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Oral Contraceptive Pill Alters Acute Dietary Protein-Induced Thermogenesis in Young Women

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Objective: There is much interest in the role of dietary protein for weight control. However, there remains a need to characterize individual determinants of the thermogenic effects of protein. This study aimed to investigate the influence of menstrual cycle phase and the combined, monophasic oral contraceptive pill on the thermogenic response to a standardized high-protein (HP) versus normal-protein (NP) meal.
Methods: Following an overnight fast, resting energy expenditure (EE) was measured in 16 healthy young women (8 taking and 8 not taking the pill) and 8 men for 30 minutes pre ingestion and 3 hours post ingestion of a NP (11%) or HP (24%) meal.
Results: There was no effect of menstrual phase or contraceptive pill use on fasting EE or NP response. However, HP increased EE significantly more than NP in women not taking the oral contraceptive pill and in men, but not in women taking the pill.
Conclusions: This study shows an absence of the greater thermic effect of HP versus NP in women taking the oral contraceptive pill and has important implications regarding the effectiveness of HP for body weight regulation in women. With current obesity treatment/prevention strategies remaining largely ineffective, understanding the relationship between oral contraceptive pill use and protein-induced thermogenesis may enable the successful recalibration of existing dietary recommendations.

Introduction

Although men and women share the same environmental risk factors, the prevalence of obesity in women now exceeds that of men in all World Bank regions (1,2). The concomitant surge in obesity-related cardiometabolic disease (type 2 diabetes, cardiovascular disease) in women of childbearing age is a growing public health burden, not only for women's health but also for the health of future children through epigenetic modification. However, existing diet intervention strategies make little or no distinction between the sexes. This "one-size-fits-all" approach is ineffective, with women generally losing less weight during such interventions than men (3).

Successful approaches to weight control should elicit effects on both sides of the energy balance equation, i.e., energy intake and energy expenditure (EE). For this reason, there has been much interest in the role of dietary protein, which has been demonstrated to favorably affect satiety (4), thermogenesis (5,6), body weight (7), and body composition (8). However, there have been a number of discrepancies in study findings (9), and as such, there is a need to characterize individual determinants of the thermogenic and satiety effects of dietary protein.

Toward this end, the goal of the present study was to determine the influence of menstrual cycle phase and oral contraceptive use on the thermogenic response to a standardized high-protein (HP) meal in healthy young women.

Methods

Participant recruitment and screening

Sixteen healthy young women of European descent participated in the present study, with a mean (± SEM) age of 23 ± 1 years, weight of 61.2 ± 2.7 kg, fat-free mass (FFM) of 46.3 ± 0.9 kg, and BMI of 22.0 ± 0.7 kg/m^2. A group of eight healthy young men (age 23 ± 1 years, weight 67.3 ± 2.2 kg, FFM 61.2 ± 2.5 kg, and BMI 21.2 ± 0.6 kg/m^2) was recruited to act as a time control. Prior to testing, participants visited the laboratory in order to complete a questionnaire regarding their lifestyle and medical history and to familiarize themselves with the experimental procedure and equipment. All participants were weight stable. Smokers, claustrophobic individuals, individuals taking medication other than the oral contraceptive pill, and those with any metabolic disease or food intolerance were excluded. The female participants were split evenly between eight women not taking the oral contraceptive pill (age 24 ± 1 years,

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weight 60.6 ± 2.1 kg, FFM 45.8 ± 1.0 kg, and BMI 22.0 ± 0.6 kg/m^2) and eight women regularly taking a combined, monophasic oral contraceptive containing 20 to 35 μg (median: 25 μg, mode: 20 μg) of ethinyl estradiol (age 23 ± 1 years, weight 61.8 ± 5.1 kg, FFM 46.8 ± 1.5 kg, and BMI 21.9 ± 1.2 kg/m^2). Each participant completed four separate experimental test visits. In women, two of these visits occurred during the follicular phase of the menstrual cycle (approximate cycle day 5-12, with cycle day 1 determined by self-report), and the other two visits occurred during the luteal phase (approximate cycle day 19-26). This timing was matched in the men, with the second two visits (control phase 2 [C2]) occurring 2 weeks after the first two visits (control phase 1 [C1]). In both men and women, experimental visits were separated by an interval of at least 2 days. The study complied with the Declaration of Helsinki and was approved by the state ethical review board; all participants gave written consent.

