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Dietary Composition in Restoring Reproductive and Metabolic Physiology in Overweight Women with Polycystic Ovary Syndrome

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Overweight women with polycystic ovary syndrome (PCOS) were randomized to a high protein (HP; 40% carbohydrate and 30% protein; n = 14) or a low protein (LP; 55% carbohydrate and 15% protein) diet (n = 14). The intervention consisted of 12 wk of energy restriction (~6000 kJ/d), followed by 4 wk of weight maintenance. Pregnancies (two HP and one LP); improvements in menstrual cyclicity, lipid profile, and insulin resistance (as measured by the homeostasis model); and decreases in weight (7.5%) and abdominal fat (12.5%) occurred independently of diet composition. Improvements in menstrual cyclicity were associated with greater decreases in insulin resistance and fasting insulin ($P = 0.011$). On the LP diet, high density lipoprotein cholesterol decreased 10% during energy restriction ($P = 0.008$), and the free androgen index increased 44% in weight maintenance stages ($P = 0.027$). Weight loss leads to improvements in cardiovascular and reproductive parameters potentially mediated by improvements in surrogate measures of insulin resistance. An HP weight loss diet may result in minor differential endocrine and metabolic improvements. (*J Clin Endocrinol Metab* 88: 812–819, 2003)

POLYCYSTIC OVARY SYNDROME (PCOS) is a common endocrine disorder in women of reproductive age with primary manifestations of infertility, menstrual dysfunction, and clinical or biochemical hyperandrogenism (hirsutism, acne, and elevated androgens). It is often associated with hyperlipidemia and impaired glucose tolerance, leading to potential increases in morbidity and mortality from cardiovascular disease and type II diabetes mellitus (1). Insulin resistance is strongly implicated in its etiology (2). Insulin directly stimulates thecal cell androgen production (either alone or synergistically with LH) (3, 4) and decreases hepatic SHBG production (5). This results in biochemical hyperandrogenism and associated clinical features of anovulation and clinical hyperandrogenism.

Obesity, particularly of the abdominal type, is present in varying degrees in women with PCOS (ranging from 10–50%) and both enhances the features of insulin resistance (6) and is associated with reproductive dysfunction (7, 8). Improvement of insulin resistance through diet- and exercise-induced weight loss has shown promising metabolic and clinical results. Modest weight loss (<10% of initial body weight) increases the frequency of ovulation, improves conception, and reduces miscarriage, hyperlipidemia, hyperglycemia, and insulin resistance in women with PCOS (9–12).

Although a low fat/high carbohydrate diet is traditionally thought to aid weight loss and improve metabolic and reproductive dysfunction, there has been increased community interest in a high protein/low carbohydrate diet (13). This may aid in increased weight loss (14) due to the increased satiating power of protein compared with carbohydrate or fat (15) and may improve insulin sensitivity through maintenance of lean body mass with weight loss (16). Although this suggests that high protein/low carbohydrate diets may be useful in the management of PCOS, there is no current supporting evidence for adoption of this dietary regimen as a treatment strategy. The objective of this study was to examine the effects of replacing dietary protein with carbohydrate in isocaloric energy-restricted diets on weight loss, body composition, glucose and insulin homeostasis, and lipid profile in overweight women with PCOS. This study also aimed to examine the influence of these diets on reproductive clinical outcomes, specifically menstrual cyclicity, ovulation, and hirsutism.

Subjects and Methods

Subjects and recruitment

Overweight women (European Caucasian) with PCOS (n = 45) were recruited through public advertisement (Fig. 1). The study was approved by the human ethics committees of the North West Adelaide Health Service and CSIRO Division of Health Sciences and Nutrition. Inclusion criteria were diagnosis of PCOS by menstrual irregularity (cycle length, <21 d or >35 d or variation between consecutive cycles of >3 d) and clinical (hirsutism/acne) and/or biochemical hyperandrogenism (17). If a definition of menstrual irregularity as fewer than 9 menses/yr is used, 10 of 22 women fulfilled this criteria. However, this is an approximation, as a 12-month retrospective menses calendar was not available for all subjects, and 8 subjects were previously using hormonal medication. Hyperandrogenism was defined as a free androgen index (FAI) more than 2.85 (FAI = testosterone/SHBG × 100). This range was obtained from a representative population of non-PCOS women (n = 80). Exclusion criteria were inability to comply with study

Abbreviations: AUC, Area under the curve; DEXA, dual x-ray absorptiometry; FAI, free androgen index; HDL-C, high density lipoprotein cholesterol; HOMA, homeostatic model assessment; HP, high protein; LDL-C, low density lipoprotein cholesterol; LP, low protein; MTT, meal tolerance test; PCOS, polycystic ovary syndrome; TC, total cholesterol.

