Relationship between insulin sensitivity and menstrual cycle is modified by BMI, fitness and physical activity in NHANES

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Abstract:

Context:

There is evidence demonstrating variation in insulin sensitivity across the menstrual cycle. However, to date, research has yielded inconsistent results.

Objective:

This study investigated variation in insulin sensitivity across the menstrual cycle and associations with BMI, physical activity and cardiorespiratory fitness.

Design:

Data from 1906 premenopausal women in NHANES cycles 1999-2006 were analysed.

Main outcome measures:

Menstrual cycle day was assessed using questionnaire responses recording days since last period. Rhythmic variation of plasma glucose, triglyceride and insulin, homeostatic model of insulin resistance (HOMA-IR) and adipose tissue insulin resistance index (ADIPO-IR) across the menstrual cycle were analysed using cosinor rhythmometry. Participants were assigned low or high categories of BMI, physical activity and cardiorespiratory fitness and category membership included in cosinor models as covariates.

Results:

Rhythmicity was demonstrated by a significant cosine fit for glucose ($p= 0.014$) but not triglyceride ($p= 0.369$), insulin ($p= 0.470$), HOMA-IR ($p=0.461$) and ADIPO-IR ($p= 0.335$). When covariates were included, rhythmicity was observed when adjusting for: 1. BMI: glucose ($p< 0.001$), triglyceride ($p< 0.001$), insulin ($p< 0.001$), HOMA-IR ($p< 0.001$) and ADIPO-IR ($p< 0.001$); 2. Physical activity: glucose ($p< 0.001$), triglyceride ($p= 0.006$) and ADIPO-IR ($p= 0.038$); 3. Cardiorespiratory fitness: triglyceride ($p= 0.041$), insulin ($p= 0.002$), HOMA-IR ($p= 0.004$) and

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ADIPO-IR (p= 0.004). Triglyceride amplitude, but not acrophase, was greater in the high physical activity category compared to low (p=0.018).

Conclusions:

Rhythmicity in insulin sensitivity and associated metabolites across the menstrual cycle are modified by BMI, physical activity and cardiorespiratory fitness.

Key words:

Menstrual cycle, insulin, glucose, triglyceride, insulin sensitivity, NHANES.

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Introduction:

The onset and severity of insulin resistance are associated with a range of modifiable and non-modifiable risk factors, including, sex, age, adiposity, physical inactivity and cardiovascular fitness (1,2). Women exhibit lower fasting plasma glucose levels, but greater impairment in glucose tolerance compared to men (3). Within adipose tissue, women have greater insulin stimulated glucose and fatty acid uptake compared to men (4). Whilst BMI and age are positively associated with insulin resistance, in women insulin resistance typically occurs at a higher BMI and higher age when compared with men (5). Moreover, low fitness has a greater association with insulin resistance in overweight women compared with overweight men (2).

Reports have demonstrated a clear mechanistic role of sex hormones underpinning sexual dimorphism in insulin resistance (6). Insulin sensitivity has been positively associated with estradiol and negatively associated with progesterone in rats (7). This suggests that hormonal fluctuations across the menstrual cycle in humans may play a role in insulin sensitivity. However, strategies targeting the prevention and treatment of reduced insulin sensitivity rarely consider sex and none consider the role of the menstrual cycle.

The menstrual cycle is a fundamental biological rhythmic cycle occurring in females of reproductive age, comprised of the ovarian and uterine cycles. The ovarian cycle, consisting of follicular, ovulatory and luteal phases is concerned with oocyte maturation and release, whilst the uterine cycle, consisting of menstruation, proliferative and secretory phases, is concerned with preparing the uterine lining for possible oocyte implantation in the event of fertilisation (8). The ovarian cycle and uterine cycle occur in a coordinated and concurrent manner; herein we will refer to menstrual cycle phase in terms of the follicular and luteal phases (8). Average

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cycle length is 29 d, although this varies between individuals, with cycle lengths of 24-35 d considered normal and healthy (8,9). Within an individual, typical cycle length declines as age increases (9). The menstrual cycle is governed by rhythmic fluctuations of hormones within the hypothalamic-pituitary-gonadal axis; gonadotropin-releasing hormone, pituitary hormones (follicle stimulating hormone (FSH) and luteinizing hormone (LH)) and ovarian hormones (estradiol, progesterone and testosterone) (8). However, the effect of the menstrual cycle on physiology is under-researched and, in fact is frequently cited as a barrier towards the inclusion of women in research projects (10).

Cyclical fluctuations in hormonal profiles across the menstrual cycle have been associated with alterations in metabolic control. During the luteal phase an increase in circulating insulin and reductions in circulating glucose and triglyceride have been observed (11,12). Correspondingly, insulin sensitivity would be expected to fluctuate across the menstrual cycle. However, studies so far have been equivocal. Reductions in insulin sensitivity during the luteal phase have been reported (12–18). However other studies document no change in insulin sensitivity across the menstrual cycle (19–22). These inconsistencies may be attributable to the relatively small sample sizes used in all but one (12) of these studies (n= 6-30), which lacked statistical power to robustly detect the small, yet clinically meaningful, changes in insulin sensitivity across the menstrual cycle. Yeung *et al.* used a large sample size (n= 259), and reported significant variation in insulin sensitivity across the menstrual cycle (12). Moreover, previous studies recruited heterogenous study populations with varying BMI and physical activity levels, in which limited adjustment or investigation into the potentially confounding effects of these modifiable risk factors was conducted (12,21,22). Examining the role of modifiable risk factors in a large cohort of women is necessary to fully understand rhythmicity in insulin sensitivity across the menstrual cycle.

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In this study we firstly aim to characterise the variation in insulin sensitivity and associated metabolites across the menstrual cycle in a large cohort of well characterised females. Secondly, we will investigate the role of BMI, physical activity and cardiovascular fitness on variation in insulin sensitivity and associated metabolites across the menstrual cycle.

