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Campolier M, Thondre SP, Clegg M, Shafat A, Mcintosh A, Lightowler H. Changes in PYY and gastric emptying across the phases of the menstrual cycle and the influence of the ovarian hormones. *Appetite*. 2016;107:106-115.

What We Know, Think We Know, or Are Starting to Know

Women are underrepresented in clinical research, particular pre-menopausal women in reproductive age, and while female participant numbers are increasing in research, they often remain lower than the proportion of women at risk of a given condition in the general population [1,2]. However, nutrition is one of the few exceptions; women have higher representation relative to the burden of disease of women, compared to men [3].

One argument often heard from researchers is that for pre-menopausal women, the menstrual may act as a potential confounder if metabolism varies over the course of the menstrual cycle [1]. However, any physiological factor *does* vary over the course of the menstrual cycle, then this itself is important to know and understand for women's health.

Numerous aspects of metabolism, from energy expenditure to postprandial metabolic responses, have been suggested to vary over the course of the menstrual cycle [4–7]. One specific area of potential sex differences in metabolism is in relation to gastric emptying, i.e., the process of delivery of food from the stomach to the small intestine for further digestion and absorption, with evidence suggesting a slower rate of gastric emptying in women compared to men [4].

However, whether gastric emptying varies according to the menstrual cycle has been controversial, with evidence both for and against this hypothesis [4,7–9]. The study we Deepdive into here used advanced techniques to measure the rate of gastric emptying in women across three phases of the menstrual cycle.

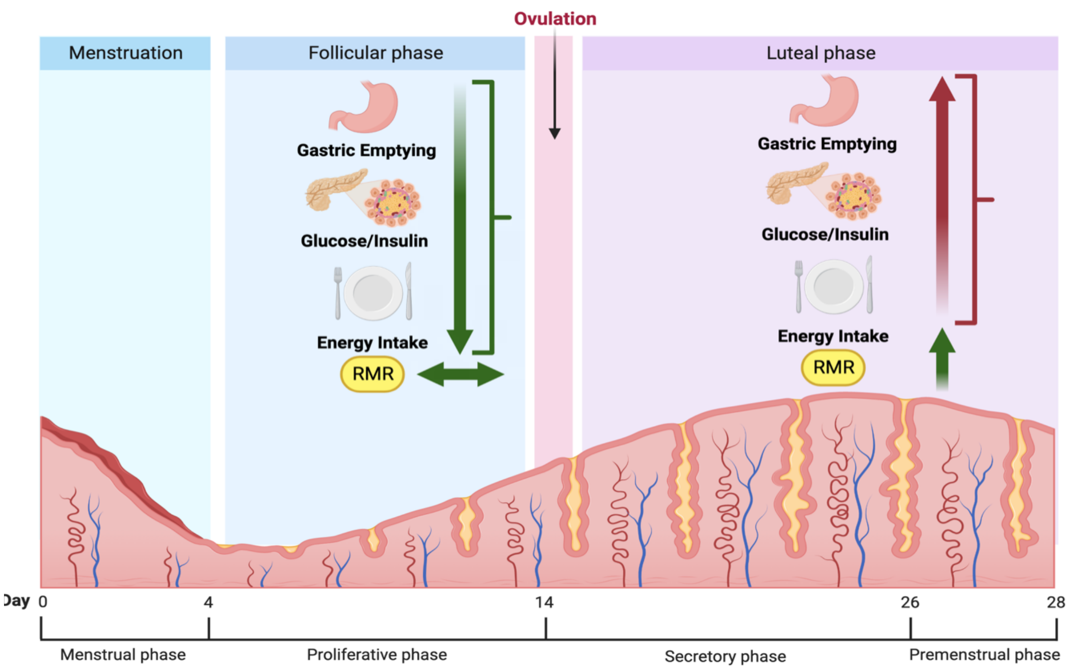


Figure *illustrating the potential variance in metabolic processes according to phases of the menstrual cycle. The arrows indicate the direction of the effect; the bracket indicates that the postprandial glucose and insulin responses, and energy intake, may relate to the rate of gastric emptying, which itself may be slower during the follicular phase, i.e., slower gastric emptying attenuates the postprandial glucose and inulin response, and modulates energy intake. Resting metabolic rate may be slightly higher during the luteal phase, while gastric emptying may be more rapid in the luteal phase, influencing glucose/insulin responses and potentially energy intake.*

The Study

The study was a non-randomised intervention trial to investigate the effects of a standardised test breakfast on gastric emptying and subsequent energy intake during an *ad libitum* buffet lunch in women at three phases of their menstrual cycle.