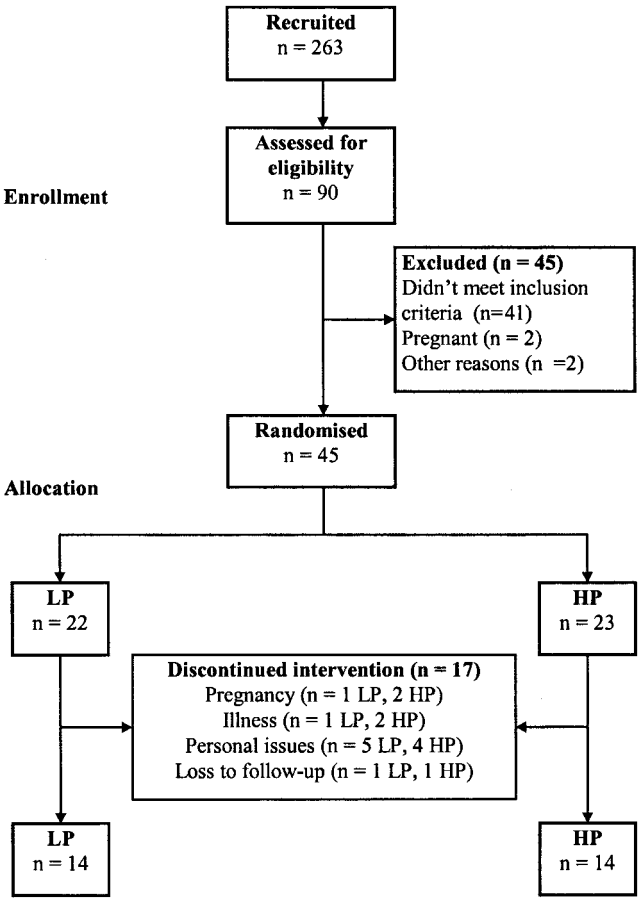

FIG. 1. Study parallel design flow chart.

requirements, weight greater than 140 kg, smoking, and use of oral contraceptives/hormone treatment/insulin-sensitizing agents. Subjects were eligible for the study if they had not been taking oral contraceptives for more than 4 wk or hormone treatment/insulin-sensitizing agents for more than 2 wk. Subjects with hyperprolactinemia, thyroid abnormalities, or nonclassic adrenal hyperplasia were excluded through appropriate hormone assessment (17).

Study design

Subjects were stratified to ensure equal distribution for known confounding factors of weight, age, and desire to conceive and then randomized by an independent observer after obtaining informed written consent. Subjects and investigators were not blinded as to the dietary intervention. The dietary interventions were low protein (LP; 55% carbohydrate, 15% protein, and 30% fat) and high protein (HP; 40% carbohydrate, 30% protein, and 30% fat) with macronutrient composition calculated as a percentage of the total calories. Both diets were nutritionally complete, and alcohol intake was not permitted throughout the 16 wk. An energy-restricted diet (6000 kJ/d) was prescribed for 12 wk, followed by a weight maintenance diet for the final 4 wk with the same dietary composition adhered to in both phases (divide by 4.186 for conversion from kilojoules to kilocalories). Subjects attended a weekly exercise/education class (11, 12, 18) and were advised to increase exercise to a minimum of three times per week. Exercise levels were categorized according to National Health and Medical Research Council standards and were documented monthly and at baseline and study completion (19).

Subjects attended monthly out-patient clinic visits at a dietary clinic on 2 consecutive d during each study period (wk 0, 4, 8, 12, and 16) for overnight fasting venous blood sampling. At all visits, subjects were weighed in light clothes with no shoes (Mettler scales, model AMZ14; A&D Mercury, Kinomoto, Japan); body mass index was calculated by weight (kilograms) divided by squared height (meters). At wk 0 and 16, a 3-h meal tolerance test (MTT) was performed with a test meal (3000 kJ) corresponding to the appropriate diet. Dietary composition was 11% protein, 14% fat, and 76% carbohydrate for the LP and 31% protein, 14% fat, and 55% carbohydrate for the HP diet. The test meal macronutrient profile was not identical to that of the study diets so that the maximum difference in postprandial glucose and insulin responses could be observed. Venous blood was assayed for glucose and insulin at 0, 60, 120, and 180 min after meal consumption. Fasting venous blood was assayed on consecutive clinic days at wk 0, 12, and 16 for reproductive hormones, insulin, lipids, and glucose. Two additional samples were obtained over 10 min to calculate an average LH value.

Dietary intervention

Subjects met with a registered dietitian fortnightly for initial education on quantification and recording of their daily food intake and to assess and modify the dietary regimen based on compliance and weight loss. Nutrient intakes were calculated with Diet 1/Nutrient Calculation software (Xyris Software, Highgate Hill, Australia) based on data from Australian food composition tables. Nutritional intake was assessed from monthly 3-d consecutive dietary food records (1 weekday and 2 weekend days) and daily dietary checklists. Dietary compliance was determined by subject adherence to the macronutrient profiles (protein, carbohydrate, and fat) and from assessment of random urine samples (wk 0, 12, and 16) for urea excretion relative to urinary creatinine.

Clinical measurements

Dual x-ray absorptiometry (DEXA; Norland Medical Systems, Inc., NY) was performed at wk 0 and 16 to assess body fat composition (fat mass of soft tissue and lean mass of soft tissue; coefficient of variation, 3–4%) (20). Abdominal fat mass was measured from the area demarcated by the ribs at the upper portion and the iliac crests at the lower portion.