Materials and methods:

Participants:

NHANES is a national, cross-sectional population-based study representative of the non-institutionalised US civilian population (NHANES, RRID:SCR_013201). Data were collected in 2-year cycles beginning in 1999, with data collection ongoing. NHANES participants completed an at-home interview and a physical examination at a mobile examination centre (MEC). A reproductive health questionnaire was included in data collection cycles between 1999-2006. This questionnaire was completed by 23,569 females. Participants were excluded if they had current diagnoses of metabolic disorder (diabetes, thyroid condition) or were taking medication that altered insulin sensitivity (Figure 1). Details of variable description and codes used in this study are provided in supplementary materials (23).

Menstrual cycle assessment:

Response to the question "number of days since last period started" was treated as day of menstrual cycle. Responses were collected once for each participant. Unfortunately data on typical menstrual cycle length were not available within the NHANES database. Participants were excluded from data analysis where reported number of days since last period started >35d. Whilst the typical menstrual cycle length is 29 d, a maximum cycle day value of 35 d was selected to encompass 95% of cycle lengths in women (9). Participants were excluded based on

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factors that influence the hormonal milieu across the menstrual cycle; <16 yr, currently taking hormonal contraceptive medication, currently pregnant or gave birth within the previous year. Final analyses were conducted on 1906 participants (Figure 1).

Anthropometric assessment:

Height was measured using a stadiometer and weight was measured using a digital scale following standard procedures at the MEC. BMI was calculated using weight in kilograms divided by height in metres squared (kg/m^2). Prior to analyses participants were assigned to BMI defined categories based on standard cut-off thresholds (Low BMI- underweight and healthy weight $\leq$24.9 kg/m^2; high BMI- overweight and obese >25 kg/m^2) (24).

Blood sampling and biochemical analysis:

Venous blood samples were collected on the same day as the menstrual cycle questionnaire following a fast of at least 9 hr, but not more than 24 hr, by a trained phlebotomist at the MEC and processed according to a standardised protocol (25). Serum FSH and LH concentrations were analysed by microparticle Enzyme Immunoassay (Abbot Laboratories, Illinois, US) (26). Plasma glucose and triglyceride concentrations were analysed enzymatically (Roche Diagnostic Systems, New Jersey, US) (27,28). Plasma insulin concentration was assessed via radioimmunoassay (Pharmacia Diagnostics AB, Uppsala, Sweden) (29). Insulin sensitivity was calculated using the HOMA-IR (30) and ADIPO-IR (31) methods.

Physical activity:

Each participant completed a physical activity questionnaire which included questions relating to all physical activity performed in the previous 30 d. Activity type, duration, intensity and number of times performed in the last 30 d were recorded. Moderate intensity activities were

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defined as inducing light sweating or a slight to moderate increase in breathing or heart rate. Vigorous activities were defined as inducing heavy sweating or large increases in breathing or heart rate. MET scores for specific activities were calculated using activity type and intensity (32). MET scores were multiplied by the average duration and number of times performed in the last 30 d to calculate MET min/30d for each activity. MET min/30d were summed for each activity then divided by 4.29 to calculate total MET min/wk. Prior to analyses participants were assigned to low and high physical activity categories based on whether they met the national physical activity guidelines (Low physical activity <500 MET/week; high physical activity ≥ 500 MET/ week) (33).

Cardiorespiratory fitness:

Participants underwent a submaximal exercise test on a treadmill to predict $\dot{V}O_2$max (34). Participants were assigned to 1 of 8 protocols, of varying difficulty, based on age, BMI and self-reported physical activity level. Each protocol included a 2 min warm-up, 2 X 2 min stages and a 2 min cool down. Heart rate was recorded throughout using an automated monitor. These exercise protocols aimed to elicit 75% of maximal heart rate by the end of the test. Predicted $\dot{V}O_2$max was estimated by extrapolating age-specific maximal heart-rate responses to the two 2-minute exercise stages, assuming a linear relation between HR and O_2 consumption during exercise (35,36). Prior to analyses participants were assigned to low and high cardiorespiratory fitness categories based on whether their V̇O2max score were below or above the age specific 50th percentile (37).

Statistical analysis:

All analyses were conducted in R (V 3.6.3) (38). Participant demographic data are presented as mean ± sd. Number of participants are shown for each analysis; this varies due to missing data. Data were tested for normality using visual inspection of histogram and Shapiro-Wilk test. Non-

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normal data were log10 transformed. Rhythmicity across the menstrual cycle was detected using the "Cosinor" and "Cosinor2" packages (39,40). Cosinor fits a cosine curve with a free phase to data and calculates MESOR (a rhythm adjusted mean), amplitude (half the predictable variation within a cycle) and acrophase (time of highest value within a cycle). Peak to peak difference (%) (P-P) was calculated using the following equation: ((2 x amplitude/mean)*100). In separate Cosinor models, we included BMI, physical activity and cardiorespiratory fitness category as a covariate. Inclusion of covariates in the Cosinor model allows the MESOR, amplitude and acrophase to differ between respective high and low covariate categories. Overall significance of the cosine model was established using the zero-amplitude test. Wald tests were conducted to test for differences in the amplitude and acrophase between respective high and low covariate categories. Cosine data are presented as MESOR ± amplitude. Data are shown as conventional boxplots.

