 (0.67-1.67) vs. 1.00 (0.67-1.67)/day, P=0.041)] and a higher BSS [3.69 (3.00-4.23) vs. 3.36 (3.00-4.00), P=0.008] during the menstrual versus the mid-follicular phase, respectively. Results indicate that i) higher fiber intake was not the reason for higher stool frequency reported by males and ii) the menstrual cycle appears to affect bowel function, but further research is needed to determine the mechanism.

Introduction

Fiber and prebiotics are under investigation for their multiple health benefits when fermented by beneficial gastrointestinal (GI) microbes (J. Slavin, 2013; Wu & Wu, 2012). Researchers are even examining the extent of a neurological connection between the mind and gut microbiota (Logsdon, Erickson, Rhea, Salameh, & Banks, 2018). These new findings have led to extensive GI health research efforts. Researchers need to identify potential extraneous and confounding variables in order to refine future research in this field.

Differences in GI function and symptoms between males and females have been observed in recent studies, and the physiological reasons for these differences are still unknown. In a recent study investigating the effect of probiotics on stress levels and stress-related GI symptoms, females reported more symptoms associated with diarrhea, constipation, abdominal pain, and indigestion than males using the Gastrointestinal Symptom Rating Scale (GSRS) (Culpepper et al., 2016). However, it should be noted that this questionnaire has not yet been validated in healthy adults. Additionally, a meta-analysis found women were more likely to report constipation-related symptoms in several general population studies (i.e. abdominal distension, bloating, and straining) (Adeyemo, Spiegel, & Chang, 2010). This may be due to a potential difference in colonic transit time between healthy men and women. Various studies in the literature have examined this, but the results appear divided. Several studies have found colonic transit and gastric emptying to be significantly faster in healthy men compared to healthy women. A study published in the *Scandinavian Journal of Gastroenterology* studied gastric emptying, colonic transit, and small bowel transit in 83 healthy subjects, and all outcomes were found to be significantly faster in men (Sadik, Abrahamsson, & Stotzer, 2003). Another study examined 32 healthy participants and used radio-opaque markers to find gastric emptying and colonic transit to be faster in males compared to females (Degen & Phillips, 1996). Interestingly, another study completed in Korea used the same method of measurement, but found no significant difference in colonic transit time between healthy men and women in a sample of 42 healthy adults (Jung, Kim, & Moon, 2003).

The Food Science and Human Nutrition Department at the University of Florida has recently completed several studies that have observed sex differences in bowel function and symptom reporting as well. Recently, an unpublished five-week, randomized, controlled clinical trial observing the effects of citrus pomace fiber in healthy adults (IRB201602073, NCT02979496) was completed. Of the individuals in the control group who received no additional fiber, males had a higher stool frequency compared to females (1.41 ± 0.08 vs. 1.15 ± 0.05, $P = 0.0063$).

There are several possible explanations as to why these sex differences in GI function exist. Fiber intake can differ between males and females and is known to affect bowel function, which could explain variation in GI symptom reporting. “Fiber” is a broad term that encompasses both dietary fiber and functional fiber (J. L. Slavin, 2008). The Institute of Medicine defines dietary fiber as “non-digestible carbohydrates and lignin that are intrinsic and intact in plants” and functional fiber as “isolated non-digestible carbohydrates that have beneficial physiological effects in human beings” (Trumbo, Schlicker, Yates, Poos, & Food and Nutrition Board of the Institute of Medicine, 2002). Dietary fiber can be further divided into soluble and insoluble fiber. Both soluble and insoluble fibers have effects on GI function. Soluble fibers tend to absorb water, delay gastric emptying and slow intestinal transit while insoluble fibers are known to decrease intestinal transit time, increase stool bulk, and increase stool frequency (Anderson et al., 2009). These are generalizations of the functions of fibers; many of these effects tend to overlap between soluble and insoluble fiber (J. Slavin, 2013). In fact, a recent study confirmed that incorporating 16 g/d of oligofructose fiber, which is considered a soluble fiber, into the diets of healthy adults increased stool frequency (Dahl et al., 2014).

Since fiber intake is known to increase stool frequency, fiber intake differences between males and females may explain the observed sex differences in bowel function (i.e. higher stool frequency in males). By analyzing data from the National Health and Nutrition Examination Survey (NHANES II) completed between 1976 and 1980, it was found that females consumed less total fiber on average compared to males (Block & Lanza, 1987). However, an international study across 23 countries used an International Health Behaviour Survey (IHBS) to find that women were more likely to report eating “high-fiber foods” than men (Wardle et al., 2004). Perhaps differences in energy intake (i.e. caloric intake) between sexes are a reason why fiber intake may vary between males and females. Men typically have a higher energy intake than women. Theoretically, consuming more calories per day likely entails consuming a higher volume of food per day, which would allow for more opportunity to consume dietary fiber. A higher energy intake may be why males appear to consume a higher total amount of fiber per day. However, it seems that women may consume a higher proportion of fiber in their diet, based on the study mentioned above. More research is needed to determine if energy and/or fiber intake explain sex differences seen in GI function.

