

Effects of caloric intake timing on insulin resistance and hyperandrogenism in lean women with polycystic ovary syndrome

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Abstract

In women with PCOS (polycystic ovary syndrome), hyperinsulinaemia stimulates ovarian cytochrome P450c17 α activity that, in turn, stimulates ovarian androgen production. Our objective was to compare whether timed caloric intake differentially influences insulin resistance and hyperandrogenism in lean PCOS women. A total of 60 lean PCOS women [BMI (body mass index), 23.7 ± 0.2 kg/m²] were randomized into two isocaloric (~1800 kcal; where 1 kcal \approx 4.184 J) maintenance diets with different meal timing distribution: a BF (breakfast diet) (980 kcal breakfast, 640 kcal lunch and 190 kcal dinner) or a D (dinner diet) group (190 kcal breakfast, 640 kcal lunch and 980 kcal dinner) for 90 days. In the BF group, a significant decrease was observed in both AUC_{glucose} (glucose area under the curve) and AUC_{insulin} (insulin area under the curve) by 7 and 54% respectively. In the BF group, free testosterone decreased by 50% and SHBG (sex hormone-binding globulin) increased by 105%. GnRH (gonadotropin-releasing hormone)-stimulated peak serum 17OHP (17 α -hydroxyprogesterone) decreased by 39%. No change in these parameters was observed in the D group. In addition, women in the BF group had an increased ovulation rate. In lean PCOS women, a high caloric intake at breakfast with reduced intake at dinner results in improved insulin sensitivity indices and reduced cytochrome P450c17 α activity, which ameliorates hyperandrogenism and improves ovulation rate. Meal timing and distribution should be considered as a therapeutic option for women with PCOS.

Key words: breakfast, hyperandrogenism, leuprolide, oral glucose tolerance test, polycystic ovary syndrome (PCOS)

INTRODUCTION

PCOS (polycystic ovary syndrome) affects 6–10% of women of reproductive age and is the most common cause of infertility [1]. PCOS is typified by chronic anovulation and hyperandrogenism. Regardless of body mass, the majority of obese as well as lean PCOS women have hyperinsulinaemia and insulin resistance, which play a central role in the pathogenesis by both stimulating ovarian androgen production [2–9] and decreasing serum SHBG (sex hormone-binding globulin) concentrations [10,11], leading to increased levels of circulating free testosterone.

Women with PCOS have increased ovarian cytochrome P450c17 α activity, as evidenced by an elevated serum 17OHP (17 α -hydroxyprogesterone) response to stimulation by GnRH (gonadotropin-releasing hormone) agonists [12]. Ovarian cyto-

chrome P450c17 α appears to be stimulated by insulin in PCOS as reducing insulin release with metformin [8], rosiglitazone [13] or weight loss [9] ameliorates ovarian P450c17 α activity resulting, in turn, in reduced free serum testosterone, improved menstrual cyclicity and increased ovulation [9,14,15]. However, interventions, such as weight loss and rosiglitazone treatment, are not good therapeutic options for lean PCOS women.

Previously, we have shown that meal timing and feeding schedule exert strong entraining effects on peripheral oscillators [16,17]. In addition, meal timing has crucial implications for mass, appetite and insulin resistance [18–23]. Moreover, circadian disruption in hormone secretion has been reported in PCOS patients [24,25]. Thus resetting the circadian clock by scheduled meals and, as a result, ameliorating insulin sensitivity and hormone secretion may have beneficial effects in PCOS patients.

Abbreviations: AUC, area under the curve; BAI, body adiposity index; BF group, breakfast diet group; BMI, body mass index; D group, dinner diet group; DHEA-S, dehydroepiandrosterone sulfate; FAI, free androgen index; GnRH, gonadotropin-releasing hormone; HOMA-B, homeostasis model assessment index of β -cell function; HOMA-IR, homeostasis model assessment index of insulin resistance; ISI, insulin sensitivity index; IU, international units; LS, least-squares; MANOVA, multivariate ANOVA; OGTT, oral glucose tolerance test; SHBG, sex hormone-binding globulin; 17OHP, 17 α -hydroxyprogesterone; PCOS, polycystic ovary syndrome.

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Recently, we have found that high-caloric intake at breakfast led to greater weight loss, improved glucose metabolism and insulin sensitivity indices in obese women with metabolic syndrome than high-calorie dinner [26]. We chose to evaluate the effect of timing of caloric intake (increased caloric intake at breakfast versus dinner) on glucose metabolism and insulin sensitivity indices without the confounding effect of weight loss in lean PCOS patients. Thus our objective was to test whether a scheduled diet with high-calorie breakfast, by improving insulin sensitivity, will reduce ovarian cytochrome P450c17 α hyperactivity and hyperandrogenism and will enhance ovulation in lean women with PCOS compared with an isocaloric diet with high-calorie dinner.

