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ABSTRACT

-luteal phases of the MC in NoOC-group (n=38) or the active and inactive phases of the COC cycle (COC, n=19). Participants recorded their food intake for 3 days after measurements. A secondary analysis was completed for the NoOC-group without REE outliers (difference between measurements >1.5 × interquartile range, n=4). **Results**: In the NoOC-group, luteal phase REE was 40 kcal higher than follicular phase REE [95% confidence interval (CI): -2 kcal/d–82 kcal/d, d=0.20, p=0.061]. Leptin (d=0.35, p<0.001), T3 (d=0.26, p=0.05) and fat intake (d=0.48, p=0.027) were lower, and T4 (d=0.21, p=0.041) was higher in the luteal phase. After excluding outliers, REE was 44 kcal higher in the luteal phase than in the follicular phase (95% CI: 12kcal/d–76kcal/d, d=0.22, p=0.007). In the COC-group, the mean difference in REE was -2 kcal (95% CI-82 kcal/d–79 kcal/d) between active and inactive phases, while T3 was higher in the inactive phase (d=0.01, p=0.037). **Conclusions:** REE increases only slightly from the follicular to the luteal phase but remains unchanged between COC phases. Increases in T3, leptin, and fat intake during the luteal phase might echo metabolic fluctuations that parallel female sex hormones during the MC.

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Key Words: FEMALE PHYSIOLOGY, ENERGY INTAKE, HORMONAL CONTRACEPTION, REPRODUCTIVE HORMONES, RESTING METABOLISM

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INTRODUCTION

The menstrual cycle (MC) is a series of finely tuned physiological processes caused by fluctuations in endogenous sex hormones. These hormonal fluctuations cause changes not only in the structure and function of the ovaries but also influence various metabolic pathways that regulate energy metabolism (1). Resting energy expenditure (REE) typically accounts for 60 to 75% of total daily energy expenditure and is reflective primarily of fat-free mass (FFM) and fat mass (FM), which are associated with functional demands of organs and tissues (2). Nevertheless, the possible influence of sex hormones on REE remains equivocal and requires more attention to improve our understanding of female physiology (3).

There is some evidence that REE increases in the luteal phase of the MC compared to the follicular phase. High levels of progesterone (P4) accompanied by estradiol (E2) during the mid-luteal phase are proposed to contribute to REE through their thermoregulatory interactions (4,5). In a recent systematic review (3), 47% of 26 studies reported higher REE in the luteal phase, although the varying quality of the studies limited the interpretation of the results. Of particular concern was the inconsistency or lack of MC phase verification in included studies as it has been proposed that the MC should be divided into four distinct phases according to hormonal profiles when assessing the influence of sex hormones and their ratios on metabolism (3,6).

In addition to sex hormones, habitual food intake may increase during the luteal phase compared to the follicular phase (7). Regrettably, not all previous research has been able to account for habitual food intake, a variable of significance when studying the relationship

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between the menstrual cycle and metabolism. Considering that food intake is closely related to REE (8), MC-dependent patterns mediating the effects of food intake on REE should not be ignored.

Sex hormones E2 and P4 may interact with metabolic hormones that are involved in the regulation of REE and food intake. For example, thyroid hormones play an important role in energy metabolism by regulating complex cell functions and contributing to thermogenesis and therefore, stimulating energy expenditure (9,10). Previous studies have not, however, consistently showed that thyroid hormone levels fluctuate during the MC (11). Adipocyte-derived leptin and the gut hormone ghrelin are considered appetite-regulating hormones but are also strongly associated with energy metabolism (12). Leptin is known to be involved in the regulation of the MC through the link between energy status and reproduction and its increase during the luteal phase have been consistently reported (13) while ghrelin does not appear to be influenced by the MC (14). There is, however, evidence that leptin does not directly influence the processes underlying REE (15) whereas ghrelin is shown to be negatively associated with REE independent of body fat (16).

The synthetic female sex hormones contained in monophasic combined oral contraceptives (COC) downregulate endogenous E2 and P4 and appear to only trivially affect other physiological processes (17). Regrettably, the interaction of COC with REE is not clear, but a recent review suggests that COC use does not affect food intake (18). Studies investigating the effects of COC phases on metabolic hormones are scarce, but currently there appears to be

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consensus on unchanged thyroid hormones (19), leptin (20) and ghrelin (21) levels during the COC cycle.

Considering that a woman has an average of 450 MCs during her lifetime (22), regular fluctuation in energy metabolism can have meaningful effects on her health. Moreover, according to United Nations (23), approximately 16% (151 million) of women use COC, which underlines the importance of further research on the metabolic effects of hormonal contraception use. As such, the aim of this study was to investigate the effects of endogenous and exogenous female sex hormones on REE and metabolic hormones in naturally menstruating women and women using COC. We hypothesized that REE, energy intake, and leptin would be higher in the luteal phase than in the follicular phase, while no changes would occur between COC cycle phases.

MATERIAL AND METHODS

Participants

Healthy untrained women (n = 77, Tier 1: recreationally active, defined according to the Participant Classification Framework (24)) aged 18-35 years with a self-reported body mass index (BMI) between 19.5 and 35 kg/m^2 at recruitment volunteered to participate in this study via the advertisement in social media, sport halls, gyms, public places, and university mailing lists. The participants were stratified into two groups, one not using any hormonal contraception with a self-reported regular 26–35-day MC over the previous six months (NoOC-group, n=58, normally menstruating), and one group using monophasic COC (COC-group,

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n=19). The participants were required to complete a health questionnaire that was screened and approved by a medical doctor prior to inclusion in the study. Participants using any medication affecting metabolism or exercise responses, that were smokers or unable to run were excluded from the study. The study followed the principles of the Declaration of Helsinki, and the Ethics Committee of the University of Jyväskylä approved the study (1519/13.00.04/2021). All subjects signed an informed consent prior to participation.

In the NoOC-group, 20 participants were excluded prior to analysis because they had incomplete data, or they did not meet the criteria for hormonal parameters or MC length (6). Of these, 11 participants had P4 concentrations below 16 mmol/l, which does not meet the recommendation for phase 4 (7 days from ovulation) indicating the possibility of an inadequate luteal phase or an error in measurement timing. One participant had a cycle length of 38 days, two participants had TSH levels above the reference range, indicating potential hypothyroidism, and 6 participants completed only the first or second measurement due to illness or personal reasons. Therefore, the final number of participants in the NoOC-group was 38.

