Effects of increased dietary protein-to-carbohydrate ratios in women with polycystic ovary syndrome1–3

Lone B Sørensen, Maibrit Søe, Kristiane H Halkier, Bjarne Stigsby, and Arne Astrup

ABSTRACT

Background: Some evidence has suggested that a diet with a higher ratio of protein to carbohydrates has metabolic advantages in the treatment of polycystic ovary syndrome (PCOS).

Objective: The objective of this study was to compare the effect of a high-protein (HP) diet to a standard-protein (SP) diet in women with PCOS.

Design: A controlled, 6-mo trial was conducted in 57 PCOS women. The women were assigned through rank minimization to one of the following 2 diets without caloric restriction: an HP diet (>40% of energy from protein and 30% of energy from fat) or an SP diet (<15% of energy from protein and 30% of energy from fat). The women received monthly dietary counseling. At baseline and 3 and 6 mo, anthropometric measurements were performed, and blood samples were collected.

Results: Seven women dropped out because of pregnancy, 23 women dropped out because of other reasons, and 27 women completed the study. The HP diet produced a greater weight loss (mean: 4.4 kg; 95% CI: 0.3, 8.6 kg) and body fat loss (mean: 4.3 kg; 95% CI: 0.9, 7.6 kg) than the SP diet after 6 mo. Waist circumference was reduced more by the HP diet than by the SP diet. The HP diet produced greater decreases in glucose than did the SP diet, which persisted after adjustment for weight changes. There were no differences in testosterone, sex hormone–binding globulin, and blood lipids between the groups after 6 mo. However, adjustment for weight changes led to significantly lower testosterone concentrations in the SP-diet group than in the HP-diet group.

Conclusion: Replacement of carbohydrates with protein in ad libitum diets improves weight loss and improves glucose metabolism by an effect that seems to be independent of the weight loss and, thus, seems to offer an improved dietary treatment of PCOS women. Am J Clin Nutr 2012;95:39–48.

INTRODUCTION

PCOS4 is the most common endocrine disorder in women and affects 6–8% of women of reproductive age (1). PCOS is associated with an increased risk of infertility, dysfunctional bleeding, obesity, type 2 diabetes, dyslipidemia, and possibly cardiovascular disease (1). The etiology of PCOS is multifactorial; the syndrome is a combination of genetic predisposition and influence from environmental factors (2). Insulin resistance and compensatory hyperinsulinemia play a significant part in the progression of the disorder. Approximately 80% of women with PCOS are insulin resistant with compensating hyperinsulinemia (3). Lean and overweight women with PCOS are more insulin resistant than are healthy women with similar BMI, even after adjustment for BMI, fat-free mass, and fat distribution (4). Insulin mediates excess androgen production directly and indirectly in PCOS women (5, 6). Insulin also inhibits the hepatic synthesis of SHBG (5, 7), which is a key circulating protein that binds to testosterone and, thus, increases the proportion of testosterone that circulates in the unbound free state.

Trials with lifestyle changes that led to weight loss in obese PCOS patients have shown beneficial effects on metabolic and endocrine abnormalities (8–11). PCOS often occurs in the presence of obesity, and therefore, most dietary intervention studies with PCOS women have focused on energy restriction as the first goal of treatment. Studies have shown that diets low in carbohydrates and high in protein can produce a clinically relevant weight loss and reduce insulin resistance and insulin concentrations in overweight and hyperinsulinemic individuals (12–14). Because insulin resistance and hyperinsulinemia play a significant role in PCOS and its metabolic and endocrine complications, both in lean and obese PCOS women, a replacement of carbohydrates with protein may be a possible strategy for the treatment of PCOS. A 24-wk pilot study investigated the effects of a low-carbohydrate diet on PCOS (15). This diet led to weight loss and improvements in both metabolic and reproductive abnormalities. Only a limited number of dietary interventions have compared the effects of an HP diet with a HC diet in PCOS women (16, 17). These studies included isocaloric energy-restricted diets, and one of their main endpoints was weight loss. Apparently no studies have investigated whether an HP/LC diet, per se, might improve reproductive and metabolic...
abnormalities in women with PCOS. The primary objective of the current study was to compare the effects of an ad libitum HP diet to those of an ad libitum SP diet on concentrations of total and free testosterone, and the secondary objective was to investigate effects on glucose metabolism, lipid profile, and body weight and composition in women with PCOS.

SUBJECTS AND METHODS

Study design

The study was a 6-mo, parallel group controlled trial. To secure similarity between groups, subjects were assigned to one of 2 diet groups by using the rank-minimization method (18). The prognostic factors used were age, height, weight, and concentrations of testosterone, SHBG, and C-peptide. For 6 mo, the women were to consume one of the following 2 ad libitum diets: an HP diet or an SP diet.