Female reproductive hormones are also theorized to have an effect on GI function, which may explain sex differences in GI symptom reporting. Females are thought to have more physiological variation than males due to fluctuations in hormone concentrations related to the menstrual cycle. The extent to which the menstrual cycle affects the GI tract has been moderately explored, however, no clear mechanism has been confirmed. The female reproductive system is in close proximity to the gastrointestinal tract. It has been proposed that hormones that affect the uterine smooth muscle tissue may affect nearby GI smooth muscle tissue as well (Heitkemper, Shaver, & Mitchell, 1988). In fact, estrogen receptors have been located throughout the GI tract, but their role has not been fully established (Eliakim, Abulafia, & Sherer, 2000). Progesterone and estrogen may decrease GI smooth muscle contractility (Eliakim et al., 2000; Heitkemper & Chang, 2009). Progesterone and estrogen concentrations drop significantly prior to menstruation in the absence of a pregnancy. This sudden drop in hormone concentrations occurs in women taking oral contraceptives (OCs) as well and corresponds with the placebo pills provided by most oral birth control manufacturers (Heitkemper et al., 2003; Heitkemper et al., 1988). Prostaglandins (i.e. hormone-like lipid compounds) are then released from the endometrium causing the contraction of uterine smooth muscle tissue and the sloughing off of dead tissue, known as menstrual bleeding (Rees, Anderson, Demers, & Turnbull, 1984). Prostaglandins have the greatest effect on smooth muscle tissue during the first three days of menstruation (referred to as Days 1, 2, and 3) (Jensen, Andersen, & Wagner, 1987), and this may explain why women may have heavier flow during the first three days of menstruation (Dasharathy et al., 2012; Heitkemper et al., 2003). It is theorized that the combined effect of the increased concentration of prostaglandins and sudden drop in estrogen and progesterone concentrations may explain potential changes in GI function experienced throughout the menstrual cycle (Heitkemper & Chang, 2009). When women enter the mid-follicular phase (Days 7, 8, and 9) of the menstrual cycle, progesterone and estrogen concentrations are still relatively low but have begun to slowly increase. Estrogen and progesterone concentrations typically rise to high levels during the mid-luteal phase (Days 19, 20, and 21; assuming a 28-day cycle) of the menstrual cycle (Wald et al., 1981). As the concentration of estrogen and progesterone increases, these hormones may slow GI tract motility and/or contractility (Bielefeldt, Waite, Abboud, & Conklin, 1996; Verrengia, Sachdeva, Gaughan, Fisher, & Parkman, 2011), resulting in more constipation-related GI symptoms.

Most published studies investigating the effect of the menstrual cycle on GI symptoms involve people with Irritable Bowel Syndrome (IBS), Inflammatory Bowel Disease (IBD), or dysmenorrhea (i.e. abnormally painful periods) most likely because both GI and menstrual symptoms are often exacerbated and amplified in these populations (Heitkemper et al., 2003). The majority of these studies acknowledge that female reproductive hormones may increase risk of developing these conditions (Adeyemo et al., 2010). While one study involving women with IBS found no significant difference in GI symptoms (i.e. abdominal pain, diarrhea, constipation, abdominal distension, tenesmus) between menstrual phases (Lee et al., 2007), another study found an increase in GI symptoms (i.e. abdominal pain, visceral sensitivity, diarrhea, constipation) around the time of menstruation (Heitkemper & Chang, 2009). A systematic review of 13 studies found an increase in IBS-related symptoms at the time of menses compared to other menstrual cycle phases in both women with IBS and healthy women from general population studies (Adeyemo et al., 2010). In a study involving three groups of women (dysmenorrheic, non-dysmenorrheic, and non-dysmenorrheic taking OCs), participants were asked to record the number of stools per day and rate stool consistency from 1, meaning “hard pellets,” to 9, meaning “watery.” Daily mean stool frequency and daily mean stool consistency scores were used in analyses. Higher stool consistency scores (i.e. “looser stools”) were observed between the first two days of menstruation compared to the other phases of the menstrual cycle in all three groups (Heitkemper et al., 1988). It is unclear if the stool consistency scale was validated. It should also be noted that this study involved a small number of women (n=34). More research is needed to determine the full effect of hormone concentration fluctuations associated with the menstrual cycle on GI symptom reporting in populations affected by IBS, IBD, and dysmenorrhea.

A handful of studies exist in the literature that examine the effect of the menstrual cycle on GI function in samples of healthy women. With regard to GI symptoms experienced throughout the menstrual cycle, 73% of healthy study participants (n = 156) experienced at least one of seven common GI symptoms (i.e. abdominal pain, diarrhea, nausea, vomiting, and/or constipation) “in conjunction with their menstrual cycle” in a study observing GI symptoms before and during menstruation (Bernstein et al., 2014). However, the previous study was exploratory in nature and used a questionnaire not yet validated in healthy adults; the questionnaire was piloted in a sample of 20 women before being used in the study. Several studies have examined the effect of menstrual phases on colonic transit time, however, the results are conflicting. One study found that the follicular and luteal phases of the menstrual cycle did not influence transit time (Degen & Phillips, 1996). In a different study, colonic transit time was longer in the luteal phase of 11 healthy female subjects when compared to the follicular phase of 10 different healthy female subjects (40.9 ± 19.0 h vs. 20.6 ± 19.2 h) (Jung et al., 2003). One study determined that gastric emptying was unaffected by the menstrual cycle in healthy females using an isotopic technique after participants consumed a radiolabeled meal (Monés et al., 1993). More research is needed to determine how and to what extent the menstrual cycle affects GI function in healthy females.

As stated above, fiber intake and/or the menstrual cycle may impact bowel function in healthy adults. The objective of this study was to investigate potential causes of sex differences in stool frequency observed in a recent fiber intervention study (IRB201602073, NCT02979496).

Specific Aims

Aim 1: Determine if observed differences in stool frequency between healthy males and females assigned to the control group of a recent fiber intervention can be explained by fiber intake.