The participants in the COC-group (n=19) took the second-, third- or fourth-generation COC pills for 21 or 24 days (active phase), followed by a 7- or 4-day hormone-free interval (inactive phase) (Supplemental Table 1, Supplemental Digital Content, Content of COC pills and brand names used by participants in the COC-

-group. The participant flow is visualized in the Supplemental Figure 1 (Supplemental Digital Content, Flowchart of participant enrollment through the study).

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Study design

The study began with a one MC or COC cycle control period in which the participants were instructed to complete training and menstrual diaries daily. Participants also received written instructions to refrain from vigorous physical activity, and alcohol 24 hours before blood and metabolic measurements. In addition, participants were instructed to avoid eating 10 hours before fasting measurements and to otherwise maintain their typical diet throughout the study period. The participants were allowed to drink a glass of water (200 ml) after waking up. Study visits were scheduled to occur twice during the MC or COC cycle. The timings of the measurements were based on current methodological recommendations (6). The first visit for the NoOC-group was scheduled during the mid-luteal phase (4–8 days after a positive ovulation test), and the second visit during the early follicular phase (1–5 days after the onset of bleeding). In the COC-group, the corresponding measurement points were scheduled during the 2nd–6th day of the inactive phase (end of the COC cycle) and the 2nd–9th day of the active phase (beginning of the COC cycle). Visits were not randomized, as participants proceeded to the training intervention after the measurements. This training intervention is not included in the present study. Both measurement sessions were identical and included indirect calorimetry assessment to determine REE, blood sampling (hormonal measurements), and bioimpedance analysis for body composition assessment.

Ovulation was determined by monitoring urinary luteinizing hormone and E2 using a Clearblue two-hormone fertility monitor (Clearblue® Advanced Digital Ovulation, SDP Swiss Precision Diagnostics GmbH (SDP), Geneva, Switzerland). The method has been shown to be

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accurate when compared to transvaginal ultrasound scans and serum hormone measurements (25). Participants in the NoOC-group were instructed to start using the ovulation test on an individually determined date predicted based on the last six MCs, as stated in the manufacturer's instructions.

Anthropometrics and body composition

The height of each participant was measured on the first study visit with a wall-mounted stadiometer. Body composition and body mass were measured using a multifrequency bioelectrical impedance device (Inbody 770 body composition analyzer, Biospace Co. Ltd, Seoul Korea), with participants wearing underwear. BMI was calculated as weight (kg) divided by height squared (m^2).

Resting metabolism

REE and respiratory exchange ratio (RER) were measured using the Vyntus CPX metabolic cart (Vyaire Medical GmbH, Hoechberg, Germany) with the canopy method. Prior to each test, the equipment was calibrated according to manufacturer instructions using standard gases. Before measurements, the participant rested in a supine position for 10 min in a dimly lit room.

The room temperature ranged from 19.5 to 24°C due to seasonal variation. Despite this variation, data collection for each participant was completed within the same season, and the variation between the two measurements was small [mean coefficient of variation (CV)% 1.5, SD 1.3]. $\dot{V}O_2$ and $\dot{V}CO_2$ were measured for 20 min and averaged at 1 min intervals. For analysis,

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the first 5 min of data were excluded and any minutes with a RER above 1.0 or below 0.7 were discarded as non-physiological (26). REE was calculated using the Weir equation (27).

The test-retest reliability in REE measurements in our laboratory was assessed in older men and women participating in the ENDURE project (28). These unpublished results from the control group participants indicated an intra-individual day-to-day variation of 5.3% in REE measurements, with a typical error of 39 kcal/d. The typical error was calculated by dividing standard deviation of the differences between scores by the square root of 2 (29).

Blood samples

Blood samples were taken from an antecubital vein (2x6 ml serum tubes, Vacuette® Tube Greiner Bio-One GmBH, Kremsmunster, Austria) using standard procedures between 6 and 11 AM after an overnight fast lasting at least 10 hours. The serum samples were centrifuged for 10 minutes at 2245g (Megafuge, 1.0R, Heraeus, Germany) after a 15 min period at room temperature. The serum was separated and stored in a freezer (-80°C) for later analysis. E2, P4, free thyroxine (T4), free triiodothyronine (T3) and thyroid stimulating hormone (TSH) were assessed with chemiluminescence immunoassays using Immulite®2000 XPi analyzator (Siemens Healthcare Diagnostics, New York, USA). Leptin (Biovendor Human Leptin ELISA, Czech Republic), acylated ghrelin (AG) (Biovendor Human Ghrelin Acylated Express ELISA, Czech Republic) and unacylated ghrelin (unAG) (Biovendor Human Ghrelin Unacylated Express ELISA, Czech Republic) were assessed with an enzyme-linked immunosorbent assay (ELISA) using Dynex DS 2-analyzator (Dynex Technologies, Chantilly, VA, USA). Inter-assay coefficients of variation (CV) and analytical sensitivities were as follows: E2: 8.7%, 55 pmol/L;

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P4: 15.5%, 0.3 nmol/L; T4: 5.8%, 1.4 pmol/L; T3: 7.6%, 1.5 pmol/L; TSH: 6.3%, 0.004µIU/mL; leptin: 6.7%, 0.2 ng/ml; AG: 6.7%, 5 pg/mL; unAG: 4.6%, 6 pg/mL. Inter-assay CV testing was performed in our laboratory.

Food and training diaries

Participants completed a food diary for 3 days during both menstrual and COC phases. On the day after the fasting measurement, participants completed performance testing that included maximal bilateral isometric leg press, a countermovement jump, and a graded treadmill test to determine maximal oxygen uptake. Food intake recordings started on the day after the performance tests, as the measurement days represented unusual conditions for participants and the aim was to capture participants' habitual diet. Therefore, participants in the NoOC-group completed a diary during the follicular phase on MC days 4–9 and the luteal phase on days 20–27. In the COC-group, all diaries of the active phase were completed between days 4–9 of the COC cycle, but some of the inactive phase diaries were started between days 6–7 of the inactive phase due to measurement timing thus carrying over into the active phase.

Participants were instructed to record all foods, drinks, and supplements consumed, including commercial names, producers, preparation methods, fat percentages and sugar or sweetener contents of the product. The participants were asked to estimate the amount of each food as accurately as possible using household measuring cups and spoons if a kitchen scale was not available. Photographing meals with a smart phone was recommended in cases where estimating or weighing was not possible. It was emphasized that the subjects maintained their

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usual diet throughout the recording period. Analysis for energy and macronutrient content were conducted by Fineli (National Institute for Health and Welfare, Helsinki, Finland).