Hirsutism was self-assessed at wk 0 and 16 by the Ferriman-Gallwey score (21). Subjects documented their menstrual cycles for the study duration and for 6 months before study commencement. First morning urine samples were collected twice weekly and assessed for total urinary pregnanediol-3-glucuronide and urinary estrone glucuronide (St. Michael's Natural Family Planning, University of Melbourne) to determine ovulation status (22). Results were compared with the menses calendars to qualitatively determine ovulation. Improvements in menstrual cyclicity were defined as a change from nonovulatory to ovulatory cycles or from irregular to regular cycles or an improvement in consecutive intercycle variation. Six subjects (two LP and four HP) had no ovulations during the study. They were classified as nonovulators, and LH, FSH, progesterone, and estradiol were analyzed for this subset of subjects at wk 0, 12, and 16.

Biochemical measurements

SHBG, total testosterone (bound and unbound), LH, FSH, progesterone, estradiol, TSH, PRL, and 17α-hydroxyprogesterone were measured as previously described (11). Total cholesterol, low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), triglycerides, insulin, and glucose were measured as previously described (23). Total areas under the insulin and glucose curves above baseline during the MTT were calculated geometrically (trapezoidal rule) (24). All inter- and intraassay coefficients of variation were less than 10%. The homeostatic model assessment (HOMA) was used as a surrogate measure of insulin sensitivity (25), calculated as fasting glucose (millimoles per liter) $\times$ log (10) fasting insulin (milliunits per liter)/22.5.

Study outcomes

Primary outcomes were weight, body composition, dietary compliance, menstrual cyclicity, ovulation, fasting and postprandial glucose and insulin, surrogate measures of insulin sensitivity (homeostasis model of assessment), and lipid profile. Secondary outcome measures were hirsutism and reproductive hormone profile.

Statistics

Data are expressed as the mean ± SEM and were log transformed before analysis where skewed. Two-tailed analysis were performed using SPSS 10.0 for Windows (SPSS, Inc., Chicago, IL). Statistical significance was set at $P < 0.05$. Baseline parametric data were assessed using a one-way ANOVA, and nonparametric data were assessed by Kruskal-Wallis test, with diet as the between-subject factor. For comparison between time points, a repeated measures ANOVA was used for parametric data, and the Mann-Whitney *U* test was used for nonparametric data, with diet as the between-subject factor. Categorical data were analyzed by nonparametric tests. Samples were adjusted for covariates of weight loss at wk 16 and variables that were significantly different at baseline [total cholesterol (TC), LDL-C, and HDL-C] or approached significant difference at baseline (fasting insulin and fasting FAI). Subjects who responded to weight loss with improved menstrual cyclicity (responders) were assessed separately from those who responded to weight loss with no improved menstrual cyclicity (nonresponders), with cyclicity as the between-subject factor. Bonferroni adjustments were performed on multiple comparisons. Data for insulin are presented for 27 subjects due to an outlier of more than 3 SD from the mean. Menstrual cyclicity and DEXA data are presented for 25 subjects due to incomplete data.

With 28 subjects, this study had 80% power at $P < 0.05$ to detect a difference of 1.34 kg in abdominal fat, 6.5–7 nmol/liter in SHBG, 0.85 nmol/liter in testosterone, and 4.3 for FAI.

Results

Subjects

Twenty-eight subjects completed the study (mean body mass index, 37.4 ± 1.24 m^2; mean age, 33 ± 0.84 yr), baseline characteristics are shown in Table 1. Three subjects (two HP and one LP) conceived of a total of 20 women who were actively trying to conceive or who were not using contraception. These subjects did not continue with the diet therapy. Study drop-outs are documented in Fig. 1.

TABLE 1. Subject baseline characteristics

Variable	LP (n = 14)	HP (n = 14)
Age (yr)	33 ± 1.2	32 ± 1.2
Body mass index (kg/m^2)	37.7 ± 1.9	37.9 ± 1.6
Weight (kg)	98.6 ± 4.6	104.2 ± 5.3
Fasting glucose (mmol/liter)	5.66 ± 0.27	5.52 ± 0.12
Fasting insulin (mU/liter)[a]	16.9 ± 2.5	23.0 ± 2.4
Fasting TC (mmol/liter)	6.1 ± 0.19	5.25 ± 0.23[b]
Fasting LDL-C (mmol/liter)	3.99 ± 0.17	3.42 ± 0.20[b]
Fasting HDL-C (mmol/liter)	1.21 ± 0.09	0.97 ± 0.08[b]
Fasting triglycerides (mmol/liter)	1.96 ± 0.30	1.87 ± 0.27
Actively trying to conceive (%)	64.3	57.1
Testosterone (nmol/liter)	1.49 ± 0.18	1.79 ± 0.17
SHBG (nmol/liter)	31.16 ± 3.97	23.29 ± 2.03
Hirsutism	20.43 ± 1.67	18.5 ± 1.49

[a] n = 27.
[b] Significant difference between LP and HP diet ($P < 0.05$).