Results:

Participant characteristics:

Participant characteristics are outlined in Table 1. As would be expected, greater weight, higher BMI, lower physical activity and lower $\dot{V}O_2$max were observed in the low physical activity category (difference= 2.0 kg, p= 0.020; 1.2 kg/m^2, p< 0.001, 2884.7 MET min/wk p< 0.001; 1.4 ml/min/kg, p= 0.019 respectively), high BMI category (26.6 kg, p< 0.001; 10.3 kg/m^2, p< 0.001; 576.4 MET min/wk, p< 0.001; 2.5 ml/min/kg, p< 0.001) and low cardiorespiratory fitness category (3.4 kg, p= 0.003; 1.3 kg/m^2, p= 0.001; 403.1 MET min/wk p= 0.028; 10.8 ml/min/kg, p< 0.001 respectively). Age was significantly greater for the low physical activity (3.1 yr, p< 0.001), high BMI (3.6 yr, p< 0.001) and high cardiorespiratory fitness (5.5 yr, p< 0.001) categories. Height was significantly greater in the high physical activity category (1.5 cm, p <0.001), but not BMI (0.4 cm, p= 0.166) nor cardiorespiratory fitness (0.2 cm, p= 0.757). No rhythmic cycling was detected across the menstrual cycle in BMI (MESOR: 26.3 ± amplitude:

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0.15 kg/m^2, p= 0.822), physical activity (1527.1± 182.1 MET min/wk, p= 0.199) or cardiorespiratory fitness (37.5 ± 0.5 ml/min/kg, p= 0.517) ([23]).

Pituitary hormone concentration across the menstrual cycle:

To demonstrate the validity of cosine analysis for analysing cyclic rhythms in variables across the menstrual cycle, pituitary hormones were analysed. Plasma FSH and LH concentrations were available for a subset of participants (Table 2 & Figure 2). FSH concentration reached a peak of 8.6 IU at day 9 falling to 3.5 IU at day 26 (p<.001). LH concentration peaked at 8.1 IU on day 12 and declined to a trough of 2.1 IU on day 29 (P<.001).

What is the effect of the menstrual cycle on insulin sensitivity:

Rhythmicity was demonstrated by a significant cosine fit for glucose (MESOR: 85.1 ± amplitude: 1.2 mmol/L; p= 0.014). No significant fit was observed for triglyceride (87.7 ± 2.8 mg/dL; p= 0.369), insulin (9.8 ± 0.4 mmol/L; p= 0.470), HOMA-IR (2.1 ± 0.1 mmol/L; p= 0.461) or ADIPO-IR, (9.7 ± 0.6 mmol/L; p= 0.335) (Table 3; Figure 3 & 4).

How does BMI affect insulin sensitivity across the menstrual cycle:

When BMI category was added as a covariate into the cosine model, significant cosine fit was observed for glucose (p< 0.001), triglyceride (p< 0.001), insulin (p< 0.001), HOMA-IR (p< 0.001) and ADIPO-IR (p< 0.001) (Table 3; Figure 3 & 4). There were no significant differences in amplitude between low and high BMI categories for glucose (0.7 vs 1.7 mmol/L, p= 0.205), triglyceride (3.4 vs 3.9 mg/dL, p= 0.889), insulin (0.3 vs 1.1 mmol/L, p= 0.486), HOMA-IR (0.1 vs 0.3 mmol/L, p= 0.318) or ADIPOIR (0.2 vs 1.9 mmol/L, p= 0.248). Nor was there a significant

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difference in acrophase between low and high BMI categories for glucose (12 vs 16 d, p= 0.335), triglyceride (28 vs 21 d, p= 0.098), insulin (15 vs 23 d, p= 0.180), HOMA-IR (15 vs 22 d, p= 0.267) or ADIPO-IR (23 vs 22 d, p= 0.902).

How does physical activity affect insulin sensitivity across the menstrual cycle:

When physical activity category was added as a covariate into the cosine model, significant cosine fit was observed for glucose (p< 0.001), triglyceride (p= 0.006) and ADIPO-IR (P= 0.038), but not insulin (p= 0.095), HOMA-IR (p= 0.061) (Table 3; Figure 3 & 4). Triglyceride amplitude was significantly lower in the low physical activity category compared to the low physical activity category (3.1 vs 7.2 mg/dl, p= 0.018). No significant differences were observed in amplitude between low and high physical activity categories across the menstrual cycle for either glucose (1.5 vs 1.1 mmol/L, p= 0.308), insulin (0.7 vs 0.3 mmol/L, p= 0.284), HOMA-IR (0.2 vs 0.1 mmol/L, p= 0.310) or ADIPO-IR (0.7 vs 0.7 mmol/L, p= 0.506). There were no significant differences in acrophase between low and high BMI categories for glucose (17 vs 12 d, p= 0.235), triglyceride (12 vs 27 d, p= 0.675), insulin (21 vs 14 d, p= 0.571), HOMA-IR (21 vs 14 d, p= 0.577), ADIPO-IR (18 vs 26 d, p= 0.423).

How does cardiorespiratory fitness affect insulin sensitivity across the menstrual cycle:

When cardiorespiratory fitness category was added as a covariate into the cosine model, significant cosine fit was observed for triglyceride (p= 0.041), insulin (p= 0.002), HOMA-IR (p= 0.004) and ADIPO-IR (p= 0.004), but not glucose (p= 0.223) (Table 3; Figure 3 & 4). No significant differences in amplitude across the menstrual cycle were observed between low and high cardiorespiratory fitness for glucose (0.4 vs 0.8 mmol/L, p= 0.460), triglyceride (6.9 vs 6.0 mg/dL, p= 0.116), insulin (1.2 vs 0.7 mmol/L, p =0.099), HOMA-IR (0.3 vs 0.2 mmol/L, p= 0.109) or ADIPOIR (1.7 vs 1.0 mmol/L, p= 0.115). There were no significant differences in

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acrophase between low and high BMI categories for glucose (20 vs 12 d, p= 0.443), triglyceride (34 vs 24 d, p= 0.271), insulin (27 vs 17 d, p= 0.290), HOMA-IR (27 vs 17 d, p= 0.282) or ADIPO-IR (30 vs 20 d, p= 0.260).