Energy availability (EA) was calculated from purposeful physical activity on days corresponding to food diaries by subtracting exercise energy expenditure (EEE) from energy intake divided by the FFM. EEE was assessed by using estimated MET-values. Subjects were asked to report daily physical activity, duration, distance, and rated perceived exertion (RPE) (30) in the training diary. Based on the type, duration, and speed of activity, MET values were determined according to the listing of Ainsworth et al. (31). Exercise energy expenditure was calculated by the formula EEE = t x MET x (REE/24) - (REE/24) x t, where t is the duration of activity and REE is the measured REE.

Nutrition data were missing completely for a total of 9 participants (NoOC, n=7 and COC, n=2). In the COC-group, one participant refused to complete a food diary and another participant did not complete a food diary during the inactive phase due to illness. In the NoOC-group, 4 participants refused to complete the food diary during both MC phases and 3 participants completed the food diary only during follicular phase or luteal phase due to personal reasons. Therefore, the total number of food diaries used in the comparison between MC or COC phases was 29 in the NoOC-group and 16 in the COC-group. In addition, EA could not be calculated for one subject in the COC-group and two subjects in the luteal phase or follicular phase due to missing training diaries.

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Statistical analysis

Statistical analyses were conducted using SPSS Statistics 28 (SPSS Inc., Chicago, IL) and figures were graphed with GraphPad Prism. Results are reported as mean ± SD or as medians with first and third quartiles. The effect size for results is expressed as Cohen's *d* (32). The normality of the data was tested by Shapiro-Wilk. Within-group changes were analyzed by Student's paired t-test for normally distributed data and Wilcoxon's signed-rank test for non-normally distributed data. Associations between variables of interest and REE were tested by multiple regression analysis using FFM and FM as covariates per recommendations (2). Consistency within resting metabolism measures were assessed using the intraclass correlation coefficient (ICC) with a one-way random-effects model (Supplemental Table 2, Supplemental Digital Content, ICCof REE and RER between two measurements in NoOC- and COC-

-determined level of statistical significance was $p \leq 0.05$.

Inspection of the data revealed four participants in the NoOC group as potential outliers because their REE change from the follicular to the luteal phase exceeded 1.5 × the interquartile range (33). Although we found no physiological or measurement-related reasons for these observations, outliers have the potential to excessively influence the results in parametric tests compared with other observations. Therefore, a secondary analysis was conducted, excluding these four participants when using REE as the outcome.

RESULTS

The physical characteristics of participants are presented in Table 1. Results for the NoOC- and COC-groups are presented separately because the groups are hormonally different,

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and the primary purpose of this investigation was to observe within-group changes in two homogeneous samples.

Differences between follicular and luteal phases in the NoOC-group

Table 2 shows the hormone concentrations, resting metabolism, body composition, and dietary measures during the follicular and luteal phases. E2 concentrations were lower in the follicular phase compared to the luteal phase ($p<0.001$, $d=1.83$) whereas all participants demonstrated P4 values > 16 nmol/l in the luteal phase, indicating expectedly higher P4 concentrations compared to the follicular phase ($p<0.001$, $d=4.79$). Leptin ($p<0.001$, $d=0.35$) and T3 ($p=0.05$, $d=0.26$) were lower in the follicular phase compared to the luteal phase, while T4 was higher ($p=0.042$, $d=0.21$) in the follicular phase (Figure 1A–C).

In the full sample, REE data were compatible with a -2 kcal/d decrease to 82 kcal/d increase (95% CI) from the follicular to the luteal phase (mean difference 40 kcal/d, Figure 2A). Additionally, RER remained similar between MC phases. However, the secondary analysis indicated significantly higher REE in the luteal phase compared to the follicular phase (mean difference 44 kcal/d, 95% CI: 13 kcal/d to 76 kcal/d, $p=0.007$) (Figure 2B). We observed a strong ICC for REE in both the full sample and after excluding outliers ($p<0.001$), whereas RER showed a weak ICC in both cases ($p=0.451$ and $p=0.311$, respectively) (Supplemental Table 2, Supplemental Digital Content).

Fat intake was found to be higher during the luteal phase compared to the follicular phase (mean difference 13 g/d, 95% CI: 2 g/d to 24 g/d, $p=0.027$) (Figure 1D). The change in

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energy intake was not statistically significant, but results favored higher energy intake during the luteal phase, with a mean difference of 147 kcal/d (95% CI: -5 kcal/d to 300 kcal/d, p=0.058).

Associations of hormones and dietary measures with FFM- and FM-adjusted REE in the NoOC-group

The associations of hormone concentrations and dietary measures with REE adjusted for FFM and FM are shown in Table 3. FFM and FM explained 48% and 58% of the variance of REE in the follicular and luteal phases, respectively. T3 was positively associated with REE in both phases, increasing the adjusted R^2 to 51% in the follicular phase and 69% in the luteal phase. Of the dietary measures, energy intake (adjusted R^2=55%), energy availability (adjusted R^2=50%), fat intake (adjusted R^2=49%), and carbohydrate intake (adjusted R^2=51%) were positively associated with REE in the follicular phase but not in the luteal phase.

Secondary analysis without REE outliers indicated that the ability of FFM and FM to explain REE variability improved (adjusted R^2 55% and 64% in the follicular and luteal phases, respectively). The reported associations between T3 and dietary measures, and REE remained similar but were stronger after exclusion of outliers (Supplemental Table 3, Supplemental Digital Content, The associations of hormone concentrations and dietary measures with resting energy expenditure, adjusted for FFM and FM, at FOL and LUT phases). T3 increased the adjusted R^2 to 64% (β=0.35, p=0.002) in the follicular phase and 75% (β=0.38, p<0.001) in the luteal phase. Associations between REE and energy intake (adjusted R^2=67%, β=0.49, p<0.001), energy availability (adjusted R^2 =60%, β=0.42, p<0.005), fat intake (adjusted

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R^2=58%, β=0.37, p<0.010), and carbohydrate intake (adjusted R^2=60%, β=0.40, p<0.005), were found in follicular phase.

Differences between active and inactive phases in the COC-group

The hormone concentrations, body composition, resting metabolism and dietary measures in the COC-group are presented in Table 4. T3 (p<0.001, *d*=0.93) (Figure 3A) and body mass (p=0.037, *d*=0.058) were higher in the inactive phase compared to the active phase, but T4 and leptin did not change from the active phase to the inactive phase (Figure 3B–C). The mean difference for REE change between phases was -2 kcal (95% CI: -82 kcal/d to 79 kcal/d, p=0.969) (Figure 3D) and for fat intake -1 g (95% CI: 17 g/d to 15 g/d, p=0.885) (Figure 3E). The ICC for both REE (p=0.005) and RER (p=0.027) was strong (Supplemental Table 2, Supplemental Digital Content).