Diet and exercise

Both diets were well tolerated, with no adverse events or effects reported. Energy intake and saturated fat intake were not different between the diets during both the energy restriction and weight maintenance phases. Protein intake was higher, and carbohydrate intake was lower on the HP diet than on the LP diet during both energy restriction and weight maintenance ($P < 0.001$; Table 2). There was a 27% difference in urinary urea/creatinine ratio at wk 16 between the HP and the LP diet ($P = 0.002$ for diet effect). Exercise levels and class attendance did not differ between the diet groups with 80% mean attendance. Overall, the dietary data and urea/creatinine data indicate good compliance.

Weight and body composition

A mean weight loss of 7.7 ± 0.7 kg occurred overall (6.9 ± 0.8 kg for the LP and 8.5 ± 1.1 kg for the HP diet). Weight changes in the weight maintenance phase were not significant for either group and were not different between the two groups with a total mean gain of 0.05 ± 0.2 kg, indicating good compliance with the weight maintenance regimen. There was an overall combined decrease of 14.4% in total fat mass, 3.4% in total lean mass, and 12.5% in abdominal fat mass ($P < 0.001$). There was no significant effect of diet composition on changes in weight, total fat mass, total lean mass, or abdominal fat mass.

Lipids

Over the 16 wk, decreases in TC (8.8%), triglycerides (12.5%), and LDL-C (9.8%) occurred independently of diet composition ($P < 0.001$). A significant time × diet interaction was observed for HDL-C ($P = 0.008$) and TC/HDL-C ($P = 0.002$). During energy restriction, HDL-C decreased by 10% on the LP diet ($P < 0.001$), but was not changed on the HP diet. Energy restriction resulted in a 12.5% decrease in TC/

TABLE 2. Calculated dietary composition of LP and HP diets from 4 × 3-d food records

Macronutrients	LP (n = 14)		HP (n = 14)	
	ER	WM	ER	WM
Energy (kJ)	6339	7704	6255	7515
CHO (% E)	57[a]	56[b]	43	44
Protein (% E)	16[a]	16[b]	27	27
Total fat (% E)	27[a]	28	28	28
Alcohol (% E)	0.2	0.2	0.01	0
SFA (% E)	8.1	8.7	8.4	9
PUFA (% E)	3.4[a]	3.9[b]	3.0	3.1
MUFA (% E)	13.1[a]	12.8	14.5	13.6
Cholesterol (mg)	95[a]	110[b]	171	222
Fiber (g)	25[a]	28[b]	20	22

ER, Energy restriction (wk 0–12); WM, weight maintenance (wk 12–16); SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; % E, percentage of total daily energy intake; CHO, carbohydrate.
[a] Significant difference from HP diet in ER ($P < 0.05$).
[b] Significant difference from HP diet in WM ($P < 0.05$).

TABLE 3. Effect of weight loss with a LP or a HP diet on fasting lipids, insulin, glucose, and HOMA

	LP (n = 14)			HP (n = 14)		
	Week 0	Week 12	Week 16	Week 0	Week 12	Week 16
TC (mmol/liter)	6.1 ± 0.19	5.56 ± 0.16***	5.49 ± 0.16	5.25 ± 0.23	4.87 ± 0.24***	4.81 ± 0.21
LDL-C (mmol/liter)	3.99 ± 0.17	3.81 ± 0.13*	3.57 ± 0.15*	3.42 ± 0.20	3.23 ± 0.21*	3.04 ± 0.14*
HDL-C (mmol/liter)	1.21 ± 0.09	1.10 ± 0.09[b]***	1.15 ± 0.09	0.97 ± 0.08	1.03 ± 0.08	1.07 ± 0.09
Triglycerides (mmol/liter)	1.96 ± 0.30	1.42 ± 0.14***	1.68 ± 0.22*	1.87 ± 0.27	1.33 ± 0.17***	1.52 ± 0.26*
TC/HDL-C	5.35 ± 0.40	5.50 ± 0.46*	5.11 ± 0.40	5.86 ± 0.49	5.07 ± 0.42[c]**	4.82 ± 0.36
Glucose (mmol/liter)	5.66 ± 0.27	5.31 ± 0.17	5.53 ± 0.21*	5.52 ± 0.12	5.42 ± 0.13	5.57 ± 0.13*
Insulin[a] (mU/liter)	16.9 ± 2.5	12.8 ± 2.0**	13.5 ± 2.1	23.0 ± 2.4	16.6 ± 2.4**	15.4 ± 1.8
HOMA[a] (μU·mol^{-1}·liter^{-3})	0.29 ± 0.03	0.25 ± 0.02**	0.27 ± 0.03	0.33 ± 0.02	0.28 ± 0.02**	0.29 ± 0.02

Significance is indicated for changes from wk 0–12 (wk 12 values) and wk 12–16 (wk 16 values).
*, Significant effect of time ($P < 0.05$); **, significant effect of time ($P < 0.01$); ***, significant effect of time ($P < 0.001$).
[a] n = 27 (LP, n = 13; HP, n = 14).
[b] Significant change in HDL-C ($P < 0.001$) for the LP compared with the HP diet ($P = 0.008$ for difference between diets).
[c] Significant change in TC/HDL-C ($P = 0.003$) for the HP compared with the LP diet ($P = 0.002$ for difference between diets).