Subjects

Sixty-one women were recruited, and 57 women were allocated to the 2 diets as follows: 29 women were allocated to the HP diet, and 28 women were allocated to the SP diet. Four women withdrew consent after recruitment. Inclusion criteria were PCOS diagnosis defined by ≥2 of 3 symptoms according to international criteria (19), and free testosterone concentrations >0.034 nmol/L. Exclusion criteria were the use of hormonal contraception or other medications that could have altered the concentration of androgens, use of hormones as medication for 3 mo before inclusion, initiation of hormonal therapy during the study intervention and observation period, type 1 diabetes, or mental, educational, or religious conditions that could have made the subject unsuitable for the study. Originally, only women who were attempting to become pregnant were recruited because pregnancy was an outcome measure. However, the recruitment rate was very low, and it was decided to include women who had no intention of becoming pregnant. Therefore, women who left the study because of pregnancy were treated as dropouts.

Approval was obtained from the Ethical Committee of Copenhagen and Frederiksberg, and the study was performed in accordance with the Helsinki II Declaration. Each subject signed an informed consent document before the start of the study. The study began in October 2005.

Diets

During the 6-mo intervention, the macronutrient composition of the diets was controlled, but otherwise, there was no focus on energy restriction. The aim for the HP-diet group was to consume a diet that consisted of >40% of energy from protein, 30% of energy from fat, and <30% of energy from carbohydrates. The goal was to replace sugar and starchy carbohydrates with vegetables, fruit, nuts, and more protein. Protein sources were mainly meat, eggs, fish, and dairy products because vegetarian protein sources such as legumes have an HC content. The high (40% of energy) protein intake was chosen to ensure that the difference between the 2 diets was sufficient to reveal any actual effect.

The SP diet was intended to consist of <15% of energy from protein, 30% of energy from fat, and >55% of energy from carbohydrates, which the latter to also include sugar and starchy carbohydrates. The SP diet was consistent with the Nordic Nutrient Recommendations 2004 because these are relatively low in protein, high in carbohydrates, and moderate in fat (20).

Both diet groups were advised to limit intakes of sweets, cakes and soft drinks according to the 8 dietary guidelines published by the Danish Nutrition Council and the National Food Institute (21). Dietary counseling was provided by 2 skilled dietitians at the Department of Human Nutrition, University of Copenhagen. The first month included 2 dietitian visits; thereafter, there was one visit per month. The dietitians instructed subjects in the SP group in the 8 dietary guidelines (21) and gave customized advice to subjects in the HP group (Figure 1). The dietitians went through diet suggestions with subjects, with a focus on the composition of fat, carbohydrates, and protein, by using the plate model (22). Initially, the dietitians examined the background and PCOS history of each subject and explained the relation between PCOS and disease risk to subjects with the intention of improving their understanding of the syndrome and thereby encouraging adherence to the diet.

Compliance with the diet was evaluated at each visit through the use of food diaries, which the subjects were asked to keep on a daily basis for as long as possible. Independent of allocation to diet, subjects were recommended to be physically active ≥30 min/d in accordance with general recommendations (23).

Measurements

Body weight, fat mass, fat-free mass, and waist and hip circumferences were measured, blood samples were taken, and subjects completed 3-d dietary records and a 24-h urine collection (to validate the dietary records) at baseline and at 3 and 6 mo.

Anthropometric measures

Height was measured to the nearest 0.5 cm with a wall-mounted stadiometer at the screening visit. Body weight was measured to the nearest 0.1 kg with a digital scale (Tanita BWB-600; Frederiksberg Vægtfabrik). Waist and hip circumferences

A

1. Eat fruit and vegetables – 6 pieces/portions per day
2. Eat fish and fish products – several times a week
3. Eat potatoes, rice or pasta, and wholemeal bread – every day
4. Limit intake of sugar – particularly from soft drinks, confectionary and cakes
5. Eat less fat – particularly fats from meat and dairy products
6. Eat a varied diet – and maintain a healthy body weight
7. Drink water when you are thirsty
8. Engage in physical activity – at least 30 minutes per day

B

- Eat lean meat every day – 2-3 times per day
- Eat fish and fish products – several times per week
- Eat lean dairy products – without added sugar
- Eat fruit and vegetables – 6 pieces/portions per day
- Eat only whole grain products – whole grain rye bread and whole grain crisp bread
- Eat varied nuts - often
- Eat vegetable oil – e.g. replace butter with margarine
- Drink water when you are thirsty
- Engage in physical activity – at least 30 minutes per day

FIGURE 1. A: The 8 dietary guidelines from the Danish Dietary Recommendations 2004 (21). B: The 8 dietary guidelines customized to the high-protein diet.
were measured with a tape measure. Body composition was estimated by using bioelectric impedance (Ani meter; HTS- Engineering Inc). Fat mass and fat-free mass were calculated as previously described (24).