Experimental design

On the day of testing, participants arrived at the laboratory at 8:00 AM following a 12-hour overnight fast. Body composition was determined using multifrequency bioimpedance analysis (InBody 720, Biospace Co., Ltd., Seoul, Korea). EE was measured using a ventilated hood system (Quark CPET, COSMED, Rome, Italy). Briefly, participants were seated comfortably in a car seat adapted for calorimetric monitoring (10-12), with metabolic measurement conducted for at least 30 minutes, after 15 minutes of rest. The ventilated hood was then removed while the subject ingested one of the two test meals described below within a 10-minute period. The ventilated hood was replaced, and calorimetric monitoring was continued for a further 180 minutes. Participants were permitted to watch a calm movie or a documentary during the metabolic measurements.

Test meals

Two isocaloric 590 kcal test meals were used in this study, differing in protein as a percentage of total energy. The composition of these meals is shown in Table 1. Each meal consisted of a simple breakfast meal base (two slices of toast and a 200 mL milk-based drink [Nestlé Resource, Nestlé Health Science, Florham Park, New Jersey]) to which protein powder (Protifar, Nutricia, Schiphol, the Netherlands), butter, and jam were added to adjust the protein content as necessary while maintaining the ratio of carbohydrate to fat as a constant at 1.41. Both test meals were given during both the follicular and luteal phases in women and during control phases C1 and C2 in men. The order of the test meals was randomized between normal-protein (NP) and HP meals, and the participants were blinded to the meal order.

TABLE 1 Meal composition

	Energy (kcal)				Energy (%)			Ratio
	Protein	CHO	Fat	Total	Protein	CHO	Fat	CHO:Fat
NP	62	308	219	589	11	52	37	1.41
HP	142	261	186	589	24	44	32	1.41

CHO, carbohydrate; HP, high protein; NP, normal protein.

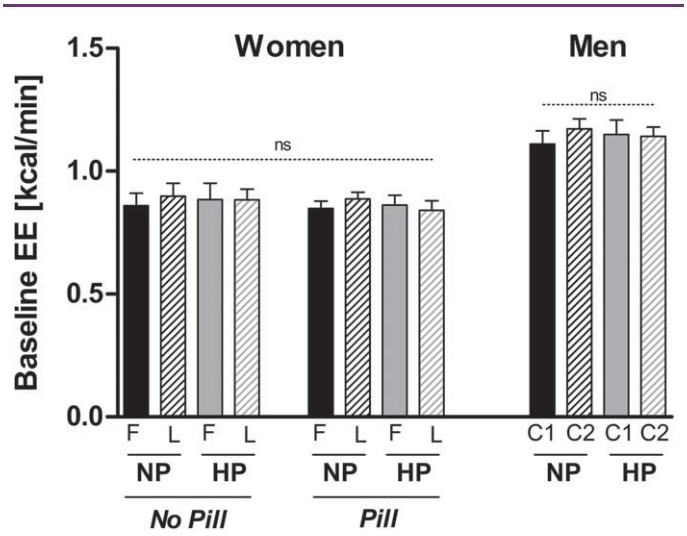

Figure 1 Baseline (fasted) resting energy expenditure (EE). EE according to combined, monophasic oral contraceptive pill use ($n = 8$ per group) and menstrual cycle phase (follicular [F] vs. luteal [L]) in young women. Values for men are shown on the right, with C1 referring to the first control phase and C2 to the second control phase (i.e., the retest after a 2-week interval). Mean ± SEM for normal protein (NP): baseline EE prior to ingestion of a NP meal; mean ± SEM for high protein (HP): baseline prior to the ingestion of a HP meal; ns: no statistically significant difference between NP vs. HP, no pill vs. pill, follicular vs. luteal, or C1 vs. C2.

Data and statistical analysis

Minute-by-minute values of the metabolic recordings were averaged in 30-minute intervals for both the baseline and 3-hour postprandial period. All data are presented as mean ± SEM. The statistical treatment of data, by repeated-measures analysis of variance (ANOVA) or three-factor ANOVA (pill, menstrual cycle phase, meal protein level) followed by post hoc multiple comparison tests, was performed using the computer software Statistix 8 (Analytical Software, St Paul, Minnesota).