Discussion

This study aimed to characterise cyclical changes in insulin sensitivity and associated metabolic parameters across the menstrual cycle and their association with BMI, physical activity and cardiorespiratory fitness. We found rhythmic cycling across the menstrual cycle for glucose, but not triglyceride, insulin, HOMA-IR or ADIPO-IR. When including selected risk factors for insulin resistance as covariates, rhythmic cycling was observed across the menstrual cycle for glucose, triglyceride, insulin, HOMA-IR, and ADIPO-IR when models included BMI; glucose, triglyceride and ADIPO-IR when models included physical activity; and triglyceride, insulin, HOMA-IR and ADIPO-IR when models included cardiorespiratory fitness. Triglyceride amplitude, but not acrophase, was significantly greater in the high physical activity category compared to the low physical activity category. No significant differences in amplitude nor acrophase were observed for glucose, insulin, HOMA-IR and ADIPO-IR between respective high and low covariate categories. These findings demonstrate changes in insulin sensitivity and triglyceride levels across the menstrual cycle are modified by BMI, physical activity and cardiorespiratory fitness status.

Previous literature reports insulin sensitivity is either reduced during the luteal phase (12–18) or remains unchanged across the menstrual cycle (19–22). Reported variation in HOMA-IR across the menstrual cycle is of a relatively small magnitude (0.3 U), although may be clinically meaningful (12). Therefore, some previous studies using small sample sizes may have lacked statistical power to detect significant variation (19–22). In contradiction to a report from

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another large study, we did not observe rhythmic variation for insulin sensitivity prior to adjusting cosine fit for BMI or cardiorespiratory fitness (12). Participants studied in Yeung *et al.* had an average lower BMI (24.1 vs 26.3 kg/m^2) which may have contributed to discrepancies in findings (12). Cardiorespiratory fitness was not assessed in their study. Following the inclusion of BMI and cardiorespiratory fitness into models, we observed rhythmic cycling for HOMA-IR with similar variability across the menstrual cycle of 0.2 U (P-P = 7.5%), to that previously reported by Yeung *et al..* This provides evidence that BMI and cardiorespiratory fitness mediate the variation in HOMA-IR across the menstrual cycle. This mediation effect may underpin inconsistencies reported in the literature.

To our knowledge, this is the first study to investigate ADIPO-IR across the menstrual cycle. We observed rhythmic variation in ADIPO-IR when adjusting for BMI, physical activity and cardiorespiratory fitness levels. Rhythmic cycling in ADIPO-IR concentration roughly coincided with rhythmic cycling of triglyceride across the menstrual cycle, which peaked at cycle day 23 declining to a trough at cycle day 5 (Table 3). This contradicts previous research, reporting elevated triglyceride concentrations during the follicular phase compared to the luteal phase (11,41). However, previous studies did not consider BMI, physical activity or cardiorespiratory fitness, which we found significantly mediated rhythmicity in triglyceride across the menstrual cycle. Additionally, we used a larger sample size in this study compared previous studies (n= 34 (41) & 259 (11) vs 869). Increases in ADIPO-IR during the luteal phase alongside concurrent elevations in triglyceride concentration, may be underpinned by a decline in insulin stimulated triglyceride uptake or suppression of lipolysis during the luteal phase. Progesterone has been shown to inhibit adipocyte insulin signalling and receptor binding (42,43). Increased circulating progesterone levels may contribute to increased ADIPO-IR observed during the luteal phase of the menstrual cycle. However, further work is required to elucidate the role progesterone plays in regulating changes in circulating triglyceride and ADIPO-IR cross the menstrual cycle.

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We observed lower mean triglyceride concentration alongside significantly greater amplitude across the menstrual cycle in the high physical activity category compared to low. The timing of the peak and trough in triglyceride concentration roughly coincided with the glucose trough and peak, respectively. Regular physical activity increases the capacity for adipose tissue and skeletal muscle lipid uptake and mobilisation (4,44). Moreover, high physical activity levels are positively associated with increased metabolic flexibility (44). Greater amplitude in triglyceride concentration across the menstrual cycle in the high physical activity category may reflect a coordinated uptake and release of triglyceride in response to fluctuations in glucose concentration.

Whilst BMI and physical activity are significantly associated with variation in HOMA-IR and ADIPO-IR across the menstrual cycle, the mechanisms underpinning this relationship are uncertain. Variation in insulin sensitivity across the menstrual cycle has been associated with progesterone and estradiol (12). Differences in BMI and physical activity are known to alter ovarian hormonal profiles. Low physical activity levels are associated with higher mean estradiol levels across the menstrual cycle and higher progesterone levels during the luteal phase (45). High BMI is associated with greater variability of estradiol, but not progesterone (46). Unfortunately, neither estradiol nor progesterone were assessed in NHANES. Future research should investigate the role of sex hormones in the relationship between insulin sensitivity and BMI and physical activity levels.

We observed significant rhythmicity in HOMA-IR and ADIPO-IR following adjustment for BMI and cardiorespiratory fitness. This suggests that menstrual cycle phase is an important consideration in the assessment of insulin sensitivity in clinical practice or research, especially

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in populations with high BMI or low cardiorespiratory fitness. Additionally, we found greater amplitude across the menstrual cycle for HOMA-IR and glucose in high compared to low BMI and HOMA-IR in low compared to high cardiorespiratory fitness. Whilst these amplitudes were not statistically significant, these data indicate individuals with high BMI or low cardiorespiratory fitness may be at greater risk of impaired insulin sensitivity and elevated glucose concentration during the luteal phase. Therefore, therapeutic strategies aiming to reduce disturbances in metabolic control across the menstrual cycle may benefit from targeting a reduction in BMI and increase in cardiovascular fitness. This is of particular clinical importance due to the role of high glucose variability and insulin resistance in the development and progression of diabetic complications (47,48). Future larger studies should further investigate the association between BMI and cardiorespiratory fitness with the magnitude of variation in insulin sensitivity and glucose concentrations across the menstrual cycle. This research is crucial to further understand the role of the menstrual cycle in diabetes.