Physical activity
Queries about physical activity were performed at baseline, 3 mo, and at the end of the 6-mo intervention period. Queries about the habitual physical activity of subjects were obtained by using the self-administered questionnaire of Baecke et al (25).

Three-day weighed dietary records
To monitor the food consumption of subjects, three 3-d weighed dietary records were completed before the intervention and at 3 and 6 mo. Digital scales were used to weigh the food, and software (DANKOST 3000; Dansk Catering Center), which is based on the Danish food-composition tables (software version 6; The National Food Institute, Technical University of Denmark), was used to calculate energy and nutrient intakes.

Urine samples
Subjects collected 24-h urine samples during the third day of every dietary record period (baseline and 3 and 6 mo) to validate the dietary records. During these 24-h periods, subjects ingested a PABA tablet at each of the 3 main meals (a total of 240 mg PABA/d) to serve as an indicator of complete urine collection (26). Urine samples that contained <85% recovered PABA were excluded from further analyses. The volume and density of each 24-h urine collection were determined, and a 2-mL sample was included from further analyses. The volume and density of each 24-

Blood samples
Blood samples were drawn into test tubes. For glucose analyses, samples were drawn into tubes that contained EDTA and fluoride, and the tubes were kept on ice. For analyses of triacylglycerol, LDL cholesterol, HDL cholesterol, and TC, C-peptide, total and free testosterone, and SHBG, samples were drawn into tubes with no additives. Analyses of plasma glucose, triacylglycerol, and HDL cholesterol and TC were carried out on a chemistry analyzer (ADVIA 2400; Siemens Healthcare Diagnostics). Plasma glucose was analyzed by using standard enzymatic methods. TC concentrations were determined by enzymatic hydrolysis and oxidation. HDL concentrations were determined through cholesterol esterase, and oxidase reactions and triacylglycerol concentrations were determined with enzymatic hydrolysis by using lipase. Finally, LDL concentrations were determined by using Friedewald’s formula as follows:

$$\text{LDL} = \text{TC} - [\text{HDL} + (0.45 \times \text{triacylglycerol})]$$

Total testosterone was measured by a radioimmunoassay after ether extraction and subsequent celite chromatography. Intra-

Statistical analyses
To detect a decrease in free testosterone at 0.02 nmol/L [on the basis of Maciel et al (31)], with 80% power and an z value of 0.05, 22 subjects in each study group were needed (32). Sixty-one subjects were originally recruited to allow for dropouts.

The unpaired t test was used to test initial group differences (Tables 1–4). ANCOVA was used to test differences in dietary intakes between diet groups (HP and SP groups), time (3 and 6 mo), and the interaction between diet groups and time. Baseline values were included as covariates, and subjects were included as random factors (Table 2). The MIXED procedure in the Statistical Analysis System software package (SAS, version 9.1; SAS Institute) was used.

The effect of diet on body weight, fat mass, lean body mass, waist and hip circumferences, waist-hip ratio, total and free testosterone, SHBG, glucose, C-peptide, triacylglycerol, LDL cholesterol, HDL cholesterol, TC, triacylglycerol:HDL-cholesterol ratio, and TC:HDL-cholesterol ratio was tested by using ANCOVA with the MIXED procedure in the Statistical Analysis System software package (SAS, version 9.1 was used; SAS Institute) with 3- and 6-mo values as responses, baseline values as covariates, and subjects as random factors (Tables 3 and 4). When body weight changes were adjusted for, ANCOVA was used with 3- and 6-mo values as responses and baseline values and body-weight changes as covariates. Log transformation of data with skewed distributions was performed when necessary. ITT analysis was done using the approach of the last observation carried forward.

RESULTS
Sixty-one subjects were recruited to the study, and 57 subjects were assigned to either the HP diet (n = 29) or SP diet (n = 28).

TABLE 1
Subject characteristics at baselinea

<table>
<thead>
<tr>
<th></th>
<th>HP-diet group (n = 29)</th>
<th>SP-diet group (n = 28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>27.7 ± 5.5</td>
<td>28.4 ± 5.8</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>85.0 ± 21.5</td>
<td>84.3 ± 23.5</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.67 ± 0.05</td>
<td>1.66 ± 0.06</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>30.6 ± 7.8</td>
<td>30.5 ± 8.5</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>36.3 ± 17.3</td>
<td>35.5 ± 8.5</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>96.3 ± 17.3</td>
<td>95.6 ± 14.3</td>
</tr>
<tr>
<td>Plasma C-peptide (pmol/L)</td>
<td>896 ± 422</td>
<td>829 ± 325</td>
</tr>
<tr>
<td>Total testosterone (nmol/L)</td>
<td>2.41 ± 0.68</td>
<td>2.56 ± 0.70</td>
</tr>
<tr>
<td>Free testosterone (nmol/L)</td>
<td>0.048 ± 0.014</td>
<td>0.054 ± 0.014</td>
</tr>
<tr>
<td>SHBG (nmol/L)</td>
<td>43 ± 20</td>
<td>39 ± 19</td>
</tr>
</tbody>
</table>