HDL-C for the HP diet ($P = 0.003$), with no change on the LP diet (Table 3).

Insulin and glucose

There was no effect of diet composition on fasting insulin, fasting glucose, or HOMA. Over the 16 wk, fasting insulin decreased by 20% ($P = 0.001$), HOMA decreased by 9% ($P = 0.001$), and fasting glucose did not change (Table 3). The changes in fasting insulin and HOMA occurred in the energy restriction phase. This decrease was maintained in the weight maintenance phase, and fasting insulin and HOMA did not return to baseline levels.

There was no effect of weight loss or diet composition on test meal area under the curve (AUC) for glucose. However, the LP test meal resulted in a 3.5 times higher AUC at wk 0 ($P = 0.02$) and a 4 times higher AUC at wk 16 ($P = 0.009$) compared with the HP test meal (Fig. 2A). There was no effect of diet composition on test meal AUC insulin. Test meal AUC insulin decreased by 30% ($P < 0.001$) over 16 wk (Fig. 2B).

Clinical parameters

The Ferriman-Gallwey score was 19.5 ± 1.12 at wk 0 and 19.7 ± 1.37 at wk 16, with no effect of weight loss or diet composition on hirsutism. With the exception of the non-ovulators, all other subjects ovulated at least once. There was an improvement in menstrual cyclicity in 11 of 25 subjects (44%), with no effect of diet composition. This occurred due to an improvement in cycle length for 6 subjects (2 LP and 4 HP), an improvement in ovulation for 3 subjects (3 LP) and 1 amenorrheic subject (HP), and a spontaneous resumption of menses in 1 amenorrheic subject (HP). Ovulatory improvements occurred at wk 4–6 and 12–13. In addition to the 28 subjects, 3 pregnancies occurred (1 LP and 2 HP). Approximate conception time was calculated as wk 4–5 with mean weight loss at this time of 5.3 kg for these subjects.

There was no difference in baseline characteristics between subjects with improved menstrual cyclicity (responders; n = 11) or those who conceived compared with those who did not have improved menstrual cyclicity (nonresponders; n = 14). However, there was a significant difference in fasting insulin (Fig. 3A) and HOMA (Fig. 3B) between the responders and nonresponders ($P = 0.011$ for cyclicity effect). In energy restriction, there was a 34.9% decrease in fasting insulin ($P = 0.006$) and a 21.3% decrease in HOMA ($P = 0.009$) for responders, with no changes for non-responders.

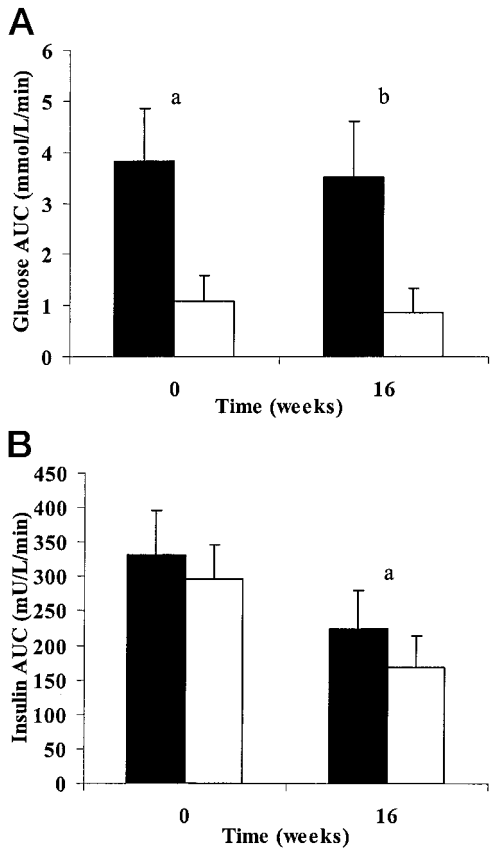

FIG. 2. Effect of the LP compared with the HP test meal on AUC insulin and AUC glucose. A, The AUC for glucose after a MTT with a LP (n = 14) or a HP (n = 14) test meal of 3000 kJ at wk 0 and 16 (n = 28). a, Significant difference between the LP and HP AUC at wk 0 ($P = 0.02$). b, Significant difference between the LP and HP AUC at wk 16 ($P = 0.009$). B, The AUC insulin after a MTT with a LP (n = 13) or a HP (n = 14) test meal of 3000 kJ at wk 0 and 16. a, Significant effect of time for both the LP and HP groups from wk 0–16 ($P < 0.001$).

Reproductive hormone profile

Energy restriction increased SHBG by 11.4% ($P = 0.028$; Fig. 4A) and decreased testosterone by 13.7% ($P = 0.01$; Fig.