MATERIALS AND METHODS

Subjects

Women were recruited from outpatient clinics by means of personal interview or advertising. Three nurses at the clinical unit were assigned to randomly enroll participants to interventions using a single allocation ratio. The study initially included a total of 60 women with PCOS (25–39 years of age, lean [BMI (body mass index) $<23.7 \pm 0.2 \text{ kg/m}^2$]. Women with a BMI $>24.9 \text{ kg/m}^2$ were excluded. PCOS was defined according to the Rotterdam criteria [27] which include the presence of two or more of the following features: chronic oligoovulation or anovulation (fewer than six menstrual periods in the previous year), androgen excess (serum total testosterone $>70 \text{ ng/dl}$), and polycystic ovaries. All women had normal serum prolactin and thyroid functions. Late-onset adrenal hyperplasia was excluded by a morning serum 17OHP level below $<200 \text{ ng/dl}$ (6 nmol/l). All women had ovarian ultrasonic findings consistent with the diagnosis of PCOS [28]. None had taken any insulin sensitizers, i.e. metformin, contraceptives, steroids or any medications known to affect glucose, insulin or reproductive hormones for at least 6 months, and none had diabetes mellitus. Individuals who were dieting, using medications affecting body mass or who had experienced a change in mass $>4.5 \text{ kg}$ or a change in physical activity within the 6 months preceding the study onset were excluded. Most women were sedentary at baseline and were asked to maintain their usual physical activity levels and to refrain from drinking more than two standard glasses of alcohol per week throughout the study. The protocol and potential risks and benefits of the study were fully explained to each subject. The protocol was approved by the Institutional Helsinki Ethics Committee and written informed consent was obtained from each subject before entry into the study (ClinicalTrials.gov Identifier no. NCT01711476).

Study design

We used a randomized open-label parallel-arm study design in which patients received dietary advice to one of two isocaloric maintenance diets of $\sim 1800 \text{ kcal}$ ($1 \text{ kcal} \approx 4.184 \text{ J}$) with the same composition but different meal timing during 12 weeks. Women were studied during the follicular phase of the menstrual cycle, as documented by serum progesterone levels below $<2 \text{ ng/ml}$ (6.4 nmol/l). On day 1, after a 12-h overnight fast, mass and height

were measured and waist/hip ratio and BAI [body adiposity index: $\text{hip (cm)/height (m)}^{1.5} - 18$] [29] were calculated. Blood samples were drawn at 08:30, 08:45 and 09:00 h and were pooled for measurement of insulin, glucose, steroid hormones and SHBG. At 09:00 h, 75 g of dextrose was given orally and blood samples were collected for determinations of serum glucose and insulin concentrations after 30, 60 and 120 min. On day 2, the women ate breakfast at 09:00 h and then fasted until 14:00 h, when a leuprolide stimulation test was performed.

Women were then placed for 12 weeks on two isocaloric maintenance diets consisting of $1800 \pm 25 \text{ kcal}$ daily based on the meal-planning exchange list of the American Diabetes Association. Total daily intake consisted of approximately 124 g of carbohydrate, 191 g of protein and 62 g of fat. The two meal plans were either high-calorie BF (breakfast diet) or high-calorie D (dinner diet) with a total energy content of $1800 \pm 25 \text{ kcal}$ for the whole day of identical macronutrient content and composition. The energy of the BF meal plan was: a large meal (980 kcal, 54%) at breakfast, medium-sized meal (640 kcal, 35%) at lunch and a small meal (190 kcal, 11%) at dinner. This was reversed in the D meal plan, i.e. a small meal at breakfast and a large meal at dinner. The subjects were asked to eat breakfast between 06:00 and 09:00 h, lunch between 12:00 h and 15:00 h, and dinner between 18:00 and 21:00h.

Serum progesterone was measured weekly with a compliance rate of 100%, and menstrual bleeding was determined monthly. Ovulation was presumed to have occurred if serum progesterone were $>2 \text{ ng/ml}$ and was followed by menstrual bleeding within 2 weeks. The women were asked to abstain from sexual intercourse or to use a barrier method during the study. The women returned for the second study after 12 weeks, and after the follicular phase of the menstrual period had been confirmed by low serum progesterone levels, all tests performed at baseline were repeated. If at the end of the study progesterone levels were $>2 \text{ ng/ml}$, all tests were postponed to after menstruation when serum progesterone values were $<2 \text{ ng/ml}$.

Dietary assessment and compliance

At baseline and every 2 weeks, participants kept a detailed, 3-day diet record, which was reviewed by a registered dietitian and analysed with the participant to facilitate understanding and adherence. Compliance assessment was based on subject adherence to dietary instruction as indicated by the assigned meal plan. Non-compliance was defined as a deviation of 10% or more from the recommended energy intake. Thus, for a diet of $\sim 1800 \text{ kcal/day}$, when energy intake on a given day exceeded 1980 kcal, a non-compliance event was recorded. During a 12-week intervention, a given individual could conceivably contribute approximately 84 non-compliance events. In each of the diets, the number of days for which participants were non-compliant, were divided by 7 during which participants were in the programme, yielding a weekly percentage of non-compliance. Those participants with weekly non-compliance equal or above 42.9% (non-compliance of >3 days per week) were withdrawn from the study. Only data from patients who completed the intervention were included at baseline. Dropout rate was 7% (2 out of 29) in the BF group and 10.3% (3 out of 31) in the D group.

Clinical and anthropometric measurements

Body mass, blood pressure and waist circumference were recorded every 2 weeks. Body mass was measured while subjects were wearing light clothing and no shoes by using a scale model Detecto Physician Beam Scale (HOSPEQ). Waist circumference was measured according to the guidelines of the National Heart, Lung and Blood Institute (NIH publication no. 00-4084). To measure waist circumference, a tape measure was placed in a horizontal plane around the abdomen at the level of the iliac crest. The measurement was made at the end of a normal expiration. Blood pressure was measured with the use of an automatic blood pressure monitor (Omron Healthcare). Participants remained comfortably seated with their legs uncrossed for ≥ 5 min before blood pressure was measured. Three blood pressure measurements were taken ≥ 1 min apart, and the second and third readings were averaged.