a All values are means ± SDs. There were no significant differences between groups (unpaired t test). HP, high protein; SHBG, sex hormone binding globulin; SP, standard protein.
During the first 3 mo, 11 subjects in the HP-diet group and 12 subjects in the SP-diet group dropped out (3 subjects in each group dropped out because of pregnancy). During the last 3 mo of the intervention, 4 more subjects in the HP-diet group dropped out (one subject dropped out because of pregnancy), and 3 more subjects in the SP-diet group dropped out. Thus, 27
subjects completed the study (14 subjects in the HP-diet group and 13 subjects in the SP-diet group) (Figure 2). Because the dropout rate was 53%, only results from the completer analysis are shown (except for in Table 1, which shows successful assignment to study groups).

For completers, there were no differences at baseline in food intakes, physical activity, and anthropometric or biochemical characteristics between the 2 groups, except for a significant higher free testosterone concentration in the SP-diet group than in the HP-diet group (P = 0.03) (Table 4).

There were no between-group differences in changes in the amount or level of physical activity during the 6-mo intervention. The physical activity score increased by ~13% in both groups.

Food intake

Records of food intake revealed no significant differences between values at 3 and 6 mo within the same diet group (Table 2).

According to the food records, the numerical mean difference in energy intake between the SP- and HP-diet groups during the intervention was 0.57 MJ (95% CI: −0.56, 1.70 MJ; P = 0.16) (Table 2). Food records showed that the HP-diet group had significantly higher intakes of protein (in both grams and percentage of energy), total fat (in both grams and percentage of energy), monounsaturated fat (g/d) and cholesterol (mg/d) and a significantly lower intake of carbohydrates (in both grams and percentage of energy) compared with intakes of the SP-diet group (Table 2). There were no differences between groups in intakes of saturated and polyunsaturated fat (g/d), alcohol (g/d and percentage of energy) or dietary fiber (g/d).

Validation of protein intake

Urinary protein excretion was estimated in 61 urine samples after exclusion of 15 samples (6 samples from the HP-diet group and 9 samples from the SP-diet group) that were incomplete, as indicated by a <85% recovery of PABA. In the HP-diet group, data were available from 14 subjects at baseline, 10 subjects at 3 mo, and 12 subjects at 6 mo. In the SP-diet group, data were available from 10 subjects at baseline, 6 subjects at 3 mo, and 8 subjects at 6 mo. The protein intake estimated from the urinary nitrogen excretion was significantly higher in the HP-diet group than in the SP-diet group (P = 0.0005) (Table 2), and overall urinary protein was significantly higher than dietary protein. Urinary protein correlated significantly with dietary protein at all 3 time points in the HP-diet group, with the strongest correlation at baseline (baseline: \( r = 0.79, P = 0.001; 3 \text{ mo: } r = 0.69, P = 0.02; \) and 6 mo: \( r = 0.64, P = 0.02 \)). In the SP-diet group, urinary protein correlated significantly with dietary protein at 3 mo (\( r = 0.91, P = 0.0008 \)), and there was a borderline significant correlation at baseline (\( r = 0.58, P = 0.06 \)), but there was no correlation between urinary protein and dietary protein at 6 mo (\( r = 0.13, P = 0.7 \)).

Body weight and composition

Body weight and fat mass decreased in both the SP- and the HP-diet groups. However, body weight and fat mass decreased more in the HP-diet group, which resulted in significant between-group differences that amounted to 4.4 kg body weight (95% CI: 0.3, 8.6 kg body weight) and 4.3 kg body fat (0.9, 7.6 kg body fat) after 6 mo. There was no difference in lean body mass between the 2 groups during the 6-mo intervention (Table 3).
TABLE 4
Total and free testosterone, SHBG, plasma glucose, C-peptide, triacylglycerol, LDL cholesterol, HDL cholesterol, and TC before (baseline) and during 3 and 6 mo of dietary intervention in the HP-diet and SP-diet groups1