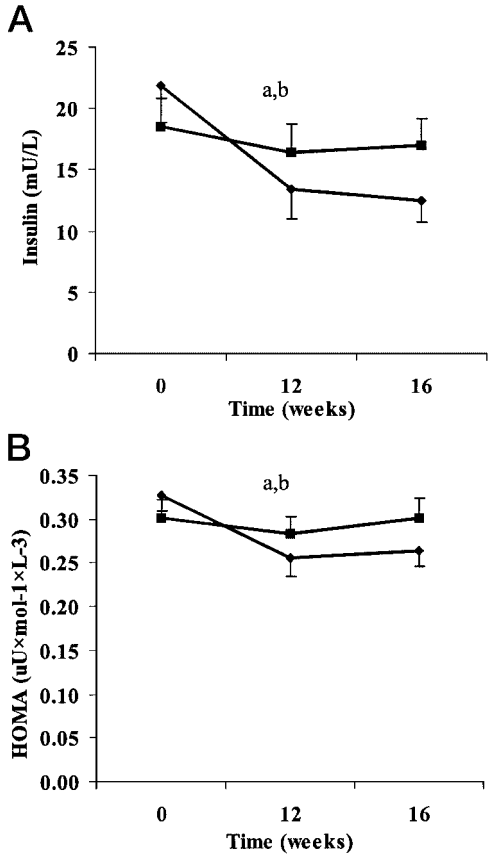

FIG. 3. Changes in fasting insulin and HOMA for responders compared with nonresponders. A, Fasting insulin at wk 0, 12, and 16 for subjects who responded to intervention with improved menstrual cyclicity (responders; n = 11) compared with those who responded to intervention with no improved menstrual cyclicity (nonresponders; n = 14; ◆, responders; ■, nonresponders). Data from wk 0–12 are during energy restriction, and data from wk 12–16 are during weight maintenance. a, Significant effect of time from wk 0–12 for responders ($P = 0.009$). b, Significant change for responders compared with nonresponders ($P = 0.011$). B, HOMA assessment at wk 0, 12, and 16 for subjects who responded to intervention with improved menstrual cyclicity (responders; n = 11) compared with those who responded to intervention with no improved menstrual cyclicity (nonresponders; n = 14; ◆, responders; ■, nonresponders). Data from wk 0–12 is during energy restriction, and data from wk 12–16 is during weight maintenance. a, Significant effect of time from wk 0–12 for the responders ($P = 0.006$). b, Significant change for responders compared with nonresponders ($P = 0.011$).

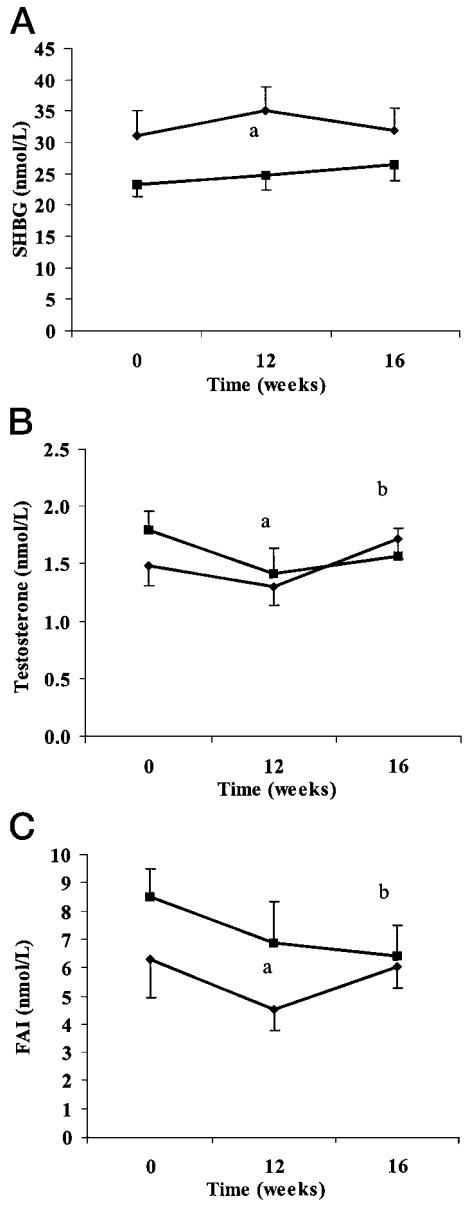

FIG. 4. Effect of weight loss with a LP compared with a HP diet on SHBG, testosterone, and FAI. A, SHBG at wk 0, 12, and 16 for subjects fed LP (n = 14) or HP diets (n = 14; ◆, LP; ■, HP). Data from wk 0–12 are during energy restriction, and data from wk 12–16 are during weight maintenance. a, Significant effect of time for the LP and HP groups from wk 0–12 ($P = 0.027$). b, Total testosterone at wk 0, 12, and 16 for subjects fed the LP (n = 14) or HP diet (n = 14; ◆, LP; ■, HP). Data from wk 0–12 are during energy restriction, and data from wk 12–16 are during weight maintenance. a, Significant effect of time for the LP and HP groups from wk 0–12 ($P = 0.010$). b, Significant effect of time for the LP and HP groups from wk 12–16 ($P = 0.038$). C, FAI at wk 0, 12, and 16 for subjects fed the LP (n = 14) or HP diet (n = 14; ◆, LP; ■, HP). Data from wk 0–12 are during energy restriction, and data from wk 12–16 are during weight maintenance. a, Significant effect of time from wk 0–12 for combined diets ($P = 0.004$). b, Significant change for LP diet compared with HP diet ($P = 0.011$; $P = 0.027$ for difference between diets).