Biochemical blood analyses

Blood sampling was conducted during the follicular phase or at any time in anovulatory women with progesterone levels < 2 ng/ml. Serum was separated by centrifugation for 15 min at 1465 g at 4°C, divided into aliquots and stored at -80°C until further analysis. To avoid inter-assay variations, all samples were analysed in duplicates in a single assay for each hormone. Serum glucose was determined by the glucose oxidase method (Beckman Glucose Analyser). Serum insulin was determined by a double antibody RIA (CIS Bio International). Serum-free testosterone and the total testosterone concentrations were measured by the Coat-A-Count Free and Total-testosterone kit (Diagnostic Products). Serum SHBG concentrations were measured by two solid-phase sandwich immunoradiometric assays (Diagnostic Products). Progesterone and androstenedione were measured by immunoluminometric assay (Immulite platform; Siemens DPC). 17β -Oestradiol, DHEA-S (dehydroepiandrosterone sulfate) and 17OHP were measured by RIA (Diagnostic Systems Laboratories). HOMA-IR (homoeostasis model assessment index of insulin resistance) and HOMA-B (homoeostasis model assessment index of β -cell function) were calculated using the following equations [30]:

$$\text{HOMA-IR} = \text{fasting serum insulin } [\mu\text{-IU (international units)/ml}] \times \text{fasting serum glucose (mmol/l)/22.5} \quad (1)$$

$$\text{HOMA-B} = 20 \times \text{fasting serum insulin } (\mu\text{-IU/ml}) / \text{fasting glucose (mmol/l)} - 3.5 \quad (2)$$

ISI (insulin sensitivity index) was calculated using the following equation [31]:

$$\text{ISI} = 10000 / \sqrt{[\text{fasting glucose (mg/dl)} \times \text{fasting insulin } (\mu\text{-IU/ml})] \times [\text{mean glucose (mg/dl)} \times \text{mean insulin } (\mu\text{-IU/ml})]} \quad (3)$$

FAI (free androgen index) was calculated using the equation:

$$\text{FAI} = [\text{testosterone (nmol/l)/SHBG (nmol/l)}] \times 100 \quad (4)$$

Leuprolide (GnRH agonist) stimulation test

After base-line blood samples were obtained at 14:00 h on day 2, leuprolide (10 $\mu\text{g/kg}$ of body mass; Lupron; Abbott Laboratories) was administered subcutaneously. Blood samples for the measurement of serum 17OHP were collected immediately before and 16, 20 and 24 h after leuprolide was administered. The women ate an evening meal but fasted thereafter until the completion of the test. The serum concentration of 17OHP measured immediately before the administration of leuprolide was considered the basal value, and the highest serum concentration of 17OHP that was measured after the administration of leuprolide was considered the peak value.

Sample size and power analysis

A sample size of 30 participants was planned (15 in each treatment group), which provided 80% power to detect a true ($P < 0.05$) between-group difference in insulin levels of 5 ± 6.5 $\mu\text{-IU/ml}$ at the end of follow-up. An additional 30 subjects were recruited to cover drop outs, which were predicted to reach approximately 50% based on diet study drop-out rates in the literature.

Statistical analysis

All results are expressed as means \pm S.E.M. For time series, a two-way ANOVA (time \times diet) was performed and a least-significant difference Student's *t* test post-hoc analysis was used for comparison between the diet at each time-point. For the comparison of end point (day 90) to baseline (day 0) parameters within a diet, a MANOVA (multivariate ANOVA) for repeated measurements was performed assessing between and within subject effects for diet and time. LS (least-squares) Tukey–Kramer post-hoc analysis was performed for multiple comparisons. Two-way tables were used to compare categorical data and the statistical significance of differences was assessed by the χ^2 test. A *P* value ≤ 0.05 was considered statistically significant. Statistical analysis was performed with JMP software (version 9; SAS Institute).

RESULTS

Patient compliance and dispensation

A total of 60 lean (BMI, 23.7 kg/m^2) women with PCOS were randomized to two meal plans (~ 1800 kcal) during 90 days. A total of 29 women were assigned to the BF meal plan and 31 women were assigned to the D meal plan. Both meal plans had the same composition, but differed in energy intake distribution throughout the day. This approach decreased the variation because of changes in food types. In the BF group, 25 completed the study, two dropped out without providing follow-up data and two were eliminated because of non-compliance with the diet. In the D group, 26 completed the study, three dropped out and two were non-compliant.

Anthropometric measurements

At baseline, the women in the BF and D groups did not differ significantly in age, BMI, waist/hip ratio and body adiposity

Table 1 Characteristics of PCOS women at baseline and after 90 days of BF or D diet

Results are means \pm S.E.M. *MANOVA for diet, time and diet by time; †BF endpoint (day 90) compared with baseline (day 0) (post-hoc LSMMeans Tukey–Kramer); ‡D end point (day 90) compared with baseline (day 0) (post-hoc LSMMeans Tukey–Kramer); §BF compared with D at baseline (day 0) (post-hoc LSMMeans Tukey–Kramer); ||BF compared with D at end point (day 90) (post-hoc LSMMeans Tukey–Kramer).