<table>
<thead>
<tr>
<th></th>
<th>Baseline2</th>
<th>3 mo</th>
<th>6 mo</th>
<th>LS means (adjusted for baseline values)</th>
<th>Rs</th>
<th>LS means (adjusted for baseline values and weight changes)</th>
<th>Rs</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total testosterone (nmol/L)3</strong></td>
<td></td>
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</tr>
<tr>
<td>HP</td>
<td>2.41 (1.90, 3.12)</td>
<td>2.45 (1.55, 2.65)</td>
<td>2.27 (1.70, 2.88)</td>
<td>2.37 (2.08, 2.71)</td>
<td>0.16</td>
<td>2.46 (2.16, 2.80)</td>
<td>0.03</td>
</tr>
<tr>
<td>SP</td>
<td>2.94 (2.39, 3.21)</td>
<td>1.70 (1.54, 2.43)</td>
<td>2.21 (1.95, 2.91)</td>
<td>2.04 (1.78, 2.34)</td>
<td>1.97 (1.72, 2.24)</td>
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<tr>
<td><strong>Free testosterone (nmol/L)3</strong></td>
<td></td>
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</tr>
<tr>
<td>HP</td>
<td>0.047 (0.035, 0.059)</td>
<td>0.038 (0.032, 0.057)</td>
<td>0.043 (0.028, 0.048)</td>
<td>0.044 (0.037, 0.052)</td>
<td>0.2</td>
<td>0.048 (0.041, 0.056)</td>
<td>0.008</td>
</tr>
<tr>
<td>SP</td>
<td>0.055 (0.049, 0.064)</td>
<td>0.033 (0.028, 0.054)</td>
<td>0.044 (0.039, 0.048)</td>
<td>0.037 (0.032, 0.044)</td>
<td>0.034 (0.029, 0.040)</td>
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<tr>
<td><strong>SHBG (nmol/L)5</strong></td>
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<tr>
<td>HP</td>
<td>43 (29, 64)</td>
<td>46 (24, 78)</td>
<td>54 (37, 67)</td>
<td>44 (37, 50)</td>
<td>0.98</td>
<td>42 (36, 48)</td>
<td>0.4</td>
</tr>
<tr>
<td>SP</td>
<td>34 (29, 45)</td>
<td>36 (35, 47)</td>
<td>43 (37, 53)</td>
<td>44 (38, 50)</td>
<td>46 (40, 53)</td>
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<tr>
<td><strong>Plasma glucose (mmol/L)7</strong></td>
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</tr>
<tr>
<td>HP</td>
<td>5.6 (5.2, 6.1)</td>
<td>5.1 (4.9, 5.4)</td>
<td>5.3 (5.1, 5.6)</td>
<td>5.2 (5.0, 5.3)</td>
<td>0.03</td>
<td>5.2 (5.0, 5.3)</td>
<td>0.03</td>
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<tr>
<td>SP</td>
<td>5.2 (5.0, 5.5)</td>
<td>5.4 (5.1, 5.6)</td>
<td>5.3 (5.0, 5.6)</td>
<td>5.4 (5.3, 5.6)</td>
<td>5.4 (5.3, 5.6)</td>
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<tr>
<td><strong>Plasma C-peptide (pmol/L)5</strong></td>
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</tr>
<tr>
<td>HP</td>
<td>908 (613, 1204)</td>
<td>711 (432, 990)</td>
<td>714 (494, 935)</td>
<td>647 (588, 707)</td>
<td>0.1</td>
<td>646 (584, 710)</td>
<td>0.1</td>
</tr>
<tr>
<td>SP</td>
<td>714 (595, 832)</td>
<td>623 (534, 711)</td>
<td>679 (559, 798)</td>
<td>719 (657, 781)</td>
<td>720 (654, 785)</td>
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</tr>
<tr>
<td><strong>Plasma triacylglycerol (mmol/L)5</strong></td>
<td></td>
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</tr>
<tr>
<td>HP</td>
<td>1.30 (0.79, 1.60)</td>
<td>0.65 (0.50, 1.00)</td>
<td>0.73 (0.60,1.03)</td>
<td>0.75 (0.64, 0.85)</td>
<td>0.3</td>
<td>0.74 (0.63, 0.86)</td>
<td>0.4</td>
</tr>
<tr>
<td>SP</td>
<td>0.86 (0.75, 1.21)</td>
<td>0.71 (0.63, 0.82)</td>
<td>0.85 (0.57, 1.01)</td>
<td>0.82 (0.71, 0.94)</td>
<td>0.81 (0.70, 0.95)</td>
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<tr>
<td><strong>Cholesterol (mmol/L)5</strong></td>
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<tr>
<td>HP</td>
<td>5.0 (4.0, 5.3)</td>
<td>4.3 (3.7, 4.7)</td>
<td>4.4 (4.4, 4.8)</td>
<td>4.5 (4.3, 4.8)</td>
<td>0.9</td>
<td>4.6 (4.3, 4.9)</td>
<td>0.3</td>
</tr>
<tr>
<td>SP</td>
<td>4.6 (4.3, 5.0)</td>
<td>4.4 (4.2, 4.5)</td>
<td>4.5 (4.2, 4.9)</td>
<td>4.5 (4.3, 4.8)</td>
<td>4.4 (4.2, 4.7)</td>
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<tr>
<td><strong>LDL cholesterol (mmol/L)5</strong></td>
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<tr>
<td>HP</td>
<td>3.1 (2.4, 3.3)</td>
<td>2.8 (2.4, 3.2)</td>
<td>2.8 (2.5, 3.2)</td>
<td>2.8 (2.6, 3.0)</td>
<td>0.9</td>
<td>2.8 (2.6, 3.0)</td>
<td>0.8</td>
</tr>
<tr>
<td>SP</td>
<td>2.7 (2.6, 2.9)</td>
<td>2.8 (2.5, 3.1)</td>
<td>2.7 (2.5, 3.1)</td>
<td>2.8 (2.6, 3.0)</td>
<td>2.8 (2.6, 3.0)</td>
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<tr>
<td><strong>HDL cholesterol (mmol/L)5</strong></td>
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</tr>
<tr>
<td>HP</td>
<td>1.30 (0.91, 1.48)</td>
<td>1.22 (1.00, 1.50)</td>
<td>1.30 (1.02, 1.70)</td>
<td>1.35 (1.23, 1.47)</td>
<td>0.3</td>
<td>1.37 (1.25, 1.50)</td>
<td>0.1</td>
</tr>
<tr>
<td>SP</td>
<td>1.46 (1.09, 1.50)</td>
<td>1.37 (1.04, 1.48)</td>
<td>1.29 (1.15, 1.53)</td>
<td>1.26 (1.15, 1.38)</td>
<td>1.24 (1.13, 1.36)</td>
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<tr>
<td><strong>Triacylglycerol:HDL-cholesterol ratio5</strong></td>
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</tr>
<tr>
<td>HP</td>
<td>1.09 (0.57, 1.50)</td>
<td>0.49 (0.34, 1.11)</td>
<td>0.55 (0.40, 1.20)</td>
<td>0.52 (0.43, 0.64)</td>
<td>0.08</td>
<td>0.51 (0.42, 0.62)</td>
<td>0.05</td>
</tr>
<tr>
<td>SP</td>
<td>0.75 (0.50, 1.06)</td>
<td>0.50 (0.45, 0.74)</td>
<td>0.65 (0.44, 0.84)</td>
<td>0.67 (0.55, 0.82)</td>
<td>0.69 (0.56, 0.85)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>TC:HDL-cholesterol ratio5</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HP</td>
<td>4.12 (3.07, 4.94)</td>
<td>3.20 (2.64, 4.44)</td>
<td>3.48 (2.73, 4.31)</td>
<td>3.30 (3.06, 3.57)</td>
<td>0.09</td>
<td>3.30 (3.04,3.58)</td>
<td>0.10</td>
</tr>
<tr>
<td>SP</td>
<td>3.47 (3.09, 4.02)</td>
<td>3.33 (3.14, 4.04)</td>
<td>3.67 (3.20, 3.90)</td>
<td>3.64 (3.37, 3.93)</td>
<td>3.65 (3.36, 3.95)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 Data were for 14 completers in the HP-diet group and 13 completers in the SP-diet group. HP, high protein; LS, least squares; SHBG, sex hormone binding globulin; SP, standard protein; TC, total cholesterol.