Associations of hormones and dietary measures with FFM- and FM-adjusted REE in the COC-group

Table 5 displays associations between hormone concentrations and dietary measures with REE adjusted for FFM and FM. FFM and FM together explained 37% and 66% of the variance in REE in the active and inactive phases, respectively. Leptin was negatively associated with REE in the inactive phase, increasing the adjusted R^2 to 71%, and unAG was negatively associated with REE in the active phase (adjusted R^2=50%). Other hormones or dietary measures did not appear to contribute to REE along with FFM and FM in either phase.

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DISCUSSION

The present study investigated REE and metabolic hormones over one menstrual or COC cycle, to our knowledge, in the largest sample to date. Our findings suggested higher REE during the luteal phase compared to the follicular phase (Figure 2), while no notable REE differences between COC cycle phases were observed (Figure 3D). In the NoOC-group, concentrations of T3, leptin, and fat intake were higher in the luteal phase compared to the follicular phase (Figure 1). Conversely, in the COC-group, only T3 and body mass were significantly higher during the inactive phase compared to the active phase. These findings underscore the intricate relationship between hormonal fluctuations and resting metabolism, emphasizing the necessity to consider menstrual cycle phase when interpreting REE measurements.

Metabolism and energy intake in the follicular and luteal phases

Our results suggested that REE was approximately 40 kcal/d higher in the luteal phase than in the follicular phase; however, the difference became statistically significant only after excluding four outliers who influenced the results (Figure 2B). Of these outliers, two had higher REE in the follicular phase, and the other two had higher REE in the luteal phase. Importantly, their exclusion improved our ability to explain REE variation using FFM and FM, justifying the value of our secondary analysis. However, despite investigating the largest sample to date, our study may still be underpowered to draw precise estimates of the MC effect on REE. The intraindividual day-to-day variation in REE has been reported to be approximately 5% (34), consistent with the test-retest reliability observed in our laboratory. This indicates that the daily

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variation in REE may be even larger than the effect of the MC. Therefore, we recommend that our results be interpreted in the light of previous literature.

Nevertheless, our findings are consistent with previous systematic review and meta-analysis by Benton et al. (3), who reported a slightly higher (d=0.33, p<0.001) REE in the luteal phase compared with the follicular phase. However, particularly the inclusion of studies published in year 2000 or after led to very similar effect size to ours (d=0.23, p=0.055). Furthermore, whether the more methodologically comparable previous studies have reported REE differences classified as statistically significant (35) or not (36), the numerical differences between early follicular and luteal phase tend to fall into the same range as observed in our study. For instance, Day et al. (35) reported a 29 kcal higher REE in the luteal phase, while Elliott et al. (36) observed variations ranging from 8 kcal to 57 kcal higher REE in the luteal phase compared to the early follicular phase across two MCs. Therefore, taking into consideration the totality of current evidence, the effect of MC on REE seems probable. Whether the effect has profound physiological significance remains uncertain.

Our findings suggest that increase of REE could be driven by T3, which is well known to stimulate energy expenditure (37). In our study, T3 levels showed MC-related fluctuations that paralleled REE and were associated with FFM- and FM-adjusted REE during both MC phases (Table 3). However, the MC-associated T3 response has been previously reported only by Lariviere et al. (38), while other studies have found more stable T3 levels during the MC (21,11,39). This discrepancy could be attributed to the functional nature of thyroid hormones (40), coupled with methodological differences such as variations in sample

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timing and assay techniques across studies. We also noted higher leptin concentrations in the luteal phase (Figure 1C), consistent with previous research (13,21,41). However, leptin levels were not associated with REE, which agrees with previous research (15). Of other potential metabolic hormones, we found stable levels of UnAG and AG throughout the MC. Moreover, their levels were not associated with REE, indicating that these hormones are unaffected by MC and are unlikely to explain MC-associated changes in REE. Nevertheless, further investigation is required to elucidate the complex interplay between female sex hormones, other metabolic hormones, and energy expenditure.

Considering energy intake as a part of the energy balance equation, we observed higher fat intake during the luteal phase compared to the follicular phase (Figure 1D), which is in line with few previous studies (42, 43). For energy intake, our data indicated it to remain constant or increase from the follicular to the luteal phase, of which the latter agrees with the widely held view that energy intake is higher during the luteal phase compared to the late follicular phase (7,44–46). The hypothesized physiological reason for these variations is that E2 may inhibit appetite in the late follicular phase (47) via a complex process involving hypothalamic E2 neurons and their regulatory role in energy homeostasis (48). Conversely, P4 is suggested to stimulate appetite in the presence of E2 during the luteal phase (47). These changes could also be a response to the possible increase in REE to maintain energy balance (49). However, an increase in energy intake during the luteal phase has not consistently been reported (21,50). It should be emphasized that the control of food intake is influenced by psychological and social factors as well as physiological mechanisms (51), which underlines the uncertainty of evaluating energy intake data.

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In this study, all dietary measures, except protein intake, contributed to the FFM- and FM-adjusted REE in the follicular phase (Table 3). In the long-term, energy intake naturally reflects body size, which explains the relationship between REE and energy intake (8,52). Diet prior to REE measurement is known to influence the results through the increased thermic effect of food (26). However, herein dietary intake was recorded for 3 days after the REE measurement for practical reasons. Nevertheless, the stable RER observed between the follicular and luteal phase indicates a lack of dramatic changes in energy balance and substrate metabolism preceding the measurement (53), which is also supported by the lack of significant changes in energy intake according to the food diaries in this study. Additionally, REE measurement was conducted in a fasted state to mitigate thermic effect of food.

The underlying reason for the associations observed only during the follicular phase, but not luteal phase, is unknown. However, it can be speculated that the energy intake replaced some of the mass-related aspects of REE in the follicular phase. This speculation is supported by our finding that FFM and FM explained a smaller proportion of REE variability in the follicular phase than in the luteal phase (48% vs. 58%). Furthermore, adding energy intake to the model increased the explanatory percentage from 48% to 59% in the follicular phase, similar to the explanatory percentage of FFM and FM (58%) in the luteal phase. This observation implies that energy intake might play a more significant role in REE variability in the follicular phase, providing insight into the complex nature of energy metabolism in women and indicating the need for further investigation.

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Metabolism and energy intake in the active and inactive phases

This study may be the first to examine REE differences between the active and inactive phase of the COC cycle while also reporting hormonal concentrations. In the present study, both endogenous E2 and P4 remained stable during the COC cycle as expected. Our results indicate no evidence of a difference in REE between COC phases (Figure 3), which agrees with previous studies (54,55).