Parameter	Group				BF compared with D				Effect P value*				
	BF		D		Baseline		Day 90		Between subjects		Within subjects		Diet
	Baseline (n=25)	Day 90 (n=25)	Baseline (n=26)	Day 90 (n=26)	%	Change value†	P	%	Change value‡	P	%	Change value	
Age	31.3 \pm 0.8	32.0 \pm 0.8	32.6 \pm 0.7	32.4 \pm 0.8	2	0.94	0.99	-1	0.63	0.97	0.69	1	1
Anthropometric measurement													
Mass (kg)	62.1 \pm 0.5	61.5 \pm 0.7	61.9 \pm 0.6	62.2 \pm 0.6	-1	0.90	0.98	0	0.99	0.84	0.89	0.34	0.009
BMI	23.8 \pm 0.2	23.6 \pm 0.2	23.6 \pm 0.2	23.6 \pm 0.1	-1	0.79	1	1	0.74	0.99	0.30	0.31	0.008
Waist (cm)	80.7 \pm 1.1	79.3 \pm 1.2	80.1 \pm 0.7	80.9 \pm 0.8	-2	0.71	0.93	1	0.96	0.64	0.87	0.59	<0.0001
Hip (cm)	101.1 \pm 1.1	100.8 \pm 1.2	100.2 \pm 0.7	100.2 \pm 0.8	0	0.99	1	1	0.89	0.97	0.62	0.02	0.66
Waist/hip ratio	0.8 \pm 0.01	0.8 \pm 0.01	0.8 \pm 0.01	0.8 \pm 0.01	0	0.87	0.95	0	0.99	0.5	0.64	0.14	<0.0001
BAI	31.3 \pm 0.5	31.1 \pm 0.5	30.6 \pm 0.4	30.4 \pm 0.5	-1	0.99	0.99	2	0.74	0.74	0.29	0.024	0.66

(Table 1). BMI, waist circumference and adiposity, as determined by BAI, did not change significantly during 90 days of intervention in the BF or D groups and there was no significance of either time or diet ($P > 0.05$, MANOVA) (Table 1).

Fasting serum glucose and insulin

At baseline, both groups were similar in serum glucose and serum insulin concentrations (Table 2). After 90 days, there was a significant effect of the diet among the subjects ($P < 0.05$, MANOVA), while there was a significant effect of time within each subject ($P < 0.05$, MANOVA) and a significant cross-reaction of the two parameters ($P < 0.05$, MANOVA). After 90 days, mean serum fasting glucose and insulin concentrations of the BF group decreased by 8% (from 89.1 ± 1 to 81.8 ± 0.9 mg/dl) ($P < 0.0001$, post-hoc LSMMeans Tukey–Kramer) and 53% (from 14.3 ± 0.9 to 6.7 ± 0.3 μ -IU/ml) ($P < 0.0001$, post-hoc LSMMeans Tukey–Kramer) respectively, whereas, in the D group, these parameters were not significantly different compared with baseline ($P > 0.05$, post-hoc LSMMeans Tukey–Kramer) (Table 2). After 90 days, HOMA-IR and HOMA-B decreased significantly only in the BF group by 56 and 35% respectively and ISI increased by 135% ($P < 0.0001$, post-hoc LSMMeans Tukey–Kramer) (Table 2).

Glucose and insulin response to OGTT (oral glucose tolerance test)

At baseline, there was no significant difference between the diet groups in fasting serum glucose and insulin response to OGTT ($P > 0.05$, two-way ANOVA) (Figures 1A and 1B). However, after 90 days, both glucose and insulin excursions were significantly lower in the BF group, but not in the D group emphasizing the effect of the diet ($P < 0.05$, two-way ANOVA) (Figures 1A and 1B). The changes were reflected in the AUC (area under the curve): on day 90, AUC_{glucose} significantly decreased from 17429 ± 155 to 13918 ± 81 mg/dl per 120 min and AUC_{insulin} significantly decreased from 7361 ± 156 to 3774 ± 94 μ -IU/ml per 120 min compared with baseline ($P < 0.0001$, post-hoc LSMMeans Tukey–Kramer) (Figures 1C and 1D). The extent of reduction in AUC_{glucose} and AUC_{insulin} in the BF group was 20 and 49% respectively (Figures 1C and 1D). In the D group on day 90, there was only a slight but significant 7% decrease in the AUC_{insulin} from 7803 ± 159 to 7193 ± 161 μ -IU/ml per 120 min ($P < 0.05$, post-hoc LSMMeans Tukey–Kramer) (Figure 1D).

Changes in SHBG and sex steroids

A significant cross-reaction of the diet and time was found for SHBG and sex steroids ($P < 0.05$, MANOVA). At baseline, the BF and D groups did not significantly differ in serum concentrations of SHBG or sex steroids ($P > 0.05$, post-hoc LSMMeans Tukey–Kramer) (Table 3). After 90 days, the levels of SHBG and sex steroids were not significantly different than the baseline in the D group ($P > 0.05$, post-hoc LSMMeans Tukey–Kramer) (Table 3). In contrast, in the BF group, the levels of SHBG concentrations significantly increased by 2-fold, from 2 ± 0.1 to 4.1 ± 0.2 μ g/dl ($P < 0.0001$, post-hoc LSMMeans Tukey–Kramer), whereas the significant decrease (50%) was observed in the levels of free testosterone (from 3.4 ± 0.2 to 1.7 ± 0.1 ng/dl) ($P < 0.0001$, post-hoc LSMMeans Tukey–Kramer). The other sex

Table 2 Fasting serum insulin, glucose and insulin resistance indices

Results are means ± S.E.M. Results marked with the same letter are not statistically different. *MANOVA for diet, time and diet by time; †BF endpoint (day 90) compared with baseline (day 0) (post-hoc LSMMeans Tukey–Kramer); ‡D end point (day 90) compared with baseline (day 0) (post-hoc LSMMeans Tukey–Kramer); §BF compared with D at baseline (day 0) (post-hoc LSMMeans Tukey–Kramer); ||BF compared with D at end point (day 90) (post-hoc LSMMeans Tukey–Kramer).