2 There were no significant differences in baseline values between the 2 groups (unpaired t test) except for free testosterone (P = 0.03).

3 P values were derived by ANCOVA with diet (HP and SP) and time (3 and 6 mo) as factors, baseline values as covariates, and subjects as random factors. There were no significant diet × time interactions.

4 P values were derived by ANCOVA with diet (HP and SP) and time (3 and 6 mo) as factors, baseline values and changes in body weight as covariates, and subjects as random factors. There were no significant diet × time interactions.

5 All data are medians (interquartile ranges) for variables with skewed distributions.

6 There was a borderline significant diet × time interaction for total testosterone (P = 0.06); post hoc tests showed a significantly lower total testosterone in the SP group than in the HP group (P = 0.02) at 3 mo. Total testosterone concentrations were significantly lower at 3 than at 6 mo in the SP-diet group (P = 0.01).

7 All data are means; 95% CIs in parentheses.

8 There was a significant time effect (P < 0.05).
Both groups had reduced waist circumferences, but there was a significant greater reduction in waist circumferences in the HP-diet group than in the SP-diet group. There were no differences in changes in either hip circumferences or waist-to-hip ratios in the 2 diet groups.

Testosterone and SHBG

After 6 mo of intervention, there were no significant differences in concentrations of total and free testosterone between the 2 groups (Table 4). However, there was a borderline significant time x diet interaction in concentrations of total testosterone ($P = 0.06$).

After adjustment for changes in body weight, total testosterone and free testosterone were significantly lower in the SP-diet group than in the HP-diet group (Table 4). In the ITT analysis, this was only a trend.

There was no significant difference in changes in concentrations of SHBG between the 2 groups, but a significant time effect was shown (Table 4). In the ITT analysis, the time effect was only a trend.

Glucose and C-peptide

After 6 mo of intervention, glucose concentrations were lower in the HP-diet group than in the SP-diet group, which was mainly because of a decrease in glucose concentrations in the HP-diet group (Table 4). The significance persisted after adjustment for changes in body weight. The same pattern was seen in the ITT analysis.