In the present study, only T3 was higher during the inactive phase of the COC cycle (Figure 3A), which contradicts with previous studies that reported stable thyroid hormone levels throughout the COC cycle (19,21). This unexpected finding indicates that further investigation of potential factors contributing this variation in thyroid hormone levels during the COC cycle might be required. However, it is noteworthy that higher T3, T4, and TSH levels have been reported in COC-users compared to non-users (19) as well as at commencement of COC use (56,57). This difference is thought to be related to the increase of thyroxine-binding globulin caused by COCs (56). Nevertheless, higher T3 in the inactive phase of the COC cycle might be attributed to individual variations in thyroid metabolism or other unexplained factors.

- and FM-adjusted REE was not associated with T3 at either phase, but leptin was inversely correlated with REE in the inactive phase while unAG had a negative correlation with REE in the active phase (Table 5). The inability of T3 to explain REE variation in the COC-group raises questions about the influence of exogenous female sex hormones on thyroid hormone metabolism. Furthermore, the underlying physiological mechanisms driving these observed negative relationships between REE and leptin, as well as REE and UnAG, remain

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inconclusive and warrant further investigation. Understanding these associations may serve as a means to optimize metabolic health of women using COCs.

Given that only few studies have addressed within-cycle changes in body composition and dietary intake among COC users, our study is a valuable contribution to this area of research. We observed higher body mass during the inactive phase, while FM, FFM, and dietary intake remained unchanged. Some previous studies have reported that body mass and body composition do not change during the COC cycle (58,59). However, our results align with Ihalainen et al. (21) who found higher body mass during the inactive phase, suggesting that COC use might have a role in body weight fluctuations. Indeed, E2 increases plasma volume, whereas P4 is also known to influence sodium and water regulation (60). Studies examining dietary intake across the COC phases have consistently reported no changes (21,54,61), which is supported by our findings. It can be speculated that in addition to inhibiting fluctuations in female sex hormones, the use of COC might also stabilize the assumed cyclic fluctuations in energy intake.

Strengths and limitations

The major strength of this study was the careful verification of the MC phases. In the NoOC-group, all participants had hormonally indicated ovulations combined with P4 concentrations over 16 nmol/l during the luteal phase, which ensured the desired context for investigating metabolism in two hormonally different environments. Furthermore, we critically assessed the sample included in the analysis, as well as controlled participants' preparation and monitored dietary intake.

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While documenting and standardizing dietary intake prior to REE measurement is known to prevent diet-

-libitum prior to measurements is beneficial for applying the results to real-life conditions. A limitation that may affect interpretation of results in the COC-group was the relatively small sample size along with heterogeneity of COCs with varying amounts of exogenous E2 and progestin components in the different generations of the drugs (62). We acknowledge that temperature range during REE measurement varied between individuals due to seasonal fluctuations. However, the CV of the temperature was small, indicating that temperature likely did not significantly influence the analysis.

We observed a strong ICC for REE in both groups, denoting a high degree of consistency between measurements (Supplemental Table 2, Supplemental Digital Content). Conversely, in the NoOC-group, the weak ICC for RER between two measurements suggests potential disparities in the reliability of these metabolic measures within the sample. It is worth noting that RER is a dynamic parameter sensitive to various factors, such as changes in energy balance (63). In conclusion, the findings of our study should be interpreted considering these limitations.

CONCLUSIONS

In line with previous literature, our study favored approximately 40 kcal/day higher REE during the luteal phase compared to the follicular phase. Additionally, higher levels of T3, leptin, and fat intake during the luteal phase suggest MC-related metabolic fluctuations due to increased female sex hormone levels. In contrast, metabolic and dietary parameters remained

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more stable among COC users, indicating distinct metabolic responses compared to naturally menstruating women. These findings highlight the complex relationships between women's hormonal status and energy balance, emphasizing the need for measurement standardization in research and consideration in designing tailored interventions, such as nutrition plans, for women.

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FIGURE LEGENDS

Figure 1. Changes in T3 (A), n=38, T4 (B), n=38, leptin (C), n=38, and fat intake (D), n=29, at follicular phase (FOL) and luteal phase (LUT). Data are presented as means with standard deviation and individual data points. NoOC. *$p \leq 0.05$, ***$p < 0.001$

Figure 2. Changes in REE in the full sample (A), n=38, and after the secondary analysis (B), n=34, at follicular phase (FOL) and luteal phase (LUT). Data are presented as means with standard deviation and individual data points. NoOC. **$p \leq 0.01$

Figure 3. Changes in T3 (A), n=19, T4 (B), n=19, leptin (C), n=19, REE (D), n=19, and fat intake (E), n=16, at active phase (ACT) and inactive phase (INACT). Data are presented as means with standard deviation and individual data points. COC. ***$p < 0.01$

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SUPPLEMENTAL DIGITAL CONTENT

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Figure 1

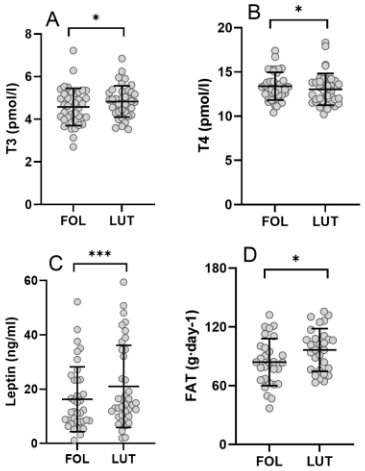

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Figure 2

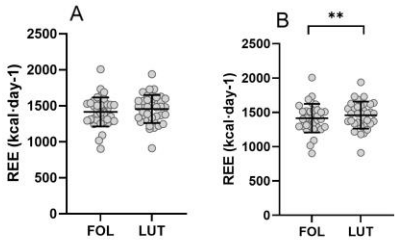

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Figure 3

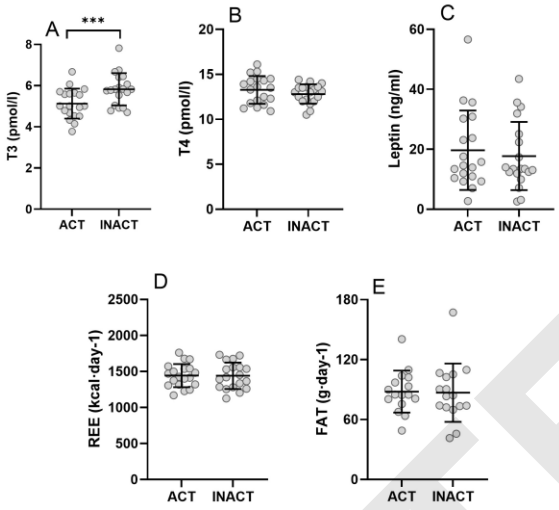

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Table 1. Mean ± SD physical characteristics of the NoOC- (n=38) and COC-group (n=19).