Parameter	Group										Effect P value*				
	BF					D					Between subjects		Within subjects		Diet by time
	Baseline (n = 25)	Day 90 (n = 25)	% Change	P value†	Baseline (n = 26)	Day 90 (n = 26)	% Change	P value‡	Baseline	Day 90	Change value§	P value	Change value	P value	
Fasting insulin (µIU/ml)	14.3 ± 0.9 ^a	6.7 ± 0.3 ^b	-53	<0.0001	15.7 ± 0.9 ^a	15.6 ± 0.7 ^a	0	0.99	-9	0.6	-57	<0.0001	<0.0001	<0.0001	<0.0001
Fasting glucose (mg/dl)	89.0 ± 1 ^a	81.8 ± 0.9 ^b	-8	<0.0001	86.7 ± 1.0 ^a	88.2 ± 1 ^a	2	0.7	3	0.4	-7	<0.0001	0.10	0.0002	<0.0001
HOMA-IR	3.2 ± 0.2 ^a	1.4 ± 0.1 ^b	-56	<0.0001	3.4 ± 0.2 ^a	3.4 ± 0.2 ^a	1	0.99	-6	0.82	-59	<0.0001	<0.0001	<0.0001	<0.0001
HOMAB	204.8 ± 14.0 ^a	133.2 ± 7.1 ^b	-35	0.001	246.8 ± 15.0 ^a	230.5 ± 13.4 ^a	-7	0.87	-17	0.1	-42	<0.0001	<0.0001	0.0006	0.0247
ISI	3.4 ± 0.1 ^b	8.0 ± 0.3 ^a	135	<0.0001	3.1 ± 0.1 ^b	3.2 ± 0.1 ^b	2	0.98	8	0.72	150	<0.0001	<0.0001	<0.0001	<0.0001

steroids decreased were androstenedione by 34%, DHEA-S by 35% and the total testosterone by 47% ($P < 0.0001$, post-hoc LSMMeans Tukey–Kramer) (Table 3). FAI decreased in the BF by 78% ($P < 0.0001$, post-hoc LSMMeans Tukey–Kramer), whereas in the D group there was a non-significant 12% increase (Table 3). Interestingly, although 17β-oestradiol levels were not affected by the BF diet, in the D group they significantly increased by 35% (from 8.8 ± 0.7 to 12.0 ± 1.0 ng/dl) ($P < 0.05$, post-hoc LSMMeans Tukey–Kramer) (Table 3).

Serum 17OHP response to leuprolide

Like other sex steroids, only after 90 days of intervention in the BF group was there a significant (35%) decrease in mean basal serum 17OHP levels (from 9.5 ± 0.8 to 6.2 ± 0.4 ng/dl; $P < 0.01$, post-hoc LSMMeans Tukey–Kramer) (Table 3). This was also reflected in the leuprolide stimulation test. At baseline, there was no significant difference between the diet groups in the response of serum concentrations of 17OHP after leuprolide administration ($P > 0.05$, Two-way ANOVA) (Figure 2A). However, only in the BF group on day 90 after leuprolide administration the peak of serum 17OHP concentration decreased significantly (from 418.8 ± 5.8 to 255.6 ± 5.7 ng/dl; $P < 0.0001$, post-hoc LSMMeans Tukey–Kramer), revealing again the effect of the diet ($P < 0.05$, two-way ANOVA) (Figure 2B). This was reflected in the AUC_{17OHP}, which was significantly decreased by 38% only in the BF group ($P < 0.0001$, post-hoc LSMMeans Tukey–Kramer) (Figure 2C).

Ovulation frequency and ovulatory cycles

During the first 4 weeks, both BF and D meal plan groups showed no ovulation. However, from week 5 both BF and D groups showed ovulation (Figure 3A). It is noteworthy that the meal timing increased ovulation rate in both groups ($P < 0.05$, χ^2 analysis). However, during the second month the percentage of ovulating women was, although not significantly, higher in the BF group than in the D group (28 and 7.6% respectively) ($P = 0.08$, χ^2 analysis) (Figure 3A). This trend increased and became significant during month 3 of the study during which almost 50% of women allocated to the BF meal plan group ovulated compared with only about 20% of women of the D group ($P < 0.05$, χ^2 analysis) (Figure 3A). At the end of the study, there were more women with at least one ovulation in the BF group. Importantly, two ovulations were seen only in women of the BF group ($P < 0.01$, χ^2 analysis) (Figure 3B).

DISCUSSION

In the present study, we tested the effect of two isocaloric maintenance diets with different timed caloric intake (high-calorie in the morning compared with the evening) in lean PCOS women. The BF group had reduced fasting serum glucose and insulin concentrations. There was also decreased response to oral glucose administration with the resulting reduction of insulin resistance indices despite no change in body mass. In contrast, in the D group no changes were observed in fasting glucose, fasting