There was no significant difference in C-peptide concentrations between the 2 groups, both before and after adjustment for changes in body weight. In the ITT analysis, a lower concentration of C-peptide was seen in the HP-diet group than in the SP-diet group ($P = 0.04$). The significance persisted after adjustment for changes in body weight.

Blood lipids

No significant differences between groups were shown in triacylglycerol, HDL-cholesterol, LDL-cholesterol, or TC concentrations, or TC:HDL-cholesterol or triacylglycerol:HDL-cholesterol ratios during the intervention (Table 4). There was a trend for the TC:HDL-cholesterol and triacylglycerol:HDL-cholesterol ratio to be lower in the HP-diet group than in the SP-diet group after the intervention. After adjustment for body weight, the triacylglycerol: HDL-cholesterol ratio was borderline significantly lower in the HP-diet group than in the SP-diet group.

In the ITT analyses, lower responses in triacylglycerol concentrations ($P < 0.05$) and the triacylglycerol:HDL-cholesterol ratio ($P = 0.02$) and a trend for a lower TC:HDL-cholesterol ratio ($P = 0.09$) were seen in the HP-diet group than in the SP-diet group. After adjustment for body-weight changes, the significant difference in the triacylglycerol:HDL-cholesterol ratio remained ($P = 0.04$), whereas the significant differences in triacylglycerol concentrations and the TC:HDL-cholesterol ratio disappeared.

DISCUSSION

The hypothesis investigated in the current study was that an HP/LC diet would cause a decrease in plasma glucose and, thereby, reduce the release of insulin, which would result in a decrease in free testosterone. In participants who completed the
study, we showed that the intake of the ad libitum HP diet for 6 mo resulted in a greater weight loss and a decrease in glucose and a trend to lower C-peptide concentrations than did the intake of the ad libitum SP diet. ITT analyses showed a significantly lower C-peptide concentration in the HP-diet group. The effect of the HP diet on C-peptide and glucose persisted after adjustment for changes in body weight. There were no consistent effects on total and free testosterone concentrations.

To our knowledge, this study was the first to investigate the long-term effect of an ad libitum HP diet in PCOS patients. Other HP-diet studies in PCOS patients have included energy restrictions. In a 1-mo study, Stamets et al (17) examined the effects of 2 energy-restricted diets that were high in either protein (30% protein, 40% carbohydrate, and 30% fat) or carbohydrates (15% protein, 55% carbohydrates, and 30% fat) with a 1000-kcal deficit per day. Both diets resulted in significant weight loss and improvements in reproductive and metabolic abnormalities. However, no significant differences between the 2 groups were shown. Dietary adherence was not evaluated in the study, so the lack of difference between the diet groups could have been due to failure of the participants to correctly implement the diet.

Moran et al (16) investigated the effect of an HP diet on PCOS patients in an intervention consisting of 12 wk of energy restriction (~6000 kJ/d) followed by 4 wk of weight maintenance. Overweight PCOS women were randomly assigned to an HP diet (30% of energy from protein and 40% of energy from carbohydrates) or a low protein diet (15% of energy from protein and 55% of energy from carbohydrates). Data from the urine samples indicated good compliance. A mean weight loss of 7.7 ± 0.7 kg occurred with no significant difference between diet groups. During the energy-restriction period, testosterone decreased, and SHBG increased, in the same manner in both groups. Furthermore, improvements in insulin resistance and lipid profiles were independent of diet, apart from minor improvements for HDL cholesterol and TC:HDL cholesterol in the HP-diet group.

In a 2-mo study, Kasim-Karakas et al (33) investigated the effects of protein compared with simple sugar intake in overweight PCOS women. In this study, a hypocaloric diet supplemented with protein caused greater weight loss than a diet supplemented with simple sugars. Testosterone, SHBG, glucose metabolism, and triacylglycerol were not significantly affected by any of the diets, whereas total and HDL cholesterol decreased in the protein group.

In the current study, the greater weight loss in the HP-diet group did not result in lower testosterone than in the SP-diet group, which might have been expected on the basis of results of several lifestyle interventions in PCOS patients (8–11) and the energy-restricted studies previously mentioned (16, 17, 33). However, an improved glucose metabolism without a resulting decrease in testosterone was also seen in a pilot study by Mavropoulos et al (15) that investigated the effects of a low-carbohydrate ketogenic diet and in a recent study by Pasquali et al (34) that evaluated responsiveness to weight loss in overweight and obese PCOS women. In the latter study, overweight and obese women diagnosed with PCOS participated in a lifestyle intervention that consisted of a 1200–1400-kcal/d diet for 6 mo followed by a mildly energy-restricted diet plus increased physical activity. After the follow-up period, the women were reclassified into 3 groups according to persistence (15.4%), partial (47.7%), or complete disappearance (36.9%) of the diagnostic criteria used to define PCOS at baseline. Weight loss, fasting and glucose-stimulated insulin, and indexes of insulin resistance were similar in the 3 groups. These results implied that overweight and obese women with PCOS may respond disparately to weight loss.