	NoOC-group	**COC-group**
Age (yr)	29.8 ± 3.9	26.1±4.0
Height (m)	1.66 ± 0.06	1.69 ± 0.05
Body mass (kg)	68.3 ± 10.9	68.5 ± 6.7
BMI (kg·m−2)	24.9 ± 4.0	24.1 ± 2.1
Body fat (%)	28.3 ± 8.3	27.7 ± 6.3
Length of MC or COC cycle (days)	27 ± 2	21–24 active + 4–7 inactive

NoOC: naturally menstruating; COC: combined oral contraceptive; BMI: body mass index; COC: combined oral contraceptive. Significant differences bolded ($p \leq 0.05$).

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Table 2. Mean ± SD or median (interquartile range) concentrations of female sex hormones and metabolic hormones, body composition, resting metabolism and dietary measures at follicular (FOL) and luteal (LUT) phases, NoOC.

	FOL	N	LUT	N	P-value	*d*	N
Hormones							
E2 (pmol/l)	117 (76.5–179.3)	38	497.5 (413–667.5)	38	**<0.001*** **	1.83	38
P4 (nmol/l)	1.4 ± 0.7	38	28.9 ± 8.1	38	**<0.001*** **	4.79	38
T3 (pmol/l)	4.6 ± 0.9	38	4.8 ± 0.8	38	**0.05***	0.26	38
T4 (pmol/l)	13.3 (12.4–14.2)	38	12.9 (11.6–13.9)	38	**0.041***	0.21	38
TSH (mIU/l)	1.9 (1.4–2.5)	38	2.0 (1.4–2.9)	38	0.369	0.13	38
Leptin (ng/ml)	11.6 (8.1–23.4)	38	14.7 (10.3–34.6)	38	**<0.001*** **	0.35	38
unAG (pg/ml)	339.5 ± 185.3	37	350.5 ± 208.3	38	0.898	0.06	37
AG (pg/ml)	45.1 (36.4–68.2)	37	43.5 (35.7–59.3)	38	0.624	0.02	37
Body composition							
Body mass (kg)	66.7 (59.4–73.6)	38	66.8 (59.7–73.7)	38	0.734	0.01	38
Fat free mass (kg)	48.6 ± 5.1	38	48.4 ± 5.1	38	0.114	0.05	38
Fat mass (kg)	16.7 (13.9–24.3)	38	18.6 (13.0–24.5)	38	0.196	0.03	38
Body fat (%)	27.8 ± 7.9	38	28.3 ± 8.3	38	0.147	0.06	38
Resting metabolism							
REE (kcal·day^{-1})	1415 ± 201	38	1455 ± 194	38	0.061	0.20	38
RER	0.82 (0.80–0.85)	38	0.84 (0.81–0.88)	38	0.193	0.30	38
Dietary measures							
EI (kcal·day^{-1})	2077 ± 388	32	2198 ± 408	31	0.058	0.31	29
EA (kcal·kg FFM^{-1}·day^{-1})	39.3 ± 6.8	31	42.5 ± 8.0	30	0.055	0.42	27
FAT (g·day^{-1})	83 ± 24	32	94 ± 23	31	**0.027***	0.48	29

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CHO (g·day^{-1})	214 ± 50	32	224 ± 51	31	0.305	0.20	29
PROT (g·day^{-1})	84 (72–103)	32	80 (69–99)	31	0.627	0.15	29

Significance of differences between FOL and LUT is presented in p-values and effect sizes (Cohen's *d*). *p≤0.05 ***p<0.001. Statistically significant p values are bolded. E2: estradiol; P4: progesterone; T3: triiodothyronine; T4: thyroxine; TSH: thyroid-stimulating hormone; unAG: unacylated ghrelin; AG: acylated ghrelin; REE: resting energy expenditure; RER: respiratory exchange ratio; EI: energy intake; EA: energy availability; FAT: fat intake; CHO: carbohydrate intake; PROT: protein intake. Note: unAG and AG values in FOL were missing from one participant because insufficient blood was drawn to perform all analyses.

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Table 3. The associations of hormone concentrations and dietary measures with resting energy expenditure (REE), adjusted for fat-free mass (FFM) and fat mass (FM), at follicular (FOL) and luteal (LUT) phases, NoOC.

	FOL					LUT				
	B	**95% CI**	**β**	**P-value**	**N**	**B**	**95% CI**	**β**	**P-value**	**N**
Hormones										
E2 (nmol/l)	0.049	-0.20 to 0.30	0.05	0.694	38	-0.16	-0.38 to 0.073	-0.15	0.175	38
P4 (nmol/l)	25.88	-47.55 to 99.32	0.09	0.479	38	-1.69	-0.07 to -0.64	-0.07	0.527	38
T3 (pmol/l)	64.19	9.84 to 118.54	0.28	**0.022**	38	91.91	43.60 to 140.25	0.36	**<0.001**	38
T4 (pmol/l)	16.37	-19.27 to 52.02	0.13	0.357	38	3.04	-23.5 to 29.58	0.03	0.818	38
TSH (mIU/l)	0.68	-54.84 to 56.2	0.003	0.980	38	8.30	-31.94 to 48.54	0.05	0.678	38
Leptin (ng/ml)	1.71	-6.31 to 9.74	0.10	0.667	38	1.41	-5.38 to 8.20	0.11	0.676	38
unAG (pg/ml)	0.21	-0.07 to 0.48	0.19	0.131	37	0.11	-0.11 to 0.32	0.12	0.307	38
AG (pg/ml)	0.12	-0.20 to 0.44	0.10	0.444	37	0.10	-0.14 to 0.34	0.10	0.394	38
Dietary measures										
EI (kcal·day^{-1})	0.17	0.06 to 0.28	0.41	**0.004**	32	0.080	-0.03 to 0.19	0.22	0.148	31
EA (kcal·day^{-1})	0.16	0.03 to 0.30	0.35	**0.019**	31	0.07	-0.05 to 0.19	0.19	0.216	30
FAT (g·day^{-1})	2.16	0.27 to 4.06	0.32	**0.027**	32	1.46	-0.61 to 3.52	0.22	0.159	31
CHO (g·day^{-1})	1.11	0.25 to 2.0	0.35	**0.014**	32	0.26	-0.60 to 1.12	0.09	0.540	31
PROT (g·day^{-1})	0.78	1.03 to 2.59	0.13	0.386	32	1.11	-0.98 to 3.17	0.17	0.287	31

P-values≤0.05 in bold. B: unstandardized coefficient; CI: confidence interval; β: standardized coefficient; E2: estradiol; P4: progesterone; T3: triiodothyronine; T4: thyroxine; TSH: thyroid-stimulating hormone; unAG: unacylated ghrelin; AG: acylated ghrelin; EI: energy intake; EA: energy availability; FAT: fat intake; CHO: carbohydrate intake; PROT: protein intake.