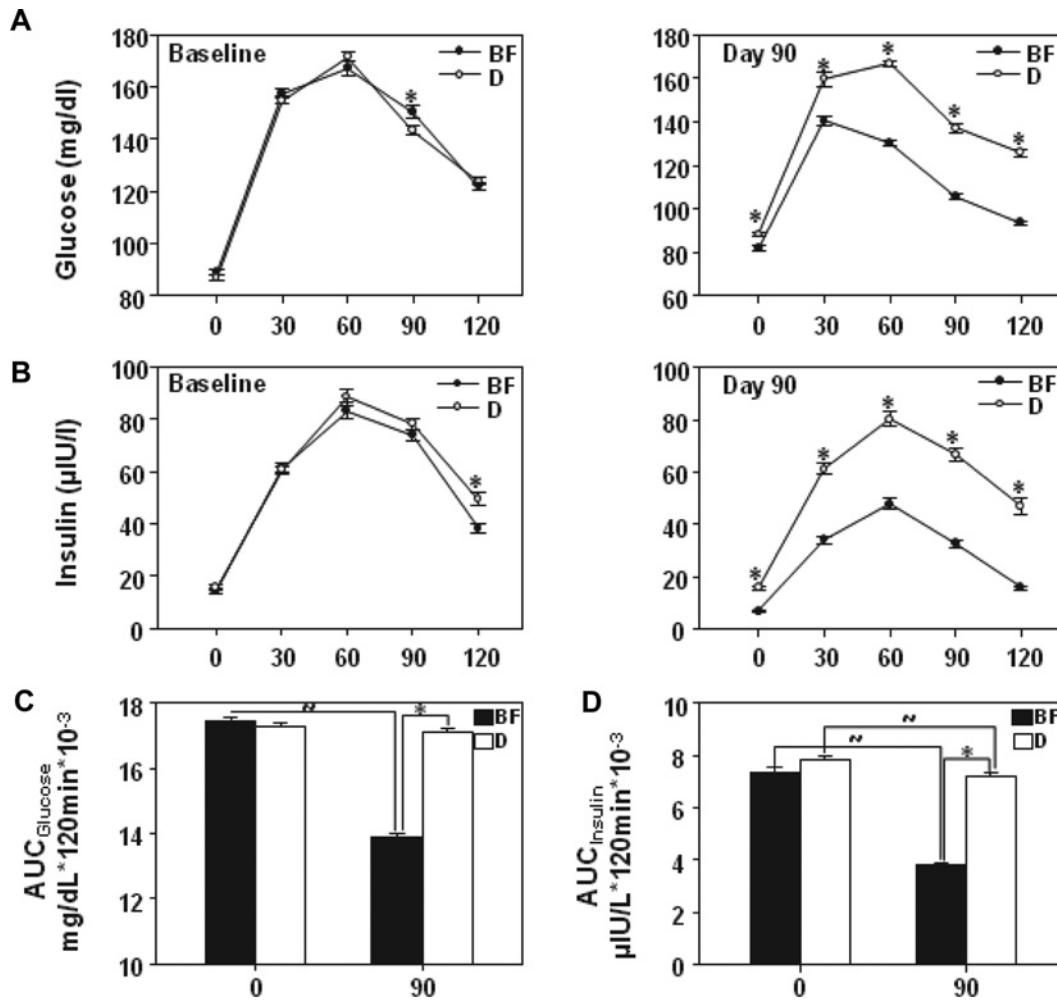


Figure 1 Effect of the timing of caloric intake on glucose and insulin levels after an OGTT

An OGTT was performed at baseline and after 90 days of follow up during the follicular phase. Blood glucose (A) and insulin (B) levels were measured 30, 60, 90 and 120 min after a 75-g oral glucose challenge. A two-way ANOVA (time \times treatment) was performed and a least-significant difference Student's *t* test post-hoc analysis was used for comparison between the treatments at each time-point. AUC of glucose (C) and insulin (D) response was calculated. For the comparison of end point (day 90) to baseline (day 0) within a diet, a MANOVA for repeated measurements was performed. An LSMeans Tukey–Kramer post-hoc analysis was performed for multiple comparisons. Values are means \pm S.E.M. **P* < 0.01 between meal plans at the same time (baseline or end point); \sim *P* < 0.01 between times (baseline compared with end point) within a meal plan.

insulin, insulin and glucose response to OGTT and insulin resistance indices. Concomitantly, in the BF group ovarian cytochrome P450c17 α activity decreased, as demonstrated by a substantial reduction in the response of serum 17OHP to the administration of leuprolide.

The reduction in cytochrome P450c17 α activity was accompanied by a decline in the serum ovarian androgen concentrations, namely total testosterone, free testosterone, DHEA-S and androstenedione only in the BF group. DHEA-S levels have been correlated with insulin resistance and it was shown that by decreasing insulin resistance, reduction of DHEA-S was also observed in PCOS women [32]. Indeed, we had significantly more ovulations in the BF group compared with the D group. The improvement of insulin resistance indices achieved in this study in the BF group correlate with reduction in ovarian cytochrome

P450c17 α activity, decrease in androgen secretion and increased ovulatory frequency. These findings are consistent with previous reports showing that the reduction in serum insulin concentrations after dietary weight loss [9] or after metformin treatment in obese [8,15] and lean PCOS women [33] was associated with decreased ovarian cytochrome P450c17 α activity, reduced ovarian androgen secretion and improved ovulatory frequency. In addition, in the BF group we observed increased serum SHBG. This finding is also supported by other reports that increased insulin levels inhibit SHBG levels [10,11]. In contrast with the BF group, serum androgens did not change in women with PCOS of the D group, most probably due to the unchanged serum insulin and/or SHBG levels.