- It was anticipated that average daily total fiber intake (g/d) was higher in males than in females.
- Energy intake may differ between sexes, altering the proportion of fiber in relation to the rest of the diet. It was anticipated that average daily total fiber intake normalized to energy intake (g/1000 kcal/d) was higher in females compared to males.
- Contingent on the results from the above analyses, it was anticipated that fiber intake was a confounding variable influencing stool frequency. By including both fiber intake and sex in the same statistical model with stool frequency as the response variable, it was possible to determine whether higher stool frequency in males was attributed to higher fiber intake.

Aim 2: Determine the effect of three different menstrual cycle phases on stool frequency, stool consistency, and bowel habit satisfaction in a sample of healthy, menstruating females.

- In the first three days of menstruation, it was expected that participants would report a higher stool consistency score (i.e. looser stools) and a higher average number of stools per day when prostaglandins have the greatest effect (Jensen et al., 1987) compared with the mid-luteal phase (Days 19-21) when estrogen and progesterone levels are typically highest and may slow GI motility (Wald et al., 1981).

- The mid-follicular phase was expected to be less affected by hormones related to the menstrual cycle (i.e. hormone concentrations remain relatively low (Heitkemper et al., 1988)). Participants were thus expected to be satisfied with bowel habits (i.e. answer “satisfied” to some degree on the daily questionnaire) on a higher average proportion of days during the mid-follicular phase compared to the menstrual phase (Days 1-3).

Methods

For the purposes of this study, a secondary analysis of data from an unpublished five-week, randomized, controlled clinical trial observing the effects of citrus pomace fiber in healthy adults was done. Participants were between 18 and 60 years of age and consumed 16 ounces of a citrus beverage daily for a three-week intervention. Each participant was randomly assigned to consume one of four possible beverages: orange juice containing 180 g pomace (10 g fiber), orange juice containing 90 g pomace (5 g fiber), orange juice containing 0 g pomace (0 g fiber), or orange-flavored, calorie-matched water. For the purposes of these analyses, participants in the calorie-matched water group were included in the 0 g fiber pomace group (i.e. control group) and are referred to as such. Participants discontinued any prebiotic, probiotic, and fiber supplements and avoided consumption of any orange, grapefruit, tomato, or V-8 juice as well as whole oranges or grapefruits. To be included in the study, participants could not be lactating, knowingly pregnant, attempting to get pregnant, receiving treatment for any physician-diagnosed diseases or conditions, following a vegetarian or vegan diet, and could not have any food allergies to citrus. Participants disclosed any medication they were currently taking at the beginning of the study, including oral contraceptives (OCs).

Participants completed daily questionnaires online regarding dietary intake, gastrointestinal habits, and general health and well-being throughout the entire five-week study (i.e. the two-week pre-baseline period and three-week intervention period) (**Figure 1**). In the daily questionnaire, participants were asked about the number of bowel movements per day and were asked to rate the consistency of each bowel movement using the Bristol Stool Form Scale (Blake, Raker, & Whelan, 2016; Lewis & Heaton, 1997). The BSFS asks participants to rate their stool form based on the following 7-point scale: 1= “separate hard lumps, like nuts,” 2 = “sausage-shaped but lumpy,” 3 = “like a sausage or snake but with cracks on its surface,” 4 = like a sausage or snake, smooth and soft,” 5 = “soft blobs with clear-cut edges,” 6 = “fluffy pieces with ragged edges, a mushy stool,” 7 = “watery, no solid pieces” (Lewis & Heaton, 1997). Using daily questionnaire responses, a Bristol Stool Score (BSS) was determined for each bowel movement each participant experienced over the course of the study. In each daily questionnaire, participants were asked if they were satisfied with their bowel habits and selected one of the following responses: very satisfied, moderately satisfied, somewhat satisfied, neither satisfied nor dissatisfied, somewhat dissatisfied, moderately dissatisfied, or very dissatisfied. Participants were also asked a “yes” or “no” question about whether they were menstruating each day. Participants completed a total of three Automated Self-Administered 24-Hour (ASA24) dietary recalls throughout the three-week intervention (Subar et al., 2012). Average daily total fiber intake (g fiber/d) throughout the study was determined for each participant by averaging data from the three recalls. Average daily total fiber intake normalized to energy intake was determined for each participant as well (g fiber/1000 kcal/d).

Fiber Intake Analyses (Aim 1)

Inclusion criteria

Analyses only included participants assigned to the control group (0 g fiber from the beverage). Participants from other interventions were not included in fiber intake analyses to ensure additional fiber from the beverage did not influence analyses. Out of the 146 participants assigned to the control group, 4 participants were excluded from fiber analyses; 2 participants did not have fiber intake data present and 2 participants had more than 9 missing stool frequency responses out of the 21 days of the intervention. Within the remaining 142 participants eligible for fiber analysis, 58 were male and 84 were female.

Statistical analyses

Average daily total fiber intake (g fiber/day) and average fiber intake normalized to energy intake (g fiber/1000 kcal/d) values did not meet the assumption of normality (Shapiro-Wilk test, $P < 0.05$). Therefore, a Mann-Whitney rank sum test was used to determine if there was a significant difference in median fiber intake values between males and females.

Since total fiber intake (g/d) tended to be higher in males than in females, both total fiber intake and sex were included as explanatory variables in the same statistical model. A general linear mixed model was used to analyze the effect of fiber intake, sex, and the interaction of these two variables on average stool frequency.