ACCEPTED

Table 4. Mean ± SD or median (interquartile range) concentrations of female sex hormones and metabolic hormones, body composition, resting metabolism and dietary measures at active (ACT) and inactive (INACT) phases, COC.

	ACT	N	INACT	N	P-value	*d*	N
Hormones							
E2 (pmol/l)	72.3 (48.8–186.0)	19	56.9 (27.4–121.0)	19	0.494	1.02	19
P4 (nmol/l)	1.1 ± 0.6	19	1.1 ± 0.7	19	0.594	0.09	19
T3 (pmol/l)	5.1 ± 0.7	19	5.8 ± 0.8	19	**<0.001*** **	0.92	19
T4 (pmol/l)	13.3 ± 1.5	19	12.8 ± 1.1	19	0.100	0.34	19
TSH (mIU/l)	2.9 ± 1.3	19	2.7 ± 1.1	19	0.412	0.20	19
Leptin (ng/ml)	13.9 (9.3–23.8)	19	13.5 (10.0–22.4)	19	0.159	0.13	19
unAG (pg/ml)	489.1 ± 354.3	19	451.9 ± 257.2	19	0.292	0.12	19
AG (pg/ml)	54.5 (41.1–112.0)	19	55.8 (38.7–97.1)	19	0.198	0.05	19
Body composition							
Body mass (kg)	68.1 ± 6.9	19	68.5 ± 6.7	19	**0.037***	0.06	19
Fat free mass (kg)	47.7 (45.6–50.9)	19	48.2 (46.8–50.9)	19	0.308	0.06	19
Fat mass (kg)	19.0 ± 5.4	19	19.2 ± 5.5	19	0.591	0.02	19
Body fat (%)	27.6 ± 6.2	19	27.7 ± 6.3	19	0.901	0.01	19
Resting metabolism							
REE (kcal·day^{-1})	1442 ± 158	19	1440 ± 183	19	0.969	0.01	19
RER	0.86 ± 0.05	19	0.85 ± 0.04	19	0.147	0.36	19
Dietary measures							
EI (kcal·day^{-1})	2158 ± 440	17	2060 ± 346	17	0.626	0.25	16
EA (kcal·kgFFM^{-1}·day^{-1})	40.3 ± 9.6	16	39.1 ± 7.1	15	0.476	0.14	14
FAT (g·day^{-1})	91 ± 23	17	87 ± 28	17	0.885	0.15	16
CHO (g·day^{-1})	230 ± 56	17	211 ± 69	17	0.302	0.31	16

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PROT (g·day^{-1})	97 ± 23	17	96 ± 21	17	0.775	0.05	16

Significance of differences between ACT and INACT is presented in p-values and effect sizes (Cohen's *d*). *p≤0.05 ***p<0.001. Statistically significant p values are bolded. E2: estradiol; P4: progesterone; T3: triiodothyronine; T4: thyroxine; TSH: thyroid-stimulating hormone; unAG: unacylated ghrelin; AG: acylated ghrelin; REE: resting energy expenditure; RER: respiratory exchange ratio; EI: energy intake; EA: energy availability; FAT: fat intake; CHO: carbohydrate intake; PROT: protein intake.

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Table 5. The associations of hormone concentrations and dietary measures with resting energy expenditure (REE), adjusted for fat-free mass (FFM) and fat mass (FM), at active (ACT) and inactive (INACT) phases, COC.

ACT						INACT				
	B	**95% CI**	**β**	**P-value**	**N**	**B**	**95% CI**	**β**	**P-value**	**N**
Hormones										
E2 (nmol/l)	0.05	-0.20 to 0.30	0.05	0.694	19	-0.12	-0.95 to 0.71	-0.048	0.714	19
P4 (nmol/l	29.55	-81.36 to 140.46	0.12	0.578	19	-16.9	-108.2 to 74.22	-0.06	0.697	19
T3 (pmol/l)	61.22	-43.35 to 165.78	0.28	0.231	19	14.5	-83.14 to 112.2	0.06	0.755	19
T4 (pmol/l)	-28.67	-71.77 to 14.42	-0.28	0.77	19	-4.9	-62.56 to 52.76	-0.03	0.859	19
TSH (mIU/l)	23.61	-28.94 to 76.17	0.20	0.353	19	-46.85	-97.82 to 5.0	-0.27	0.069	19
Leptin (ng/ml)	-1.40	-11.47 to 8.65	-0.12	0.770	19	-9.10	-16.87 to -1.33	-0.57	**0.025**	19
unAG (pg/ml)	-0.23	-0.41 to -0.05	-0.52	**0.014**	19	-0.04	-0.28 to 0.2	-0.05	0.753	19
AG (pg/ml)	-0.51	-1.05 to 0.04	-0.41	0.07	19	-0.25	-0.79 to 0.28	-0.18	0.333	19
Dietary measures										
EI (kcal·day^{-1})	0.10	-0.07 to 0.26	0.27	0.228	17	-0.01	0.20 to 0.19	-0.02	0.928	17
EA (kcal·day^{-1})	0.07	-0.13 to 0.28	0.19	0.446	16	-0.08	-0.33 to 0.18	-0.14	0.519	15
FAT (g·day^{-1})	2.21	-0.71 to 5.14	0.33	0.126	17	-0.057	-2.45 to 2.33	-0.01	0.960	17
CHO (g·day^{-1})	1.07	-0.17 to 2.4	0.38	0.084	17	-0.10	-1.05 to 0.85	-0.04	0.823	17
PROT (g·day^{-1})	0.47	-3.16 to 4.1	0.07	0.783	17	1.02	-2.26 to 4.30	0.12	0.513	17

P-values≤0.05 in bold. B: unstandardized coefficient; CI: confidence interval; β: standardized coefficient; E2: estradiol; P4: progesterone; T3: triiodothyronine; T4: thyroxine; TSH: thyroid-stimulating hormone; unAG: unacylated ghrelin; AG: acylated ghrelin; EI: energy intake; EA: energy availability; FAT: fat intake; CHO: carbohydrate intake; PROT: protein intake.

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Supplemental Digital Content 1

Supplemental Table 1. Content of COC pills and brand names used by participants in the COC-group.