Meal composition appears to influence satiety. Specifically, protein consumed at breakfast (compared with lunch or dinner)

Table 3 Serum sex steroids and SHBG

Results are mean \pm S.E.M. Results marked with the same letter are not statistically different. *MANOVA for diet, time and diet by time; †BF endpoint (day 90) compared with baseline (day 0) (post-hoc LSMean Tukey–Kramer); ‡D endpoint (day 90) compared with baseline (day 0) (post-hoc LSMean Tukey–Kramer); §BF compared with D at baseline (day 0) (post-hoc LSMean Tukey–Kramer); ||BF compared with D at end point (day 90) (post-hoc LSMean Tukey–Kramer).

Parameter	Group				D				BF compared with D				Effect P value*				
	BF		% Change	P value†	D		% Change	P value‡	Baseline		Day 90		% Change	P value	Between subjects diet	Within subjects time	Diet by time
	Baseline (n = 25)	Day 90 (n = 25)			Baseline (n = 26)	Day 90 (n = 26)			% Change	P value§	% Change	P value					
Free testosterone (ng/dl)	3.4 \pm 0.2 ^a	1.7 \pm 0.1 ^b	-50	<0.0001	3.6 \pm 0.1 ^a	3.4 \pm 0.1 ^a	-5	0.87	-5	0.81	-50	<0.0001	<0.0001	<0.0001	<0.0001		
Total testosterone (ng/dl)	141.2 \pm 6.7 ^a	74.5 \pm 6.7 ^b	-47	<0.0001	139.9 \pm 9.0 ^a	145.3 \pm 6.4 ^a	4	0.95	1	0.99	-49	<0.0001	0.0001	<0.0001	<0.0001		
Androstenedione (ng/dl)	237.1 \pm 12.8 ^a	156.3 \pm 7.1 ^b	-34	<0.0001	249.9 \pm 8.6 ^a	248.0 \pm 7.4 ^a	0	0.99	-5	0.76	-37	<0.0001	<0.0001	<0.0001	0.0002		
DHEA-S (μ g/dl)	351.1 \pm 11.4 ^a	226.8 \pm 10.9 ^b	-35	<0.0001	356.3 \pm 22.2 ^a	359.2 \pm 20.9 ^a	1	0.99	-1	0.99	-37	<0.0001	<0.0001	0.0001	<0.0001		
17 β -Oestradiol (ng/dl)	9.2 \pm 0.6 ^{ab}	10.4 \pm 0.8 ^{ab}	14	0.67	8.8 \pm 0.7 ^b	12.0 \pm 1 ^a	35	0.03	3	0.99	-13	0.55	0.56	0.0001	0.08		
17OHP (ng/dl)	9.5 \pm 0.8 ^a	6.2 \pm 0.4 ^b	-35	0.0033	8.3 \pm 0.6 ^{ab}	9.1 \pm 0.7 ^a	10	0.8	15	0.56	-32	0.0109	0.24	0.036	0.0008		
17OHP (ng/dl) (peak response to leuprolide test)	418.8 \pm 4.9 ^b	255.6 \pm 5.7 ^c	-39	<0.0001	436.0 \pm 4.8 ^{ab}	445.5 \pm 3.6 ^a	2	0.5	-3	0.06	-43	<0.0001	<0.0001	<0.0001	<0.0001		
SHBG (μ g/dl)	2.0 \pm 0.1 ^b	4.1 \pm 0.2 ^a	116	<0.0001	2.2 \pm 0.2 ^b	2.2 \pm 0.2 ^b	0	0.99	-14	0.74	86	<0.0001	<0.0001	<0.0001	<0.0001		
FAI	34.8 \pm 4.5 ^a	7.5 \pm 0.8 ^b	-78	0.0012	32.1 \pm 4.0 ^a	35.8 \pm 7.5 ^a	12	0.95	8	0.98	-79	0.0006	0.0125	0.02	0.0027		

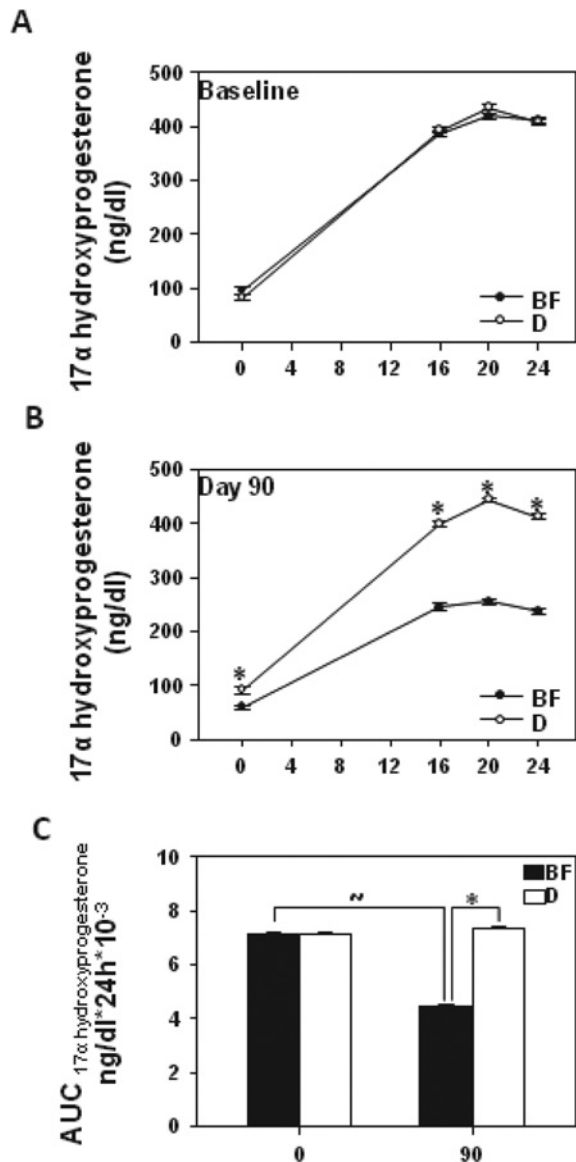


Figure 2 Effect of timing of caloric intake on 17OHP levels after leuprolide stimulation