Participants used a fertility monitor to determine the laboratory test day for each phase of the cycle based on oestradiol and progesterone levels:

- Menstrual Phase [MPh]; oestradiol and progesterone levels both low.
- Follicular Phase [FPh]; oestradiol high and progesterone low.
- Luteal Phase [LPh]; oestradiol and progesterone levels both high.

The testing day for MPh occurred within four days of a new menstrual cycle, with the fertility monitor used to schedule the subsequent visits.

Participants were asked to record three days of dietary intake around the test day, i.e., the day before, the day of testing, and the day after the testing.

On testing days, participants consumed a standardised breakfast of ~375-395kcal [35% fat, 38% carbohydrate, 23% protein], to which a stable isotope tracer***** [see ***Geek Box** below for further detail] was added to measure gastric emptying using breath tests and breath samples were collected every 15min for 4hr following the breakfast. Blood samples and subjective appetite were also measured every 15min for the first hour post-breakfast, then every 30min thereafter.

At the 3 h mark following the measures, participants were offered an *ad libitum* [i.e., no restrictions on energy intake] lunch and invited to eat until satisfied. Food intake was determined by weighing back uneaten foods.

The analysis tested differences in gastric emptying, PYY [an appetite regulatory hormone], subjective appetite, and energy intake, between each phase of the menstrual cycle, and their respective correlations with oestradiol and progesterone.

*Geek Box: Stable Isotope Tracers

So, what is a "stable isotope"? When talking about chemical elements, like nitrogen, carbon, or hydrogen, these elements exist in a form that is abundant in nature. For example, about 99% of the carbon is ^{12}C, which reflects the fact that it has 6 protons and 6 neutrons [adding the protons and neutrons give the element its 'atomic mass', in this carbon has an atomic mass of 12, thus '^{12}C'. However, around 1% of the carbon on Earth has an extra neutron, i.e., with 7 neutrons and 6 protons it has an atomic mass of 13 and is written as ^{13}C.

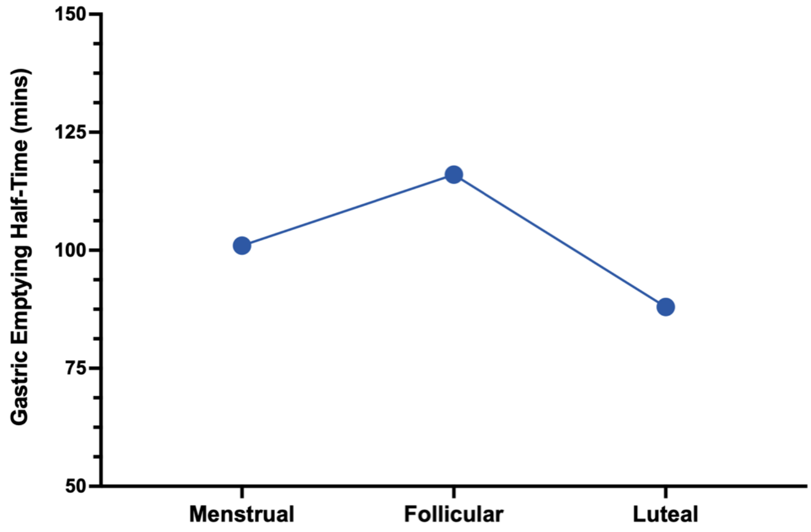
Now, what does this have to do with nutrition research? Well, recall that carbon is an element in each macronutrient; fats, carbohydrates, and proteins. As such, it is possible to chemically enrich nutrients with less abundant stable isotopes. For example, you could take a fatty acid, and substitute the ^{12}C for a ^{13}C isotope [this would all be done in the lab]. Substituting the more abundant ^{12}C for the less abundant ^{13}C in the fatty acid would then create a 'tracer', meaning that it has the same chemical properties of the original compound, but the appearance of the ^{13}C in the body is much more readily identifiable because of its scarcity.

This is possible for each macronutrient. For example, if the substrate you intended to 'trace' was a protein you could create a 'tracer' for the amino acid leucine, and you could do it for glucose if the substrate you intended to 'trace' was a carbohydrate. There are other methods, too, that depend on the type of measurement being undertaken.

The use of stable isotopes in nutrition research is a fascinating area and provides nutrition science with a highly accurate methodology to precisely trace the metabolic fate of nutrients through the body.

Results: Nine women completed the study, with an average age of 31yrs, BMI of 22.6kg/m^2, and cycle length of 29 [± 3] days.

- ***Gastric Emptying*:** The gastric emptying half-time, i.e., the time taken for 50% of the meal to empty the stomach, was significantly different between phases of the menstrual cycle; 101min during the MPh, 116min during the FPh, and decreasing to 88min during the LPh [see **figure** below for illustration]. Thus, gastric emptying half-time during the luteal phase was 28min and 13min faster compared to the menstrual and follicular phases, respectively.