Menstrual Cycle Analysis (Aim 2)

Inclusion criteria

Data from male as well as non-menstruating and/or post-menopausal female participants were excluded. Participants from all fiber interventions were included in order to increase the statistical power of this pilot study, as differences in outcomes between menstrual phases were not expected to be large. From the 206 female study participants, 35 withdrew from the study early. Data from remaining participants were evaluated for irregular or missing menstrual cycles:

- Participants without any menstrual events during the study were excluded. A menstrual event was considered to be two consecutive days of answering "yes" to the question "did you menstruate yesterday?" with at least one day of "no" before and a clear stop in menstruation afterwards (e.g. at least two days of answering "no"). Accordingly, participants who had only one day of answering "yes" surrounded by "no" on days before and after were excluded.
- Participants who had irregular menstrual bleeding patterns (i.e. intermittent spotting throughout the course of the study with no clear menstrual events as defined above) were excluded.
- If a single "no" was recorded during a menstrual event, it was assumed to be either an accident or misinterpretation of the word "menstruating;" this term was never defined in the questionnaire and could be interpreted in different ways (i.e. if a participant did not have a particularly heavy flow for one of the days during a menstrual event and considered that "not menstruating" when in fact this was still during the same menstrual event).

After these exclusions, 125 participants had menstrual cycles that were suitable for analysis.

Designation of menstrual cycle phases

Aspects of bowel function were analyzed within three distinct menstrual phases: menstrual (Days 1, 2, and 3), mid-follicular (Days 7, 8, and 9), and mid-luteal (Days 19, 20, and 21). Previous studies examining the possible effect of hormone and prostaglandin concentrations on the digestive tract were considered in delineating these phases (Heitkemper et al., 1988). Days of menstruation were numbered with the first day of the first menstrual event referred to as “Day 1”. Subsequent days were consecutively numbered after Day 1 to determine mid-follicular and mid-luteal phases. Many participants had two separate menstrual events during the course of the study; this was expected given the fact that the study took place over the course of five weeks. If the start of the first menstrual event was unclear (i.e. the very first questionnaire response indicated that the participant was already menstruating), numbering did not begin there. Instead, “Day 1” was designated as the start of the second menstrual event and days were numbered in both forward and reverse orders from Day 1 assuming a 28-day cycle (i.e. the day before Day 1 was designated as Day 28) in order to determine the mid-luteal phase and mid-follicular phases if they could not be determined by forward numbering. Previous studies with large samples of females, including those taking OCs, have determined a mean menstrual cycle length of 28 days in both IBS and healthy control groups (Farland et al., 2017). In the current study, average cycle length was also determined to confirm that the 28-day assumption was appropriate.

Some participants had two complete mid-follicular phases represented in the data since forward and backward numbering occurred for some participants. Typically, this took place when the participant had a menstrual event closer to the middle of the five-week study, causing two separate occurrences of a mid-follicular phase. In order to choose the most accurate mid-follicular phase represented, forward-numbered mid-follicular phases were preferred over backward-numbered phases.

Statistical analyses

To be included in the menstrual cycle analyses, data were required for all three of the menstrual cycle phases for each participant because the outcomes were expected to be highly correlated within each participant. Average phase BSS (n = 122) and average phase stool frequency (i.e. average number of bowel movements per day) (n = 114) were calculated for each menstrual cycle phase by averaging the three daily averages within each phase. The average phase BSS did not include any days in which no bowel movement occurred. Average stool frequency did not include days in which the participant left a blank response (i.e. this was not assumed to be no bowel movements for that day).

Because not all women had all three menstrual cycle phases, multiple paired t tests were used between the three phases instead of an ANOVA. This allowed more women to be included in the analyses. Neither the average phase stool frequency data nor the average phase BSS data met the assumption of normality (Shapiro-Wilk test, $P < 0.05$), so a Wilcoxon signed-rank test for paired data was used to determine if there was a significant difference in median phase stool frequency and median phase BSS between i) the menstrual phase (Days 1-3) and mid-luteal phase (Days 19-21) and ii) the menstrual phase and the mid-follicular phase (Days 7-9).

For each participant, the number of days "satisfied" with bowel habits to some degree (either very, moderately, or somewhat satisfied) was determined for each of the three menstrual cycle phases. The average proportion of days "satisfied" out of the three days of the phase was compared between menstrual phases. The data did not meet the assumption of normality (Shapiro-Wilk test, $P < 0.05$), therefore a Wilcoxon signed-rank test was used to determine if there was a significant difference in the median proportion of "satisfied" days between the menstrual phase (Days 1-3) and mid-follicular phase (Days 7-9).

Analyses were completed using statistical software [Sigma Plot Version 13 (Systat Software Inc., San Jose, CA, USA) and SAS version 9.4 (SAS Institute Inc., Cary, NC, USA)]. *P* values less than 0.05 were considered significant. All values are reported as medians (interquartile range) or means ± SEM as appropriate.

Results

Fiber intake analyses

Average age and body mass index (BMI) were determined for the 142 males and females included in the fiber intake analysis (**Table 1**). Average BMI values fell within the healthy range of 18.6 to 24.9 (Gilmore, 1999). Daily total fiber intake (g fiber/day) trended toward being higher in males than in females ($P = 0.077$; Table 1). When normalized to energy intake, daily total fiber intake (g fiber/1000 kcal/day) was not significantly different between males and females (Table 1). Males trended toward a higher average stool frequency than females ($P = 0.078$; **Figure 2**). Fiber intake did not appear to influence stool frequency reporting ($P = 0.541$).