4B). In weight maintenance, testosterone increased by 25.6% ($P = 0.038$). There was no significant effect of diet composition on testosterone or SHBG. Energy restriction decreased FAI by 18.2% ($P = 0.004$), with no significant differential effect of diet composition. A significant time × diet interaction was observed for FAI in weight maintenance ($P = 0.027$). In weight maintenance, FAI increased by 44% for the LP group ($P = 0.011$) to prebaseline levels and remained stable for the HP group (Fig. 4C). This change in FAI correlated with the change in testosterone ($r = 0.777$; $P < 0.001$), but not the change in SHBG ($r = 0.169$; $P = 0.389$). The FAI changes that occurred in weight maintenance were heavily dependent on a small number of individuals (two subjects), although these results were not classified as outliers. With these subjects removed, the effect of diet composition on change in FAI in weight maintenance was not significant ($P = 0.094$). There was no significant effect of diet or weight loss on LH, FSH, progesterone, or estradiol for the subset of nonovulators (n = 6).

Study power

The effect of diet composition showed nonstatistically significant effects for abdominal fat from wk 0–16 ($P = 0.094$). To confirm the observed differences between the groups of 0.8 kg to statistical significance of $P = 0.05$ and 80% power, 84 people would be needed. Although a significant effect of diet composition on FAI was observed in wk 12–16, the contributing factor for this finding cannot be determined because there was no significant effect of diet composition on SHBG ($P = 0.056$) or testosterone ($P = 0.252$) for wk 12–16. For the current number of people, this study had 80% power to detect the changes in SHBG. To confirm the observed differences in SHBG of 4.7 nmol/liter to 100% power and $P < 0.05$, 36 people would be needed. To confirm the observed differences in testosterone of 0.28 nmol/liter to 80% power and $P < 0.05$, 58 people would be needed.

Discussion

This study aimed to examine the effects of varying diet composition in energy restriction and weight maintenance on metabolic, endocrine, and clinical variables in PCOS. An HP diet resulted in minor differential improvements for HDL-C, TC/HDL-C, AUC for glucose, and FAI. However, there was no association between dietary composition and reproductive clinical parameters. Menstrual improvements appear to be correlated with changes in surrogate measures of insulin sensitivity independently of dietary composition.

Weight loss, energy restriction, and metabolic parameters

As previously shown, caloric restriction reduces abdominal fat (9, 10, 26) and improves hyperlipidemia (10) and insulin sensitivity (9–11, 27) in overweight women with PCOS. Abdominal fat has a strong association with insulin resistance, hyperandrogenism, and PCOS (9). Indeed, Huber-Buchholz *et al.* (11) found that abdominal fat loss was correlated with the restoration of ovulation. The reduction of abdominal fat observed in this study, independently of diet composition, thus has important implications for the improvement of metabolic and reproductive risk.

Weight loss, energy restriction, and clinical and endocrine parameters

We have confirmed that modest weight loss in overweight women with PCOS increases SHBG (9, 18, 27, 28), decreases FAI and testosterone, and improves menstrual cyclicity (9, 11, 12, 18, 26). The reduction in biochemical hyperandrogenism and the improvements in menstrual cyclicity, fertility, and insulin sensitivity are consistent with the postulated relationship between insulin resistance and hyperandrogenism (2).

Approximately 50% of subjects did not respond to treatment with improved menstrual cyclicity (nonresponders), as noted by other investigators (9, 11, 26). The observed disparity in HOMA and fasting insulin changes between the responders and nonresponders further supports the relationship between insulin resistance and hyperandrogenism. Although insulin sensitivity improvements in responders have been associated with changes in abdominal fat loss (11), we observed similar abdominal fat losses in both responders and nonresponders. Despite similar total weight (5%) and abdominal fat loss (11%) in this study comparison with our results, the small sample size (n = 21), more sensitive measure of insulin sensitivity by euglycemic hyperinsulinemic clamps, and measurement of abdominal fat by DEXA between the L2 and L4 vertebrae may partially account for the divergent results.

This is the first study that has examined the relative effects of energy restriction compared with weight loss on reproductive parameters. Clark *et al.* (12, 18) found fertility and menstrual improvements after weight loss, whereas Kiddy *et al.* (27) noted improvements during 4–6 wk of a very low calorie diet. We found that endocrine improvements occurred maximally during energy restriction corresponding to maximal changes in insulin sensitivity, suggesting a relationship between the two. Although strategies that maximize energy restriction may be optimal for restoring reproductive function, the effect of weight loss *per se* is more ambiguous. Short-term energy restriction studies in PCOS report decreases in fasting insulin, increases in SHBG, and decreases in testosterone as early as after 4 wk of energy restriction (27, 28). It is possible that the family of IGFs and their binding proteins may be involved, as they have been shown to be differentially affected by energy restriction (28) and weight loss (27). Specifically, IGF-binding protein-1 increases during short-term energy restriction mediated by decreased insulin levels, but remains unchanged with long-term weight loss. Increased IGF-binding protein-1 decreases free IGF-I, which down-regulates androgen synthesis through the cytochrome P450c17 system (29).