- ***PYY and Subjective Appetite*:** Fasting PYY levels were significantly different between phases of the menstrual cycle, with lower fasting PYY levels in the LPh compared to the MPh, but not compared to the FPh. For postprandial PYY levels, between 2-4hrs postprandial a hierarchy was evident with PYY levels lowest during the LPh, followed by the FPh, and highest during the MPh. There was no differences in subjective appetite between menstrual phases.

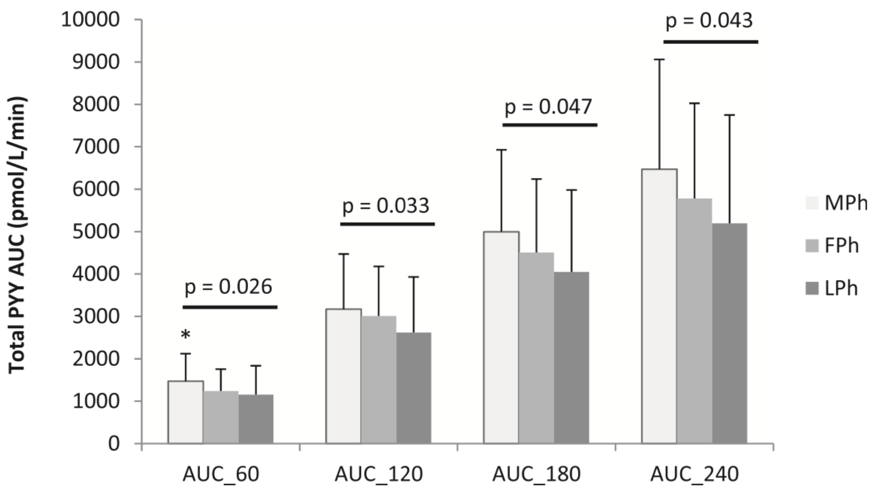


Figure from the paper illustrating the differences in PYY area-under-the-curve assessed at each hour of the postprandial period following the standardised test breakfast. It should be noted that the p-values above each time point are the differences between time-points, not between phases of the menstrual cycle, which were not statistically significantly different, with the exception of the asterix above the AUC_60 mark, which indicates that PYY at this point was lower during the luteal phase compared to the menstrual phase. However, the bar graphs indicate that this pattern held across each time point, and the lack of statistical significance may reflect a lack of adequate power in this small trial.

- ***Energy Intake***: Energy or macronutrient intake in response to the ad libitum lunch was not significantly different at any phase of the menstrual cycle, nor was average energy and macronutrient intakes assessed from the three day diet diaries.
- ***Correlations Between Measures***: Peak PYY levels showed a moderate positive correlation [r = 0.39] with the gastric emptying half-time, indicating that as PYY levels increased the gastric emptying half-time increased. Conversely, the gastric emptying half-time showed a moderate inverse correlation [r = -0.49] with progesterone levels, i.e., the gastric emptying half-time increased as progesterone decreased [more under ***Interesting Finding***, below].

The Critical Breakdown

Pros: The study was the first to consider gastric emptying and other related appetite responses across three phases of the menstrual cycle. The investigators attempted to test each menstrual phase at a time corresponding to distinct oestrogen/progesterone profiles, assessed using a urinary fertility monitoring device. Gastric emptying was assessed using advanced and accurate stable isotope tracer techniques [more under ***Key Characteristic***, below). The same standardised breakfast meal was used as the test meal at each phase of the cycle. Although non-randomised, each participant served as their own control for each phase of the menstrual cycle.

Cons: Primary and secondary outcomes not stated, and there is no clear hypothesis stated. The trial does not appear to have been pre-registered. The study was a non-randomised trial with a very small sample size [n = 9]. The participants were young and fit a homogenous body composition and demographic profile, thus the findings may not extrapolate to women of different age and body composition. The small sample size, if trying to detect small-medium effect size differences, opens the possibility for "false negatives" in the outcomes. Ultimately, as a very small, non-randomised intervention, the study should not be taken as confirmatory of its outcomes, but as providing additional context and supporting evidence to the overall evidence-base in this area of research.

Key Characteristic

Although this study is limited by the small sample size, it does have one important characteristic; the use of stable isotope tracers to assess gastric emptying, which the researchers did by adding a stable isotope of a fatty acid, ^{13}C octanoic acid, to egg [10].

^{13}C octanoic acid is added to eggs because of the binding affinity between the fatty acid and the eggs. Because ^{13}C octanoic acid is not digested in the stomach, it passes into the small intestine, where it is rapidly broken down and absorbed. In the process of metabolism of the ^{13}C stable isotope tracer fatty acid, $^{13}CO_2$ is created [a 13-carbon dioxide], which is released in the breath. The rate at which the $^{13}CO_2$ appears in the breath reflects the rate at which the food that the ^{13}C octanoic acid was bound to leaves the stomach into the small intestine.