Menstrual cycle analyses

Average age and BMI were determined for the 125 healthy, menstruating females included in the menstrual cycle analysis (**Table 2**). Average BMI values fell within the healthy range of 18.6 to 24.9 (Gilmore, 1999). Stool frequency was not significantly different between the menstrual phase and the mid-luteal phase (P = 0.109); however, stool frequency during the menstrual phase (Days 1-3) was higher than during the mid-follicular phase (Days 7-9) [1.33 (0.67 - 1.67) vs. 1.00 (0.67 - 1.67), P = 0.041]. Similarly, BSS was not significantly difference between the menstrual phase and the mid-luteal phase (Days 19-21) (P = 0.167); however, BSS was higher during the menstrual phase than during the mid-follicular phase [3.67 (3.00 - 4.23) vs. 3.36 (3.00 - 4.00), P=0.008]. The average proportion of days "satisfied" with bowel habits did not differ between the menstrual phase and the mid-follicular phase (P = 0.518).

Of the 125 participants analyzed in this study, 22 reported using some form of birth control. Of these 22 women, 18 reported using OCs, 1 reported having an intrauterine contraceptive device (IUD), and 3 did not specify what form of birth control was being used. Of the 19 females who had at least one complete and continuous menstrual cycle during the study, the average cycle length was 26.6 ± 0.7 days. Out of those 19 women, 4 were on some form of birth control. The average cycle length of these 4 females was 25.8 ± 1.7 days. The remaining 15 females who did not report using birth control had an average cycle length of 26.8 ± 0.8 days.

Conclusion

The current study sought to explain observed differences in stool frequency between males and females using data from a recent clinical trial. Before this study, there was limited research that investigated the potential reasons behind differences in stool frequency observed between males and females. Studies involving healthy samples often focus on differences in gastric emptying and colonic transit times between males and females, and this study assessed the effect of both sex and dietary fiber intake on stool frequency in the same statistical model. Additionally, research on the effect of the menstrual cycle on bowel function in healthy women was limited. This study presents differences in bowel function between different phases of the menstrual cycle.

Daily total fiber intake trended toward being higher in males as was expected; however, total fiber intake did not contribute significantly to stool frequency when fiber and sex were accounted for in the same general linear model. These trends may indicate that the differences in stool frequency between sexes may be explained by something other than fiber intake. Both sexes were found to have inadequate fiber intake according to the current recommendations for fiber in America. Currently, the adequate intake (AI) for dietary fiber is 14 g/1000 kcal, or approximately 38 g/d for adult men and 25 g/d for adult women (Trumbo et al., 2002). In fact, males and females in this study were consuming roughly half of the current recommendation (Table 1). Perhaps this analysis would have shown an effect of fiber or an effect of an interaction between fiber intake and sex on stool frequency if participants consumed higher amounts of fiber. Regardless, the observed trends in stool frequency by sex were not explained by fiber intake.

Further investigation was warranted to determine why sex-related trends in stool frequency were observed in the fiber intervention study. This study aimed to investigate if the menstrual cycle significantly influences aspects of bowel function in healthy females, as this may relate to the reason for sex differences in bowel function. While differences were not seen between the menstrual and mid-luteal phases as hypothesized, both stool frequency and BSS were significantly higher in the menstrual phase compared to the mid-follicular phase. This still aligns with what is known about prostaglandin concentrations during the first three days of menstruation: prostaglandins may increase contraction of GI smooth muscle tissue, thus increasing motility and increasing stool frequency (Jensen et al., 1987). Symptoms associated with diarrhea have been shown to increase around the time of menses (Adeyemo et al., 2010) in healthy women. Females experiencing looser stools (i.e. higher BSS) during the menstrual phase was expected as well. Bowel habit satisfaction was not different between the menstrual phase and mid-follicular phase. Perhaps the question regarding bowel habit satisfaction could have been worded differently. Participants may have been "satisfied" with their bowel habits if nothing out of the ordinary occurred (i.e. no notable change in stool frequency or consistency and/or no notable increase in undesired GI symptoms). Healthy, menstruating females may be familiar with how their GI function changes across their menstrual cycle (i.e. this may not be "out of the ordinary" for these participants). In future studies, if participants were aware that the menstrual cycle may influence bowel function, they may answer this question more perceptively.