In our study, the HP diet had a positive influence on glucose metabolism independent of weight loss. In contrast, Layman et al (35) observed that fasting glucose was unchanged after consumption of an HP diet for 10 wk, whereas glucose decreased in the HC-diet group. In the study, there was no difference in weight loss between groups. Other HP-diet studies have shown no effect of diet on glucose when there was a greater weight loss in the HP-diet group than in the HC-diet group (33, 36) and when there was no difference in weight loss (16, 17, 37). An explanation for these conflicting results in our study and the study of Layman et al (35) is not immediately evident. However, some differences between the studies may play a role. For example, the participants in our study were PCOS women, whereas the subjects in the study of Layman et al (35) were overweight but otherwise healthy women, and the trials differed greatly in length (ie, 6 mo in our study compared with 10 wk in the study of Layman et al (35)).

In the current study, we did not focus on the glycemic index, but whereas the participants in the HP-diet group were encouraged to avoid white and whole-grain bread, participants in the SP-diet group were allowed to eat both types of bread. In addition, carbohydrate intake was ~40% lower in the HP-diet group and the glycemic load was, therefore, lower in the HP diet than in the SP diet. In a recent study by Marsh et al (38), an ad libitum low–glycemic index diet improved insulin sensitivity more than a macronutrient-matched healthy diet, and the effect of diet remained after adjustment for weight changes.

In other ad libitum HP/LC studies, beneficial effects on triacylglycerol and HDL cholesterol have been seen (39). In our study, we only showed significant differences in blood lipids between the 2 groups during the intervention in the ITT analyses.

The lack of significant differences between groups in some of the variables in this study may have been due to the high dropout rate and the consequent reduction in power to detect changes. In the power calculations, a dropout rate of only ~30% was expected. Attrition rates were high in the 3 HP-diet studies with PCOS women previously mentioned, as well as in the current study. The dropout rates in these studies were 26% in the 1-mo study, 27% in the 2-mo study, and 37% in the 16-wk study (16, 17, 33). In a more recent 12-mo study that investigated glycemic index diets, the attrition rate was 49% (38). The high dropout rate in studies in PCOS women seems to be a general phenomenon, and the reasons for this phenomenon are not clear. Only 23% of the women who withdrew from the current study did so because of pregnancy.

In the current study, participants in the HP-diet group lost 7.7 kg, and participants in the SP-diet group lost 3.3 kg, although weight loss was not an aim according to the study protocol. Weight losses were achieved without focus on energy restriction and by using only an introduction to dietary guidelines, instructions on how to use the plate model, a talk about meal patterns, and a monthly visit to a skilled dietitian. However, the HP-diet group lost more than twice as much weight as the SP-diet group.
group, which suggested that the participants in the HP-diet group responded to something more than the extra focus on weight and a healthy lifestyle. In 1999, Skov et al (40) were the first to show how effective an HP/LC ad libitum diet is in producing weight loss, and other authors (39) have since confirmed this effect. Recently, Larsen et al (41) showed that even a modest increase in dietary protein was sufficient to minimize weight regain or promote further weight loss in obese patients after a successful weight-loss diet. These results suggested that it is not necessary for PCOS patients to follow energy-restricted diets because protein satiates so well that an HP diet will induce a spontaneous reduction in energy intake.

In conclusion, the replacement of carbohydrates with protein in ad libitum diets improves weight loss and improves glucose metabolism by an effect that seems to be independent of weight loss and, thus, seems to offer an improved dietary treatment of PCOS women.

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The authors’ responsibilities were as follows—BS, MS, and AA: designed research; MS and KHH conducted research; LBS: performed statistical analysis, wrote the manuscript, and had primary responsibility for the final content of the manuscript; and all authors: read and approved the final manuscript. LBS, MS, KHH, and AA reported that their department, the Department of Nutrition, University of Copenhagen, has received research support from more than 100 food companies for studies. AA served as an executive board member of Obesity International Trading (United Kingdom), Beer Knowledge Institute (Netherlands), Global Dairy Platform (United States), and Nordic Food Laboratory (Denmark), serving on the European Almond Advisory Board and the boards of 7TM Pharma, NeuroSearch, Basic Research, Merck, Johnson & Johnson Pharmaceutical Research & Development, Jenny Craig, and Kraft, acted as a consultant or advisory board member for 7TM Pharma, NeuroSearch, Basic Research, Merck, Johnson & Johnson Pharmaceutical Research & Development, Pfizer, Vivus, Jenny Craig, Almond Board of California, and Kraft, and received lecture fees from the Almond Board of California, Aria, Campina, and Astellas Pharma. BS declared no conflict of interest.

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