Diet composition and metabolic parameters

The few studies that have compared isocaloric replacement of protein for carbohydrate in weight loss have shown no differential changes in weight or abdominal fat loss, lipid and glucose metabolism, or fasting insulin (14, 16). Conversely, 12% increases in HDL-C (30) and 10% decreases in TC/HDL-C (31) have been reported for HP compared with the LP weight maintenance diets. HDL-C is known to decrease with caloric restriction and increase upon weight stabilization (32). This did not occur in the HP group, resulting in an improvement in the HP atherogenic profile (indicated by the TC/HDL-C ratio). Although substituting carbohydrate for dietary fat may lower HDL-C (32), exercise (33) counteracts this. The mechanism for these observed changes is unclear. However, low fat, high carbohydrate diets have previously been reported to decrease HDL-C through decreased production of its core protein, apolipoprotein AI (34). The dietary differences between groups (total fat, dietary fiber, and cholesterol) are of minimal clinical significance (35, 36).

The HP test meal decreased the postprandial glucose response compared with the LP test meal diet, probably due to the 38-g reduction in carbohydrate load. The MTT data are a reflection of the metabolic and pancreatic responses to a typical daily mixed food load. This may be of significance, as hyperglycemia, as measured by elevated glycosylated hemoglobin, is positively associated with cardiovascular dis-

ease morbidity and mortality (37). Additionally, hyperglycemia in pregnancy is associated with an increased risk of congenital abnormalities (38), although this has not been extensively studied in women without diabetes.

The MTT insulin results indicate a clear insulinemic effect of both test meals. Acute (39) and long-term (40) studies demonstrate that protein or amino acids stimulate insulin release. The protein source in this study, predominantly dairy, must be taken into consideration, as other protein sources exert different insulinemic responses (39) that may affect postprandial glucose and insulin homeostasis. The use of higher protein levels may modify the results (16). The use of an oral glucose tolerance test may have allowed us to directly compare glucose and insulin responses between subjects, although we have previously found that altering dietary composition had no differential effect on the oral glucose tolerance test (23).

Diet composition and clinical and endocrine parameters

In weight maintenance, some deterioration of the endocrine profile was observed on the LP diet. Conversely, the HP diet maintained the improvements that occurred in energy restriction. Whether or not this effect would be observed in a larger study requires further attention. No significant effect of diet composition was found for testosterone or SHBG; however, the significant correlation of changes in FAI with changes in testosterone indicates that this is probably the mediating factor. Additionally, this relationship is unlikely to be mediated by changes in abdominal fat or insulin sensitivity, as these parameters did not differ between the LP and HP groups.

There is a paucity of data examining the relationship between dietary factors and reproductive hormones. In a cross-sectional study, Longcope *et al.* (41) reported a positive correlation between dietary fiber and SHBG and a negative correlation between dietary protein and SHBG in men. An LP diet (10% protein and 70% carbohydrate) was associated with increased testosterone and SHBG concentrations compared with an HP (44% protein and 35% carbohydrate) diet in males (42). These associations may not be mirrored in females or subjects with PCOS, and further research is warranted.

The high drop-out rate, consequent reduced study power, and poor matching at baseline may have reduced the sensitivity of the results. Due to the small sample size, we cannot eliminate the possibility that no difference in diet composition was detected due to a type II error. DEXA does not distinguish between abdominal sc and visceral fat depots, which may be associated with differential metabolic risks (43). The use of more sensitive imaging techniques (*e.g.* magnetic resonance imaging) and exercise assessment tools might have clarified more subtle metabolic and anthropometric changes. As such, the above results must be interpreted with caution. A larger study with matching at baseline between a number of variables would aid in elucidating more accurate detail with increased power.

Conclusion

This unique study confirmed the effect of weight loss on improving metabolic, endocrine, and clinical parameters in overweight women with PCOS. Improvements occurred maximally in energy restriction and were maintained or reversed in weight maintenance, in some cases to prebaseline levels. Replacing protein for carbohydrate resulted in minor cardiovascular and reproductive improvements that did not appear to be mediated through differences in weight or abdominal fat loss or insulin sensitivity. Although enhanced reproductive function may be induced by caloric deficit and relatively small weight loss, the maintenance of reduced weight may be critical for reduced complications during pregnancy and birth and for reduction of cardiovascular and diabetic morbidity and mortality. Current dietetic and medical advice should continue to focus on weight loss as an important treatment goal in overweight women with PCOS with regard to reducing long-term risk. Conversely, maximizing caloric deficit may be a treatment strategy to achieve conception, and altering dietary composition may result in minor differential metabolic and reproductive improvements.

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