There were several limitations of this study, as it was a pilot study in many aspects. This study retrospectively analyzed data from a previous study that had an entirely different purpose. Questionnaires were used as the method of data collection to determine fiber intake, menstrual cycle phases, bowel habit satisfaction, stool frequency, and stool consistency. This may be a limitation because responses are subjective and only as accurate as the recall capability of the participants. Participants were asked to complete questionnaires about the previous day and were also encouraged to keep a diary or take notes throughout the day to help them complete questionnaires accurately. Some participants may have rushed through questionnaires and/or not answered them honestly (i.e. fear of embarrassment). Questionnaires were answered remotely, and completion was not supervised. It can be assumed that variability in questionnaire responses occurred equally between males and females; therefore, differences in stool frequency observed in the fiber intervention study are still relevant. This secondary analysis was not planned at the start of the fiber intervention study, so participants would not have been informed about any menstruation-related analyses when completing questionnaires. If participants were aware that symptoms related to their menstrual cycle were being studied, perhaps they would have answered the questionnaires more insightfully or, alternatively, with more bias. Menstruation was not defined in any way on the questionnaire and participants were not instructed beforehand on how to answer the question “Did you menstruate yesterday?” Menstruation could have been defined to give participants a clearer understanding of how to answer the question. Menstrual cycle phase analyses in this study did not exclude women taking OCs. The extent to which OC use contributes to GI symptom reporting has not been fully explored. Women taking OCs experience the same drop in steroid hormone levels prior to menstruation that non-OC users experience (Heitkemper et al., 2003; Heitkemper et al., 1988). If changes in female reproductive hormone levels affect GI function, it would be expected that women on OCs would still experience these symptoms, at least to some degree. A study found that the pattern of symptoms throughout the menstrual cycles of women affected with IBS did not differ with OC use (Heitkemper et al., 2003). Hormone concentrations were not tested in the fiber intervention study and study coordinators had no previous knowledge of each participant’s menstrual cycle (i.e. there was no prior screening process that would have ruled out women who typically did not menstruate because of their method of birth control or who had severely irregular menstrual cycles). Testing for hormone concentrations would have been a more accurate way to number the days in the cycle and monitor the menstrual cycle phases of each female participant. Although hormone concentration testing was not used in this study, an average 28-day cycle was deemed appropriate for the following reasons. Several studies with substantial samples of diverse participants have confirmed an average 28-day menstruation cycle. For instance, a recent study analyzed data from a prospective cohort study of 2,745 premenopausal women, aged 32–52 years (Farland et al., 2017). This study recruited women not taking OCs and women taking OCs. The study determined a mean menstrual cycle length of 28 days in both IBS and control groups. An average 28-day cycle was found to be appropriate for this study as well. Future studies may consider separately analyzing results from women taking OCs and women not taking OCs. Participants who completed the fiber intervention study self-selected to enroll, which may create sampling bias. For instance, individuals who enrolled in the study may have decided to participate because they have a personal interest in GI health, causing the study sample to not be truly representative of the population being studied. The average age of the participants who completed the study was relatively young (Table 1, Table 2), as this clinical trial was completed on a university campus and most participants were undergraduate or graduate students of the university. In future studies, larger study samples of healthy males and females achieved through more inclusive sampling methods (e.g. wider age range) could increase the generalizability of results.

The results of this study may indicate that variation in bowel function between males and females could be due to the effect of the menstrual cycle on the GI tract. Future studies are needed to determine the mechanism of how steroid sex hormones and prostaglandins may influence bowel function. By fully understanding the impact of menstruation on GI symptoms, researchers can determine if menstruation should be measured and accounted for statistically in future GI studies. Additionally, intervention studies could be designed in the future to explore the effects of commercially available probiotic and/or fiber supplements in alleviating undesirable GI symptoms reported throughout the menstrual cycle.

Supporting Figures/Tables

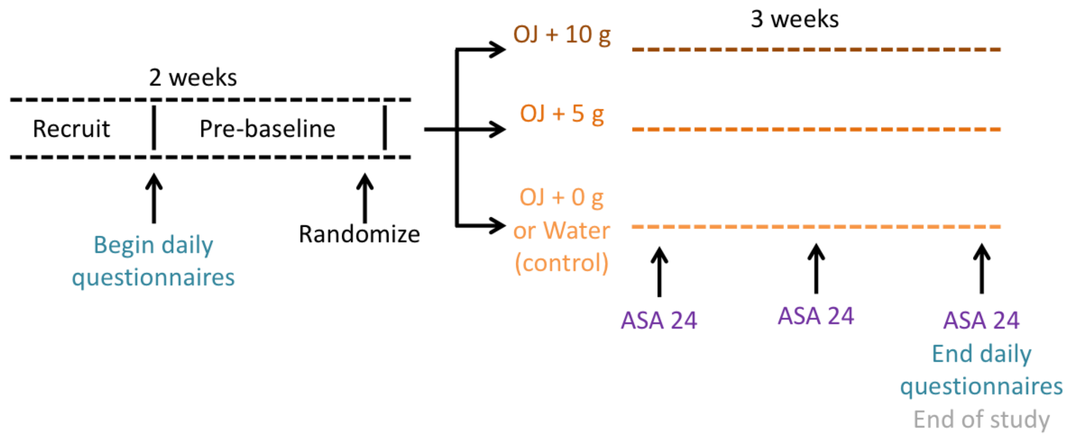

Figure 1: Study design (IRB201602073, NCT02979496) with a two-week pre-baseline and three-week intervention. Three fiber interventions are represented (i.e. 0 g, 5 g, or 10 g pomace fiber added to orange juice (OJ) beverage). Daily questionnaires were completed for the entirety of the five-week study.