Leuprolide stimulation test was performed at baseline (A) and after 90 days (B) of follow up during the follicular phase (progesterone <2 ng/dl). 17OHP was determined immediately before and 16, 20 and 24 h after 10 μ g of leuprolide/kg of body mass was administered. A two-way ANOVA (time \times treatment) was performed and a least-significant difference Student's *t* test post-hoc analysis was used for comparison between the treatments at each time-point. AUC (C) was calculated and for the comparison of end point (day 90) to baseline (day 0) within a diet, a MANOVA for repeated measurements was performed. An LSM-Eans Tukey–Kramer post-hoc analysis was performed for multiple comparisons. Values are means \pm S.E.M. **P*<0.01 between meal plans at the same time (baseline or end point); ~*P*<0.01 between times (baseline compared with end point) within a meal plan.

leads to greater initial and sustained feeling of fullness, increased satiety and reduced concentrations of the appetite-regulating hormone ghrelin [34–36]. The high protein content used in this diet was similar in its concentration (1.4 g/kg) to other studies in the field [34]. Indeed, the compliance rate was high and showed no significant preference between the BF or D meal plans, as in

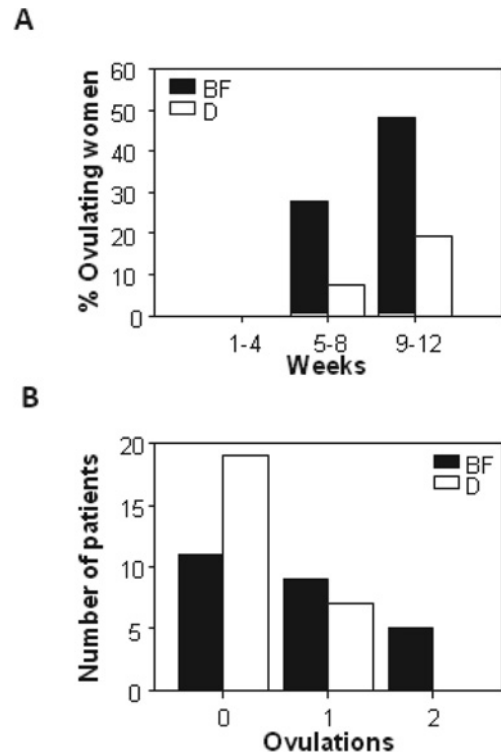


Figure 3 Ovulation incidence

Serum progesterone was measured weekly and ovulation was presumed to have occurred if serum progesterone were >2 ng/ml. (A) Percentage of ovulating women in each group was calculated every 4 weeks. (B) Patients in each group were categorized according to the number of ovulations (0, 1 or 2) during 90 days of follow-up. Two-way tables were used to compare categorical data and the statistical significance of differences was assessed by the χ^2 test.

the BF group only 7% dropped out and from the D group only 10% dropped out.

The improvement in insulin resistance indices in the BF group is consistent with previous reports that showed that the morning period is most critical in terms of maintaining optimum metabolism. Previous epidemiological studies have shown that high-calorie breakfast is related to lower daily energy intake, reduced BMI, improved nutrient intake [37,38] and lower serum cholesterol concentrations [39]. The reduction in insulin resistance indices in the BF group is also congruent with a recent study that showed that increasing carbohydrate intake at breakfast could be protective against long-term development of the metabolic syndrome [40]. In addition, a meal tolerance test in the evening showed that the levels of both glucose and insulin were higher in the D group compared with the BF group (results not shown).

Meal timing and feeding schedule have been shown to exert strong entraining effects on peripheral oscillators [16,17] and it was recently shown that the meal timing and circadian misalignment of the time of food intake is able to adversely affect several hormones involved in the control of metabolic processes including fasting serum insulin and glucose and the response to OGTT [18–23]. Thus the improvement of insulin resistance indices found in the BF group suggests that the high caloric intake in the morning might represent a schedule more synchronized

with the circadian pacemaker. This clock resetting could be protective also against the disruption in hormone secretion reported in PCOS patients [24,25]. Nevertheless, the high-caloric intake at dinner did not result in worsening of insulin resistance indices in the D group. We, therefore, suggest that the timing of caloric intake, i.e. high-calorie intake at breakfast with reduced intake at dinner, leads to reduced overall insulin levels, which lead to the reduction of ovarian P450c17 α activity and, as a result, to decreased ovarian testosterone synthesis.

CLINICAL PERSPECTIVES

- Two features of the PCOS, insulin resistance and increased ovarian cytochrome P450c17 α activity, can be attenuated by the timing of meals.
- In lean PCOS women, a high caloric intake at breakfast with reduced intake at dinner results in improved insulin sensitivity indices and reduced cytochrome P450c17 α activity, which ameliorates hyperandrogenism and improves ovulation rate.
- Meal timing directed at lowering insulin secretion in lean women with the PCOS could ameliorate their hyperandrogenism and increase ovulation rate.

AUTHOR CONTRIBUTION

Daniela Jakubowicz and Julio Wainstein conceived and carried out experiments, and Oren Froy and Maayan Barnea analysed data. All authors were involved in writing the paper and had final approval of the submitted and published versions.

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