Results

As shown in Figure 1, there was no effect of either menstrual cycle phase ($P = 0.6$) or oral contraceptive use ($P = 0.6$) on baseline (fasted) resting EE in women, and there was no effect of time on baseline (fasted) resting EE in men (i.e., no difference between control phases C1 and C2; $P = 0.6$).

Following ingestion of the NP test meal, EE increased significantly from baseline ($P < 0.001$) in women both taking and not taking the oral contraceptive pill and in men, and there was no effect of menstrual cycle phase in either group of women ($P = 0.13$; Figure 2) and no time effect in men (i.e., control phase C1 vs. control phase C2; $P = 0.4$).

However, while ingestion of the HP test meal further increased resting (postprandial) EE beyond the level of the NP diet in women not taking the oral contraceptive pill ($P < 0.001$; Figure 2A) and in men ($P < 0.001$; Figure 2C), no such increase in EE was observed in women taking the pill ($P = 0.14$; Figure 2B). This lack of potentiation in EE can also be observed in the calculated 3-hour thermic effect of each test meal (Figure 2D-2F). A three-factor ANOVA, with pill, meal protein level, and menstrual cycle phase as main

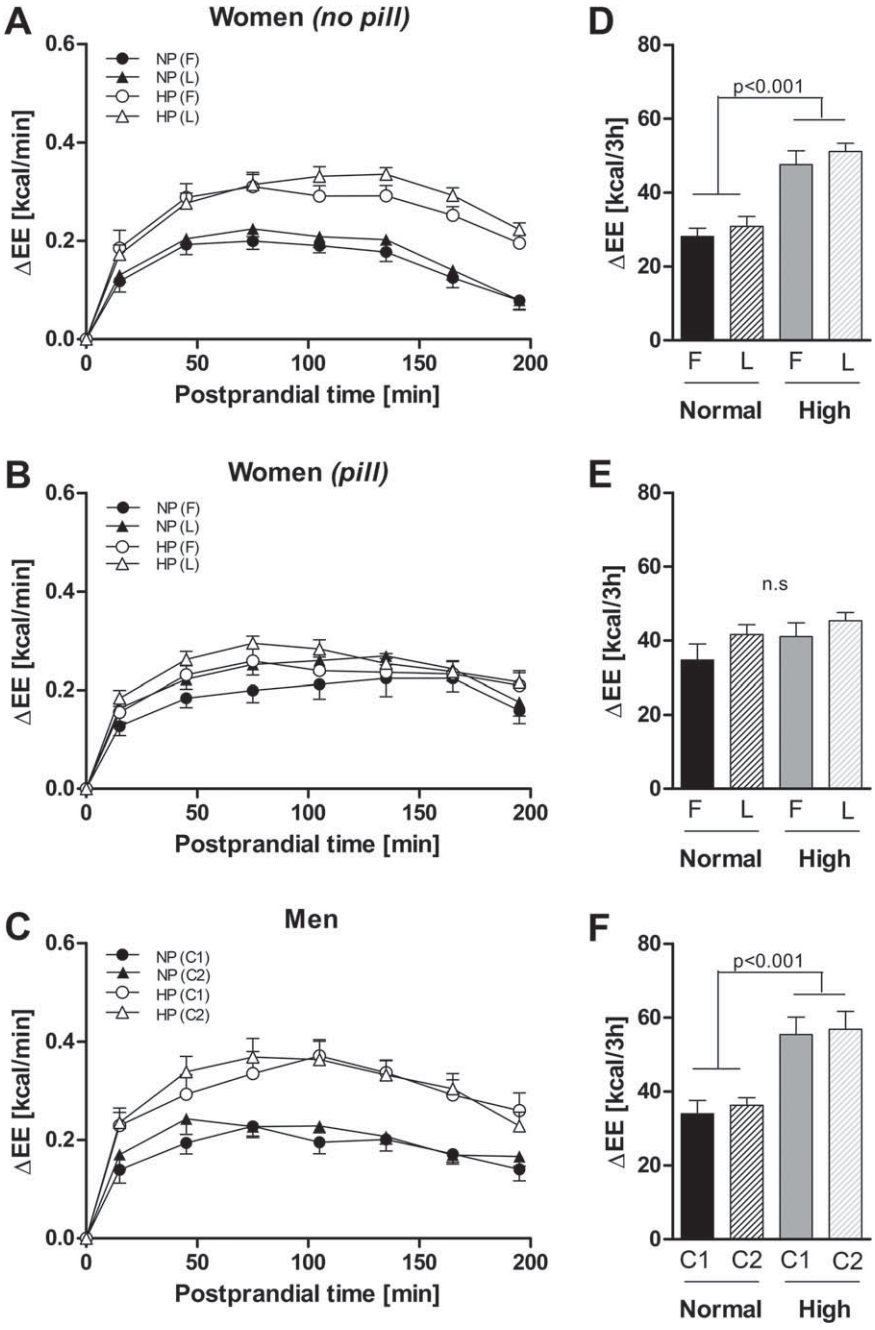

Figure 2 Change in resting energy expenditure (EE) following ingestion of an isocaloric meal containing either normal protein (NP; 11% of total energy) or high protein (HP; 24% of total energy). (**A**) Women not taking the combined, monophasic oral contraceptive pill. Mean ± SEM; repeated-measures ANOVA (effect of meal): $P < 0.001$; F, follicular phase; L, luteal phase. (**B**) Women regularly taking the combined oral contraceptive pill. Mean ± SEM; repeated-measures ANOVA (effect of meal): $P = 0.14$. (**C**) Men. Mean ± SEM; repeated-measures ANOVA (effect of meal): $P < 0.001$; control phase 1, C1; control phase 2, C2. (**D-F**) Area-under-curve (i.e., thermic effect [kilocalories per 3 hours]); mean ± SEM; statistical significance of NP vs. HP meal (by two-way ANOVA) indicated on graph; ns, not significant.

factors, revealed a significant pill and meal protein level interaction ($P < 0.005$). A subgroup analysis of women taking the pill (i.e., women taking 20 μg/d [$n = 4$] compared to those taking 30-35 μg/d [$n = 4$]) found no relationship between ethinyl estradiol dosage and the EE response to the NP versus HP meal in either cycle phase.

Discussion