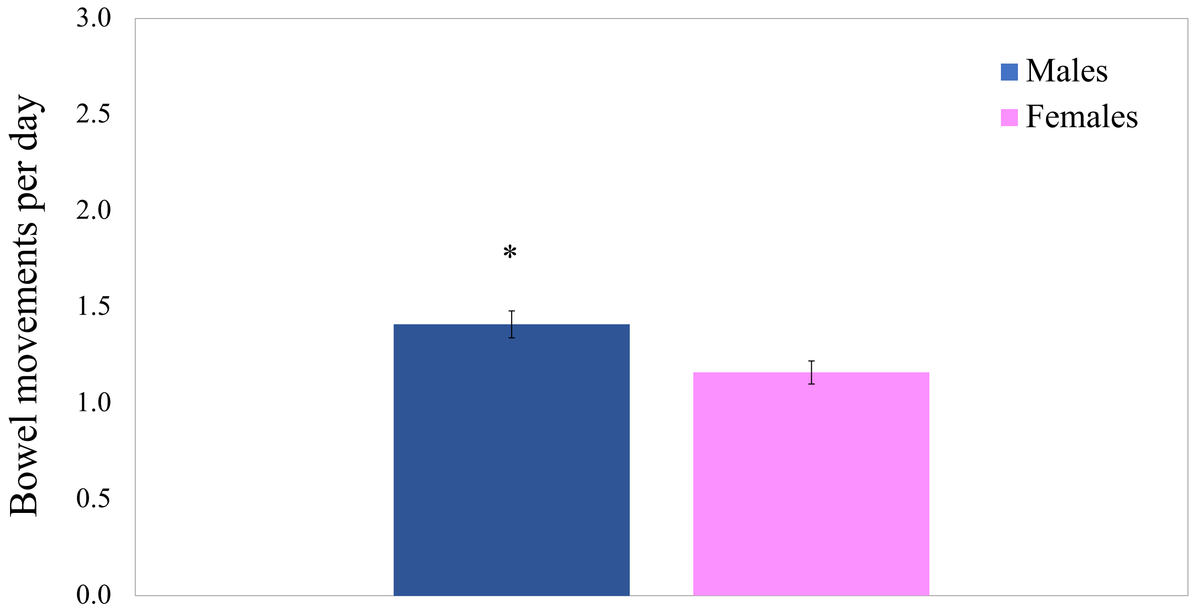

Figure 2. Average stool frequency of males compared to females during the intervention. Participants from only the control group were included in a general linear mixed model to analyze fiber intake, sex, and the interaction of the two on stool frequency (daily total number of stools averaged across the 21 days of intervention). Values are least squares means ± SEM. Males, n = 58; females, n = 84. *P = 0.078.

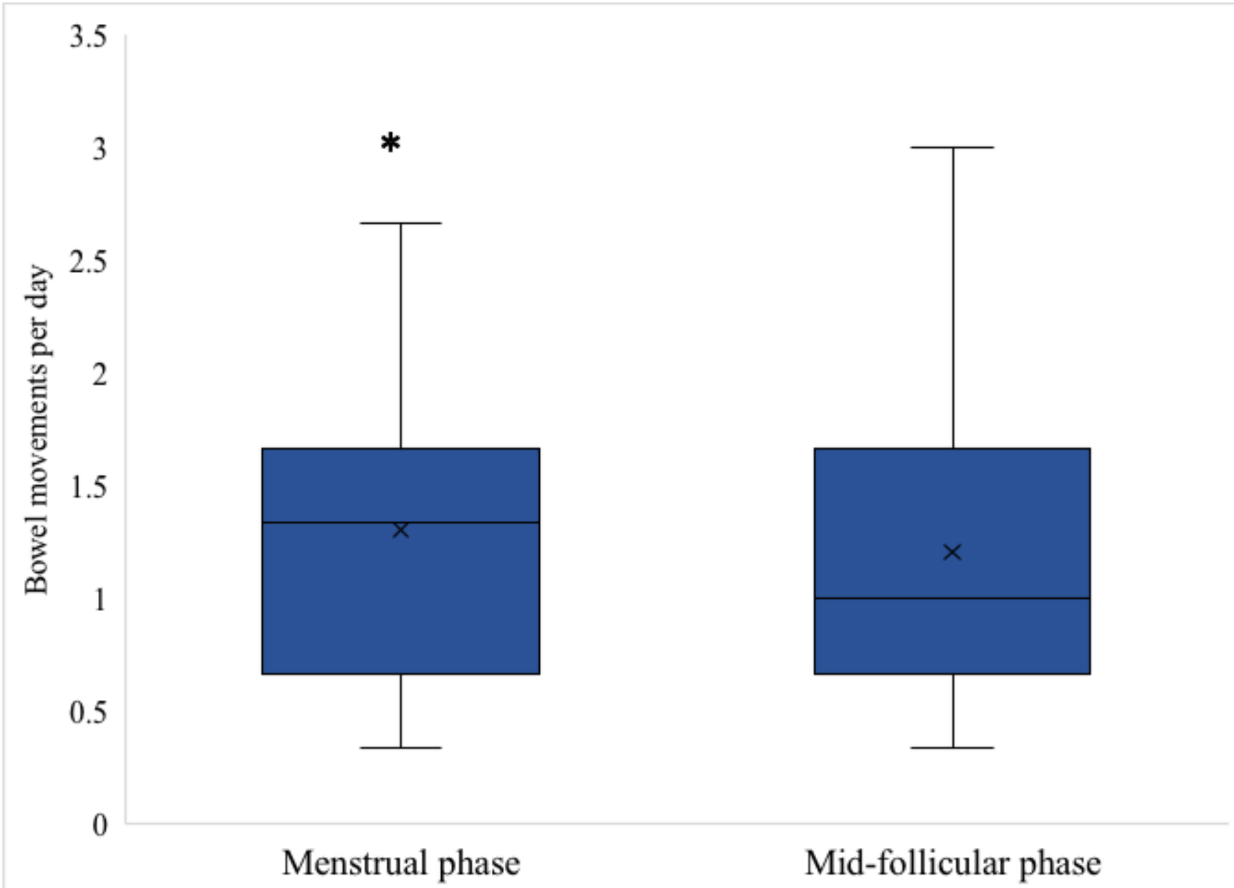

Figure 3. Stool frequency (number of bowel movements per day) was significantly higher in the menstrual phase compared to the mid-follicular phase in healthy menstruating women (n = 114). A Wilcoxon signed-rank test was used to determine a significant difference between medians. *P = 0.041.