Thus, the production and expiration of the $^{13}CO_2$ provides a means of measuring the rate of gastric emptying, in a way that is non-invasive for participants. The participants breathe into a test tube, which is sealed. The levels of $^{13}CO_2$ in each breath sample are then analysed using mass spectrometry, an advance laboratory technique. Thus, by labelling a nutrient with the less abundant ^{13}C stable isotope rather than ^{12}C, it was possible to 'trace' the metabolic fate of the ^{13}C octanoic acid through the stomach, providing an accurate measure of gastric emptying.

Interesting Finding

With any small study the potential for bias due to the particular characteristics of the study sample is always increased. This is why looking for congruity and consistency in the data becomes important. In this regard, two findings in the present study are interesting: the correlations between gastric emptying half-time and PYY, and with progesterone.

Recall that both correlations were different in their respective directions of effect; as PYY levels increased the gastric emptying half-time increased, while the gastric emptying half-time increased as progesterone decreased. To think about this in more specific context, you should interpret an increase in gastric emptying half-time as a *slower* transit of food from the stomach, while a decrease in gastric emptying half-time represents a *faster* transit of food from the stomach.

This is consistent with prior evidence. We know that two hormones – glucagon-like peptide-1 [GLP-1] and PYY – both exert regulatory effects on gastrointestinal motility, acting through feedback to the stomach, slowing the rate of GE, reducing gastric acid secretions, and slowing the overall rate of intestinal transit [11–13].

However, experimental evidence suggests that increased progesterone slows the speed at which food leaves the stomach, which may explain – at least in part – gastrointestinal distress during the third trimester of pregnancy [14,15]. However, the precise mechanisms by which progesterone may exert effects on gastric emptying remain speculative. Further, the present study limited itself by not analysing the correlations between gastric emptying half-time and progesterone at each phase of the cycle. Which leads us to ***Relevance***…

Relevance

Can we say that there are differences in gastric emptying according to menstrual cycle phase? As stated under ***What We Know***, above, there is previous evidence of sex differences in gastric emptying rate, being slower in women compared to men [4]. It has been suggested that the phase of the menstrual cycle may, at least in part, explain sex differences in gastric emptying, however, early research found no significant differences in gastric emptying of solids and/or liquids between the follicular and luteal phases of the menstrual cycle [8,9].

More recent research has suggested modest differences, with gastric emptying rate slower during the follicular phase compared to the luteal phase shown using 3D ultrasonography [7] and stable isotope tracers in the present study. The largest trial to date [n = 50] found no differences in gastric emptying rate between the follicular and luteal phases, however, the study compared two groups of women in each menstrual phase, rather than consider within-person effects at difference stages of individual cycles [4].

It is important to note that there are other nutritional factors – meal energy content/size, solid or liquid composition, viscosity of food fibres, protein content and macronutrient sequence – which may also influence gastric emptying [16]. The present study used a food-based standardised test meal of ~380kcal, and so it is possible that the 28min faster gastric emptying rate during the luteal phase compared to the follicular phase may also reflect the energy and macronutrient content of the meal [16]. Nevertheless, the evidence for variation in gastric emptying according to the menstrual cycle remains somewhat ambiguous [4,7–9].

The present study found no difference in energy intake during the *ad libitum* test lunch. Previous research showed that energy intake in response to an *ad libitum* test meal was 167kcal greater during the luteal compared to the follicular phase [7]. However, that study used a liquid 50g oral glucose test, which may not have provided any degree of satiation compared to the ~385kcal whole-food standardised test breakfast in the present study. In the largest prospective study to date, the BioCycle cohort found a 71kcal higher energy intake during the luteal phase, suggesting a trivial difference . [17].

Application to Practice

The present study has the advantage of stable isotope tracers repeated across phases of the menstrual cycle, and does lend some weight, despite the small sample size, to the finding. Nevertheless, it is difficult to come to a firm conclusion as to whether, and to what magnitude of difference, there may be changes in gastric emptying according to menstrual phase.

Were the findings of changes in gastric emptying to be confirmed, it would have important implications for metabolic health given the strong correlation between gastric emptying and postprandial glycaemia [18].

It is also important to note that research in women, there is evidence that RMR may be higher during the luteal phase, albeit the magnitude of difference is small [5]. However, there is no evidence that TEF responses differ according to menstrual cycle phase, irrespective of the method used to calculate TEF [6].

Cumulatively, it is difficult to make targeted, specific recommendations for altering diet according to menstrual phase where factors like gastric emptying are concerned.

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