Young women, particularly those of childbearing age, are developing obesity and its related comorbidities at a rapidly increasing rate compared with men of the same age (see, for example, Ford, Giles, and

Mokdad (1)). Furthermore, it appears that current diet intervention strategies to treat and prevent obesity are largely ineffective (13), particularly in women (3). In this context, there is a considerable need to investigate the sources of interindividual variability in our metabolic responses to dietary modification. Dietary protein may provide an important tool to facilitate weight control through increased satiety and thermogenesis. However, in the present study, we observed an abolishment of the greater thermic effect of a HP versus NP meal in women taking the combined, monophasic oral contraceptive pill.

As no effect of menstrual cycle phase on the acute thermic response to dietary protein was observed, no significant differences were found in body composition between women taking and women not taking the oral contraceptive pill, and as women not taking the oral contraceptive pill responded comparably to men, these results indicated an effect of exogenous rather than endogenous sex hormones (estrogen and progesterone). However, whether this effect resides in the digestive and absorptive processes or in the protein metabolism itself (e.g., the rate of protein turnover) remains to be determined. The combined oral contraceptive pill has previously been associated with an increased gut transit time (14), decreased absorption of a variety of vitamins and minerals (15,16), and decreased serum albumin levels (15). It has been found, as well, to increase both endogenous creatinine clearance and the nitrogen excretion rate/daily protein intake ratio (17) and to inhibit myofibrillar protein synthesis (18).

Regardless of the underlying mechanism(s), given that ∼9% of the world's women aged between 15 and 49 who are married or living with a partner are using the contraceptive pill (and given usage as high as 37.5% in Western Europe) (19), this finding has important implications for the effective use of HP for body weight regulation in women. With current "one-size-fits-all" obesity treatment and prevention strategies being generally ineffective, understanding the relationship between sex hormone status and our metabolic responses to dietary factors, such as protein intake, is key to enabling the successful recalibration of existing diet/lifestyle recommendations.

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