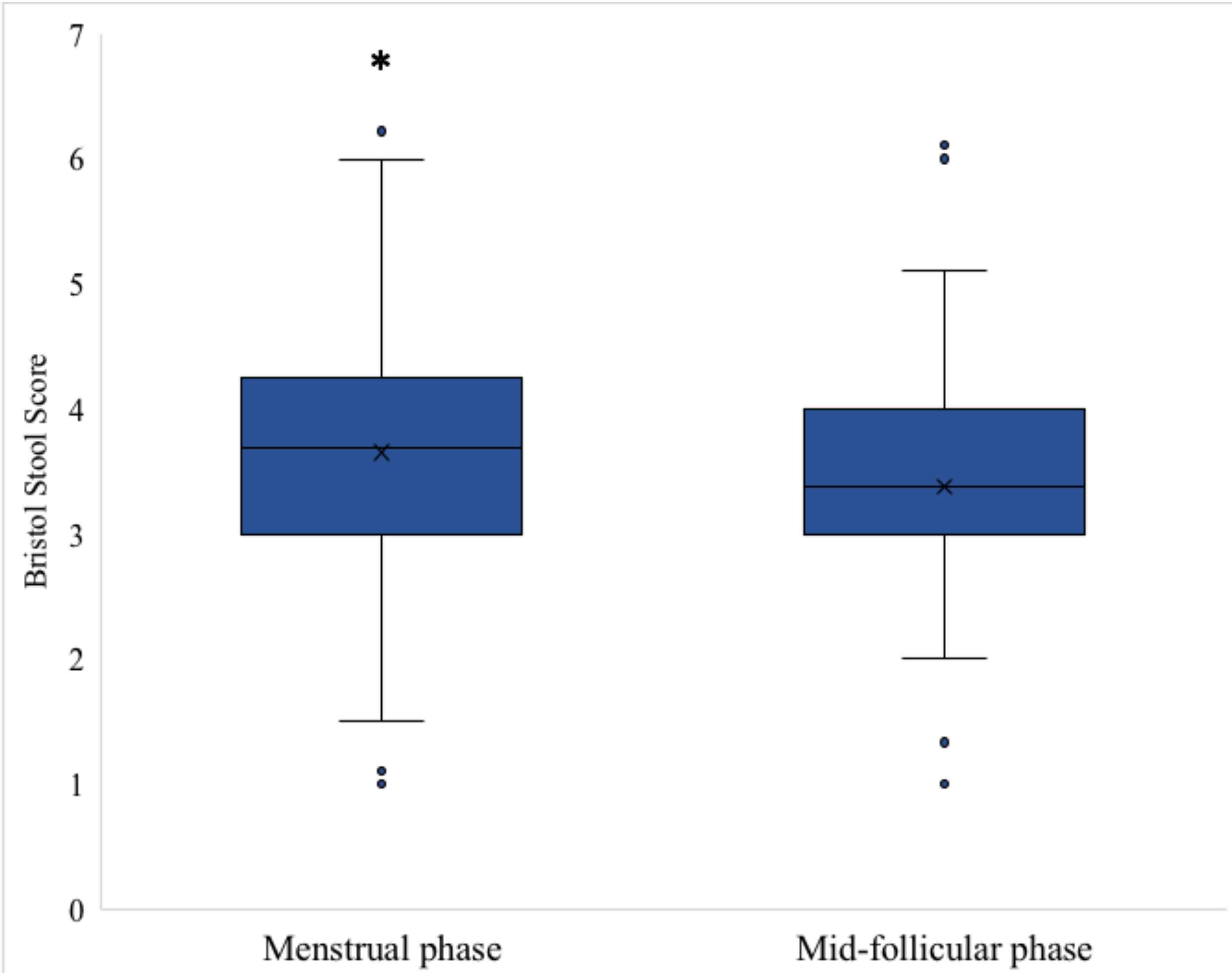

Figure 4. Bristol Stool Score was significantly higher during the menstrual phase than during the mid-follicular phase in healthy menstruating women (n = 122). A Wilcoxon signed-rank test was used to determine a significant difference between medians. *P = 0.008.

Table 1. Demographic characteristics and fiber intake by sex for participants included in the fiber intake analysis

	Males (n = 58)	**Females (n = 84)**	***P* value[1]**
Age, y	22.4 ± 0.9[2]	22.8 ± 0.7	
Body mass index, kg/m^2	25.1 ± 0.5	23.7 ± 0.4	0.029
Median total fiber intake[3], g/d	16.2 (11.4 – 20.2)[4]	14.1 (9.8 – 17.9)	0.077
Median normalized fiber intake including energy from study beverage, g/1000 kcal/d	6.8 (5.8 – 9.9)	7.6 (6.3 – 9.5)	0.272
Median energy intake, kcal/d	2019 (1544 – 2301)	1623 (1278 – 1960)	<0.001
Median energy intake including energy from study beverage, kcal/d	2446 (2022 – 2782)	2108 (1749 - 2460)	<0.001

[1]Mann-Whitney rank sum tests were performed to determine significant differences in median total fiber intake (g/d), median normalized fiber intake including energy from study beverage (g/1000 kcal/d), and median energy intake, both including the beverage and without, between males and females. A t-test was performed to determine significant differences in body mass index values between males and females.

[2]Mean ± SEM; all such values

[3]Values shown are averages of three Automated Self-Administered 24 h dietary recalls completed during each week of the intervention.

[4] Median (Interquartile range); all such values

Table 2. Demographic characteristics for female participants included in menstrual cycle analysis

	n = 125
Age, y	22.0 ± 0.5[1]
Body mass index, kg/m^2	23.7 ± 0.4

[1]Mean ± SEM; all such values

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