Unexpectedly, some participant demographics were significantly different between respective low and high demographic categories. Therefore, some caution should be applied when interpreting these findings. Age was significantly greater in high BMI, physical activity and cardiorespiratory fitness categories compared to low. However, previous research has reported the positive relationship between age and insulin resistance is associated with concurrent increases in adiposity and decreases in physical activity (49), which were included in the cosinor analysis as covariates. Height was significantly greater in the high physical activity group. We performed regression analysis to assess the relationship between height and metabolic outcome parameters whilst accounting for menstrual cycle day and found no significant associations (23). This statistically significant effect may simply be due to the number of participants in the study (50). Similarly, a previous large study reported no association between variation in HOMA-IR across the menstrual cycle and height (12). We

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would expect there to be overlap in participants within covariate categories, which may have confounding effects. For example, commonality between participants in the high BMI, low physical activity and low cardiorespiratory categories. Future studies should investigate whether there is a cumulative effect of BMI, physical activity and cardiorespiratory on rhythmic cycling in insulin resistance across the menstrual cycle.

The large, prospective nature of the NHANES data set represents a major strength of this study. Our analyses were conducted in 1906 female participants with detailed questionnaire data available for reproductive and general health. These data permitted the exclusion of women with conditions that alter metabolic control or hormonal concentrations. The indirect assessment of insulin resistance using surrogate measures (HOMA-IR and AIDIPO-IR) was a limitation. However, HOMA-IR and ADIPO-IR have been validated against the gold standard hyperinsulinaemic euglycaemic clamp ($r= 0.82$, $p< 0.001$) and the multi-step pancreatic clamp ($r= 0.86$, $p< 0.001$) respectively, demonstrating strong correlations (45, 57). Physical activity levels were determined using questionnaire. However, reports from the NHANES data set demonstrate similar amounts of self-reported physical activity and objectively measured physical activity via accelerometer in those either meeting or not meeting PA guidelines (53). Nonetheless, future studies may benefit from collecting objectively measured physical activity across the menstrual cycle. This study used number of days since last menstrual period started as a proxy for phase of menstrual cycle and was limited by a lack of data regarding participants' typical menstrual cycle length. These data would allow greater accuracy in determining menstrual cycle phase. However, that the analysis of FSH and LH displayed expected fluctuations with significant rhythmicity across the menstrual cycle supports the use of "number of days since last menstrual period started" for statistical analysis in this data set. Ovarian hormone concentrations across the menstrual cycle were not measured, which would allow further exploration into the relationship between insulin sensitivity and risk factors for

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metabolic dysregulation. Future studies should obtain further data to allow thorough characterisation of participants' menstrual cycles, including typical menstrual cycle duration, ovulation date and ovarian hormones.

In conclusion, our study confirms previous reports showing insulin sensitivity undergoes small, yet statistically and clinically significant, rhythmic cycling across the menstrual cycle. This is the first study to demonstrate a modifying effect of BMI, physical activity and cardiorespiratory fitness on variation in insulin sensitivity and associated metabolites across the menstrual cycle. These findings provide a basis for further research to explore the mediatory role of BMI, physical activity and cardiorespiratory fitness on variation in insulin sensitivity across the menstrual cycle. Furthermore, this provides direction for investigation into the therapeutic benefit of targeting BMI and physical activity to mitigate disturbances in insulin sensitivity across the menstrual cycle.

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Data availability:

All data generated or analysed during this study are included in this published article or in the data repositories listed in References (54,55)

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Legends for Figures and Tables:

Figure 1: Flowchart depicting participant selection in the study. Dotted lines represent participant exclusion.

Figure 2: Boxplot with cosine wave showing pituitary hormone concentration across the menstrual cycle. A, follicle stimulating hormone. B, luteinizing hormone.

Figure 3: Changes in glucose, triglyceride and insulin across the menstrual cycle with low and high categories of BMI (left), cardiorespiratory fitness (middle) and physical activity (right). Boxplot represents all participants data for respective variable. Cosinor model fits are shown for all participants (blue), low covariate category (green) and high covariate category (red). Low BMI, ≤24.9 kg/m^2; high BMI > 25 kg/m^2; low cardiorespiratory fitness, ≤50th age specific percentile; high cardiorespiratory fitness >50th age specific percentile; low physical activity, ≤500 MET min/wk; high physical activity, > 500 MET min/wk.

Figure 4: Changes in HOMA-IR and ADIPO-IR across the menstrual cycle with low and high categories of BMI (left), cardiorespiratory fitness (middle) and physical activity (right). Boxplot represents all participants data for respective variable. Cosinor model fits are shown for all participants (blue), low covariate category (green) and high covariate category (red). Low BMI, ≤24.9 kg/m^2; high BMI > 25 kg/m^2; low cardiorespiratory fitness, ≤50th age specific percentile; high cardiorespiratory fitness >50th age specific percentile; low physical activity, ≤500 MET min/wk; high physical activity, > 500 MET min/wk.

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Table 1: Data are presented as mean ± standard deviation. N-values are presented as total (%) for each demographic category. PA, physical activity; BMI, body mass index; CRF, cardiorespiratory fitness. * represents p <0.05 following independent samples t test.

Table 2: FSH, follicle stimulating hormone; LH , luteinizing hormone; P-P (%), difference between peak and trough. P-value from zero amplitude test for model fit.