Brand name		Progestogen component (generation)	N
Gestinyl	Ethinylestradiol 20 µg	Gestoden 75 µg (3rd)	3
Gestinyl	Ethinylestradiol 30 µg	Gestoden 75 µg (3rd)	2
Microgynon	Ethinylestradiol 0,15 mg	Levonorgestrel 30 µg (2nd)	1
Tasminetta/Yasmin	Ethinylestradiol 0,03 mg	Drospirenone 3 mg (4th)	2
Daisynelle	Ethinylestradiol 20 µg	Desogestrel 150 µg (3rd)	1
Dienorette	Ethinylestradiol 0,03 mg	Dienogest 2 mg (4th)	5
Yasminelle/Dizminelle/Stefaminelle	Ethinylestradiol 0,02 mg	Drospirenone 3 mg (4th)	3
Mercilon	Ethinylestradiol 20 µg	Desogestrel 150 µg (3rd)	1
Levesia	Ethinylestradiol 20 µg	Levonorgestrel 100 µg (2nd)	1

COC: combined oral contraceptive

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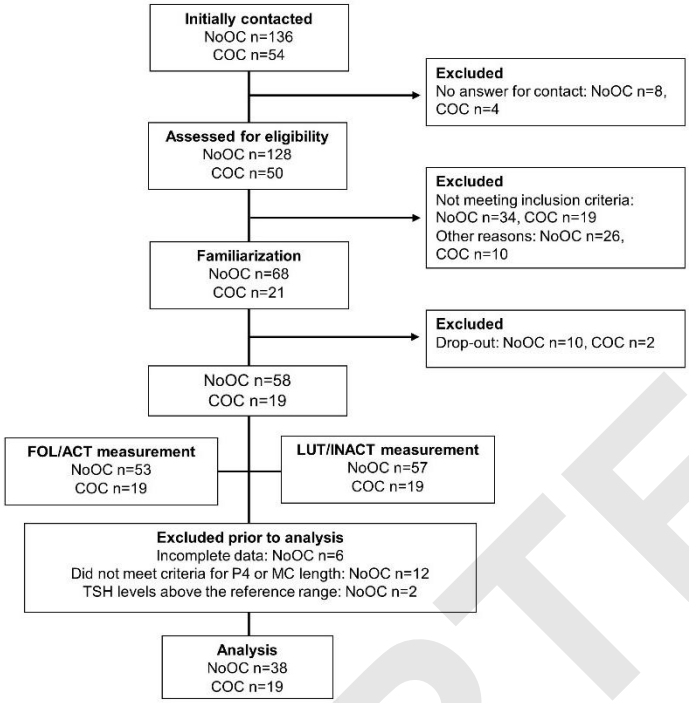
Supplemental Figure 1.



Supplemental Figure 1. Flowchart of participant enrollment through the study. FOL: follicular phase; ACT: active phase; LUT: luteal phase; INACT: inactive phase; P4: progesterone; MC: menstrual cycle; TSH: thyroid-stimulating hormone.

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Supplemental Table 2. Intraclass correlation coefficients (ICC) of REE and RER between two measurements in NoOC- and COC-groups. Results of the secondary analysis in italics.

Variable	Group	ICC (95%CI)		P-value	
REE	NoOC	0.88 (0.76–0.94)	*0.94 (0.87–0.97)*	**<0.001*** **	**<0.001*** **
	COC	0.71 (0.26–0.89)		**0.005***	
RER	NoOC	0.04 (-0.84–0.50)	*0.16 (-0.68–0.58)*	0.451	*0.311*
	COC	0.60 (-0.01–0.85)		**0.027***	

REE: resting energy expenditure; RER: respiratory exchange ratio. *p≤0.05 ***p<0.001. Statistically significant p-values are bolded.

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Supplemental Table 3. The associations of hormone concentrations and dietary measures with resting energy expenditure, adjusted for fat-free mass (FFM) and fat mass (FM), at follicular (FOL) and luteal (LUT) phases. Results of the secondary analysis. NoOC.

	FOL					LUT				
	B	**95% CI**	**β**	**P-value**	**n**	**B**	**95% CI**	**β**	**P-value**	**n**
Hormones										
E2 (nmol/l)	0.04	-0.21 to 0.28	0.04	0.757	34	-0.07	-0.35 to 0.21	-0.07	0.608	34
P4 (nmol/l)	22.12	-62.55 to 106.78	0.07	0.598	34	0.04	-0.21 to 0.28	0.04	0.757	34
T3 (pmol/l)	81.99	31.58 to 132.41	0.35	**0.002**	34	95.56	50.16 to 140.96	0.38	**<0.001**	34
T4 (pmol/l)	22.31	-12.52 to 57.14	0.17	0.201	34	1.88	-23.78 to 27.53	0.018	0.882	34
TSH (mIU/l)	-14.98	-74.38 to 44.43	-0.06	0.610	34	39.22	-7.11 to 85.56	0.18	0.094	34
Leptin (ng/ml)	3.32	-4.84 to 11.47	0.19	0.413	34	2.42	-4.31 to 9.15	0.18	0.468	34
unAG (pg/ml)	0.12	-0.16 to 0.41	0.11	0.387	33	0.14	-0.07 to 0.36	0.15	0.182	34
AG (pg/ml)	0.11	-0.21 to 0.42	0.09	0.494	33	0.08	-0.15 to 0.31	0.08	0.486	34
Dietary measures										
EI (kcal·day^{-1})	0.21	0.10 to 0.32	0.49	**<0.001**	29	0.08	-0.03 to 0.18	0.22	0.133	27
EA (kcal·day^{-1})	0.21	0.07 to 0.35	0.42	**0.005**	27	0.08	-0.03 to 0.19	0.22	0.132	26
FAT (g·day^{-1})	2.52	0.65 to 4.39	0.37	**0.010**	29	1.89	-0.12 to 3.90	0.29	0.065	27
CHO (g·day^{-1})	1.30	0.43 to 2.17	0.40	**0.005**	29	0.34	-0.46 to 1.14	0.12	0.394	27
PROT (g·day^{-1})	0.53	-1.43 to 2.50	0.08	0.582	29	0.41	-1.64 to 2.46	0.07	0.681	27

P-values <0.05 in bold. B: unstandardized coefficient; CI: confidence interval; β: standardized coefficient; E2: estradiol; P4: progesterone; T3: triiodothyronine; T4: thyroxine; TSH: thyroid-stimulating hormone; unAG; unacylated ghrelin; AG: acylated ghrelin; EI: energy intake, EA: energy availability; FAT: fat intake; CHO: carbohydrate intake; PROT: protein intake.

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