Table 3: BMI, body mass index; CRF, cardiorespiratory fitness; PA, physical activity. Low BMI, ≤24.9 kg/m^2; high BMI > 25 kg/m^2; low CRF, ≤50th age specific $\dot{V}O_2$max percentile; high CRF >50th specific $\dot{V}O_2$max percentile; low PA, ≤500 MET min/wk; high PA, > 500 MET min/wk. Cosine fit p-value represents zero amplitude test for model fit. Amplitude difference p-value represents difference in amplitudes between respective low and high covariate categories. Acrophase difference p-value represents difference in acrophase between respective low and high covariate categories. Bold font indicates a p-value < 0.05.

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Tables:

Table 1: Participant characteristics split by demographic category.

Demographic	All	Low MET	High MET	Low BMI	High BMI	Low CRF	High CRF
n	1906	946 (49.6)	960 (50.4)	1021 (53.6)	885 (46.4)	451 (46.7)	514 (53.2)
Age (yr)	25.4 ± 9.4	27.0 ± 10.0	23.9 ± 8.6*	23.8 ± 8.5	27.4 ± 10.1*	21.3 ± 5.9	26.8 ± 10.0*
Height (cm)	161.8 ± 6.9	161.1 ± 6.9	162.6 ±6.8*	162.0 ± 6.8	161.6 ± 7.0	162.0 ± 6.7	162.2 ± 6.7
Weight (kg)	69.0 ± 19.0	70.0 ± 19.9	68.0 ± 18.0*	56.6 ± 7.2	83.2 ± 18.3*	69.5 ± 19.5	66.1 ± 15.1*
BMI (kg/m^2)	26.3 ± 6.8	26.9 ± 7.2	25.7 ± 6.3*	21.5 ±2.1	31.8 ± 6.1*	26.4 ± 7.0	25.1 ± 5.4*
VO2max (ml/kg/min)	37.5 ± 9.0	36.8 ± 9.5	38.2 ± 8.6*	38.6 ± 8.8	36.1 ± 9.1*	31.8 ± 3.8	42.6 ± 9.3*
MET (min/wk)	1548.3 ± 3112.5	95.3 ± 144.7	2980.0 ± 3884.4*	1815.9 ± 3479.9	1239.5 ± 2593.5*	1407.9 ± 2498.3	1811.0 ± 3200.0*

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Table 2: Pituitary hormone concentrations across the menstrual cycle.

Variable	N	MESOR (IU)	Amplitude (IU)	P-P (%)	Peak (d)	Trough (d)	p-value
FSH	218	5.5	2.5	91.9	9	26	< 0.001
LH	219	4.2	3.0	144.0	12	29	< 0.001

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Table 3: Variation in insulin sensitivity and associated metabolites across the menstrual cycle.

Variable	Category	n-val	Mean (IU)	Amplitude (IU)	P-P (%)	Acrophase (d)	p-value	Amplitude difference p-value	Acrophase difference p-value
Glucose	All	1903	85.09	1.15	2.70	15	**0.014**		
	Low BMI	1019	83.41	0.67	1.62	12			
	High BMI	884	87.07	1.69	3.88	16	**0.000**	0.205	0.335
	Low PA	944	85.82	1.46	3.41	17			
	High PA	959	84.35	1.10	2.61	12	**0.000**	0.308	0.235
	Low CRF	451	84.16	0.44	1.05	20			
	High CRF	513	84.44	0.78	1.86	12	0.223	0.460	0.443
Triglyceride	All	872	87.67	2.79	6.37	26	0.369		
	Low BMI	482	77.26	3.39	8.78	28			
	High BMI	390	102.55	3.85	7.51	21	**0.000**	0.889	0.098
	Low PA	404	89.83	3.07	6.85	12			
	High PA	468	86.39	7.22	16.73	27	**0.006**	**0.018**	0.675
	Low CRF	192	88.14	6.87	15.58	34			
	High CRF	254	83.39	6.03	14.47	24	**0.041**	0.116	0.271
Insulin	All	872	9.75	0.37	7.63	20	0.470		
	Low BMI	483	7.49	0.30	8.14	15			
	High	389	13.55	1.09	16.0	23	**0.000**	0.486	0.180

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	BMI				5				
	Low PA	405	10.06	0.67	13.32	21			
	High PA	467	9.45	0.26	5.59	14	0.095	0.284	0.571
	Low CRF	194	10.89	1.22	22.49	27			
	High CRF	252	9.04	0.71	15.60	17	**0.002**	0.099	0.290
HOMA-IR	All	871	2.09	0.09	8.34	20	0.461		
	Low BMI	482	1.56	0.05	6.90	15			
	High BMI	389	3.01	0.28	18.91	22	**0.000**	0.318	0.267
	Low PA	404	2.18	0.15	14.18	21			
	High PA	467	2.01	0.06	6.03	14	0.061	0.310	0.577
	Low CRF	194	2.30	0.27	23.21	27			
	High CRF	252	1.92	0.16	16.75	17	**0.004**	0.109	0.282
ADIPO-IR	All	868	9.67	0.59	12.24	23	0.335		
	Low BMI	480	6.53	0.23	7.09	23			
	High BMI	388	15.71	1.89	24.09	22	**0.000**	0.248	0.902
	Low PA	403	10.24	0.72	14.02	18			
	High PA	465	9.22	0.68	14.82	26	**0.038**	0.506	0.423
	Low CRF	192	10.81	1.71	31.64	30			
	High CRF	252	8.54	1.04	24.47	20	**0.004**	0.115	0.260

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Figure 1

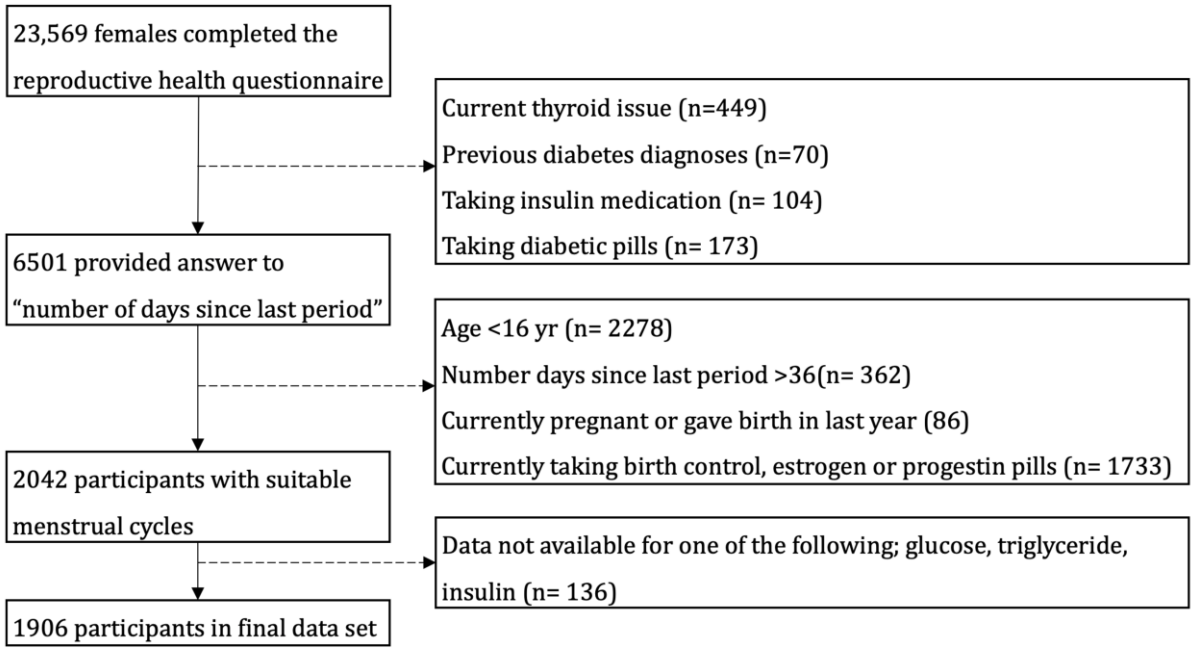

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Figure 2

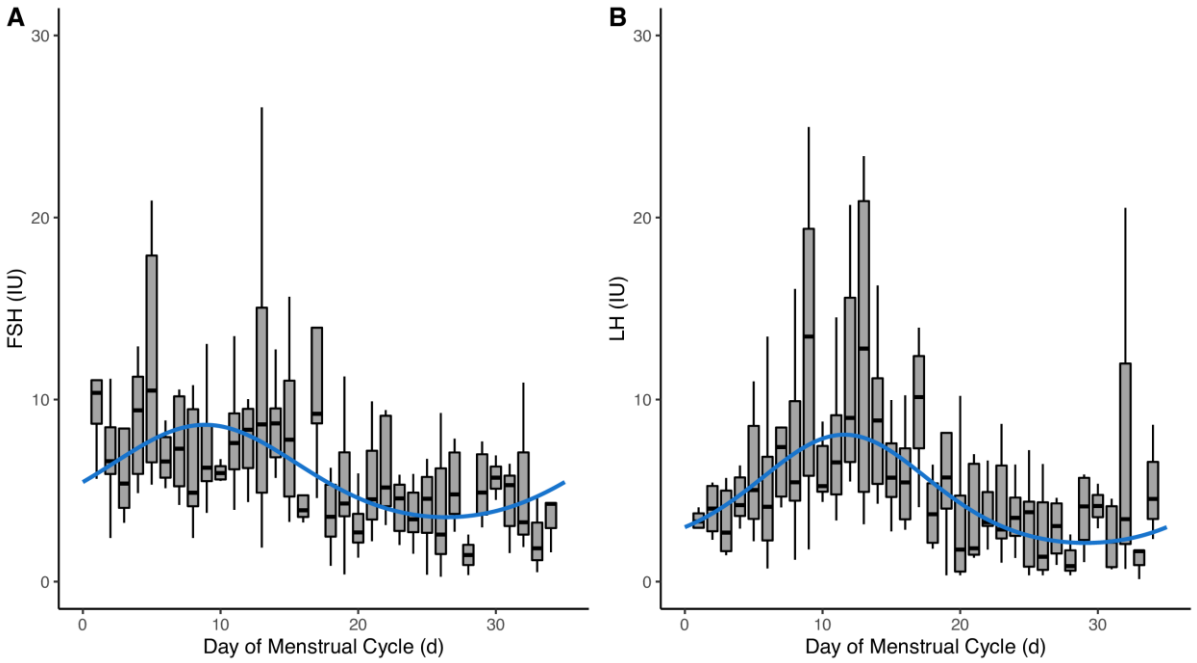

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Figure 3

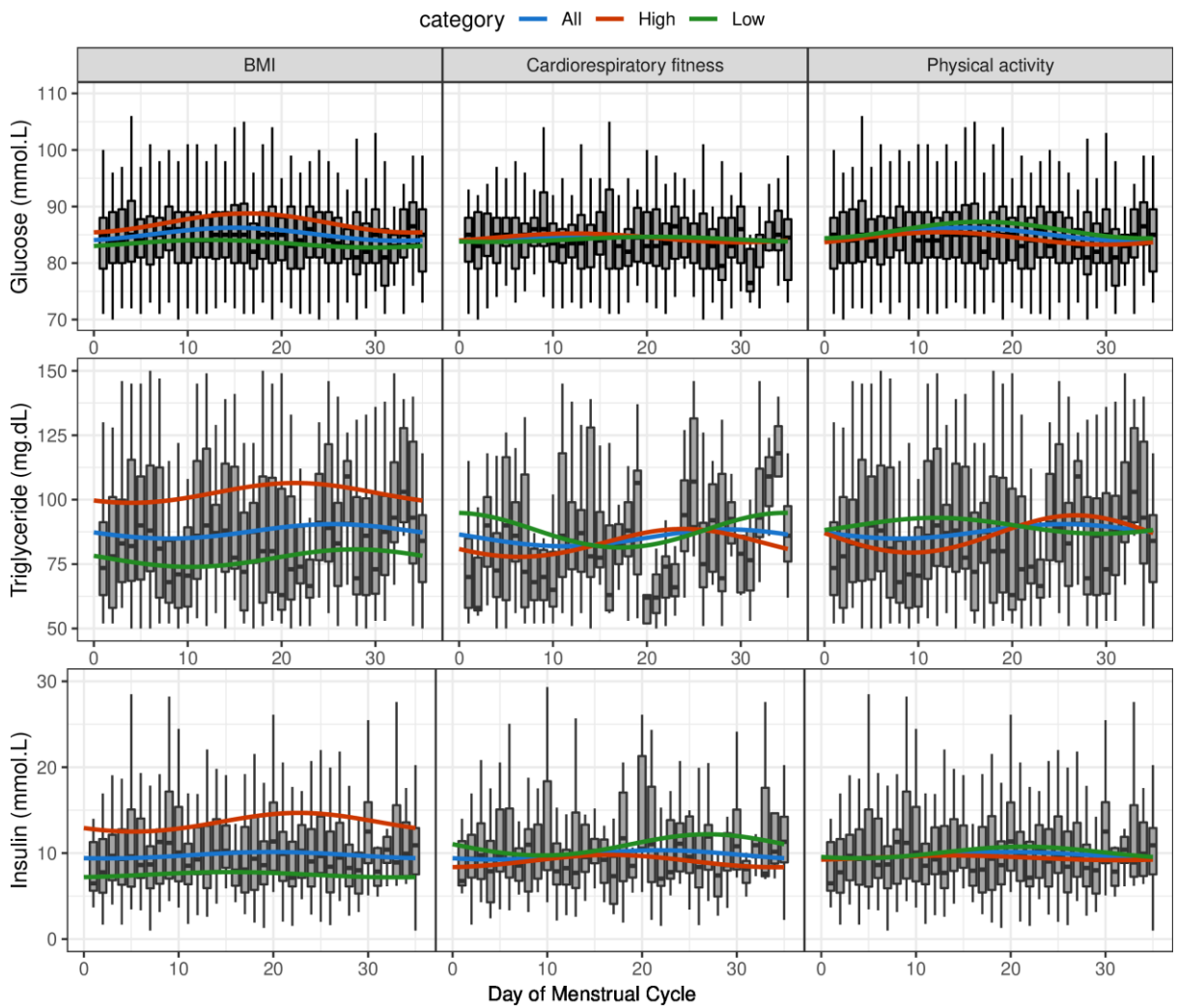

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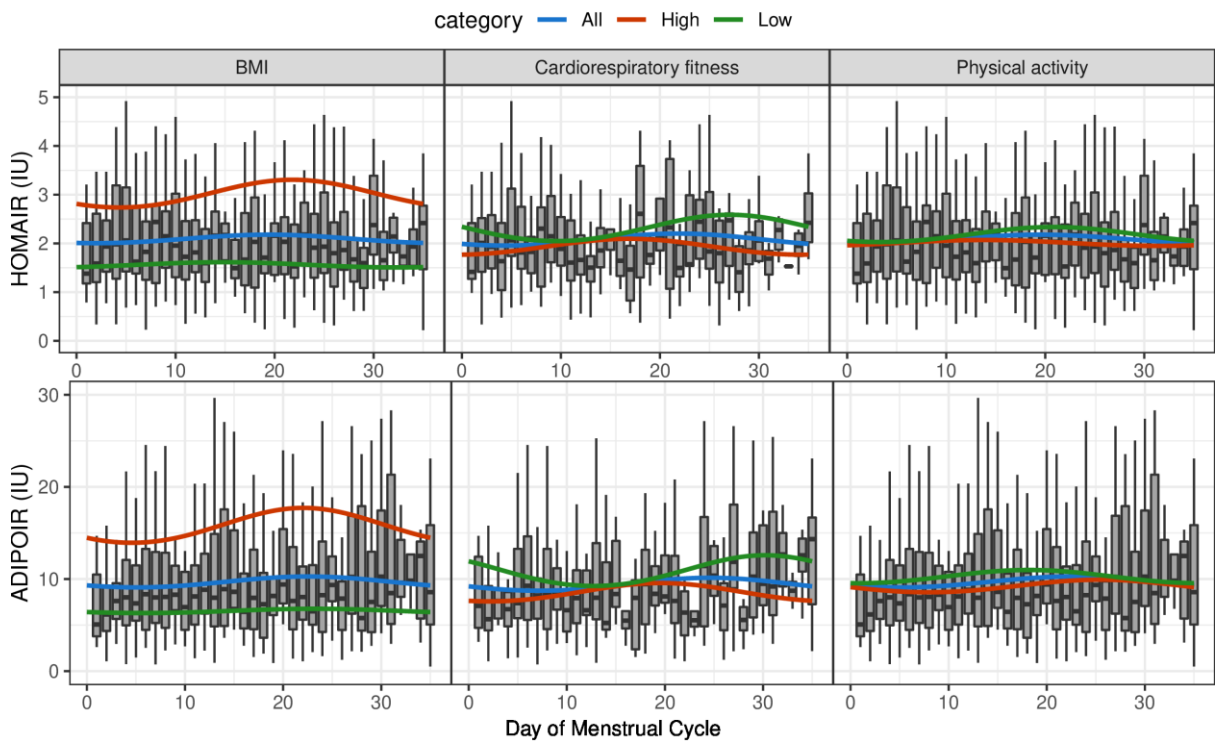
Figure 4



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