ORIGINAL ARTICLE

Favourable metabolic effects of a eucaloric lower-carbohydrate diet in women with PCOS

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Summary

Objective Diet-induced reduction in circulating insulin may be an attractive nonpharmacological treatment for women with polycystic ovary syndrome (PCOS) among whom elevated insulin may exacerbate symptoms by stimulating testosterone synthesis. This study was designed to determine whether a modest reduction in dietary carbohydrate (CHO) content affects β-cell responsiveness, serum testosterone concentration and insulin sensitivity in women with PCOS.

Design In a crossover design, two diets ('Standard,' STD, 55:18:27% energy from carbohydrate/protein/fat; lower-carbohydrate, 41:19:40) were provided for 8 weeks in random order with a 4-week washout between.

Patients Thirty women with PCOS.

Measurements β-cell responsiveness assessed as the C-peptide response to glucose during a liquid meal test; insulin sensitivity from insulin and glucose values throughout the test; insulin resistance (HOMA-IR); and total testosterone by immunoassay.

Results Paired *t*-test indicated that the lower-CHO diet induced significant decreases in basal β-cell response (PhiB), fasting insulin, fasting glucose, HOMA-IR, total testosterone and all cholesterol measures, and significant increases in insulin sensitivity and dynamic ('first-phase') β-cell response. The STD diet induced a decrease in HDL-C and an increase in the total cholesterol-to-HDL-C ratio. Across all data combined, the change in testosterone was positively associated with the changes in fasting insulin, PhiB and insulin AUC ($P < 0{\cdot}05$).

Conclusions In women with PCOS, modest reduction in dietary CHO in the context of a weight-maintaining diet has numerous beneficial effects on the metabolic profile that may lead to a decrease in circulating testosterone.

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Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women, with a prevalence of 6–10% based on the National Institutes of Health 1990 criteria and up to 15% using the Rotterdam 2003 criteria.[1] Principal features of the syndrome include biochemical or clinical hyperandrogenism, ovulatory dysfunction and polycystic ovaries.[2] At least two-thirds of women with PCOS also demonstrate concomitant insulin resistance, hyperinsulinism and metabolic dysfunction.[3,4] In these women, the higher concentration of insulin stimulates androgen production,[5] which in turn elicits the many characteristic features of PCOS. Treatment with metformin, which increases insulin sensitivity and decreases circulating insulin, has beneficial effects on both glucose metabolism and reproductive function.[6,7] However nonpharmacological therapies are limited to weight loss, which is difficult to achieve and maintain in the PCOS population.[8,9]

Reduction in insulin through dietary means may be an attractive nonpharmacological alternative. Reduction in dietary carbohydrate (CHO) would decrease the glucose stimulus to the β-cell and may thereby reduce the amount of insulin secreted on an acute basis. It is also possible that, on a chronic basis, a lower-CHO diet may reduce β-cell responsiveness to a fixed glucose stimulus. Although several studies have investigated the effect of diet quality in the context of weight loss,[10–14] few studies have probed the possible effect of diet composition on β-cell responsiveness and insulin sensitivity under weight-stable conditions. In a pilot study, we observed that 16 days of a eucaloric lower-CHO

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diet (43% energy from CHO) resulted in lower fasting insulin concentration and lower acute insulin response to intravenous glucose when compared with other experimental diets.[15] In this earlier study, we did not examine measures of β-cell function, which are defined as the C-peptide response to a given amount of glucose. Because C-peptide is secreted with insulin but is not cleared by the liver, it provides a more direct measure of β-cell responsiveness than do measures of peripheral insulin. Further, longer-term effects of the lower-CHO diet have not been investigated.

The objective of this study was to examine the effects of 8 weeks of controlled treatment with two eucaloric diets differing in CHO composition (41% *vs* 55%) on β-cell responsiveness, serum testosterone concentration and insulin sensitivity in women with PCOS. Secondary outcomes were other measures of the metabolic and reproductive endocrine profiles, and the lipid profile. We tested the hypothesis that a reduced-CHO diet would result in lower β-cell responsiveness to a fixed (standardized) liquid meal stimulus, lower circulating insulin, improved insulin sensitivity and lower testosterone when compared with a standard, higher-CHO, diet.

Methods

Participants

Thirty women with PCOS were enrolled in the study. The criteria for diagnosis of PCOS were consistent with the NIH 1990 criteria and included (i) hyperandrogenism and/or hyperandrogenaemia, (ii) oligo-ovulation and (iii) the exclusion of any existing disorders such as Cushing's syndrome, hyperprolactinaemia or congenital (nonclassic) adrenal hyperplasia. Inclusion criteria were BMI $\leq$45 kg/m^2, body weight <136 kg, age 21–50 years, nondiabetic and no weight change >2·3 kg over the past 6 months. Exclusion criteria included regular exercise >2 h per week, pregnancy, current breastfeeding, use of medication that could affect body composition or glucose metabolism (including oral contraceptives, cholesterol medications and blood pressure medications), current use of tobacco, use of illegal drugs in last 6 months, major food allergies or food dislikes, and a medical history that contraindicated inclusion in the study. Participants were evaluated for glucose tolerance using a 2-h, 75-g, oral glucose tolerance test. Participants were informed of the experimental design, and oral and written consent was obtained. The study was approved by the Institutional Review Board for Human Use at the University of Alabama at Birmingham (UAB).

Protocol

The study was conducted as a crossover design. Comprehensive metabolic testing was conducted before and after each arm, with a 4-week washout period in between arms (Fig. 1). All testing was conducted on an outpatient basis at UAB's Clinical Research Unit (CRU). After completing baseline testing, participants were assigned, using a randomization scheme, to one of two diets.

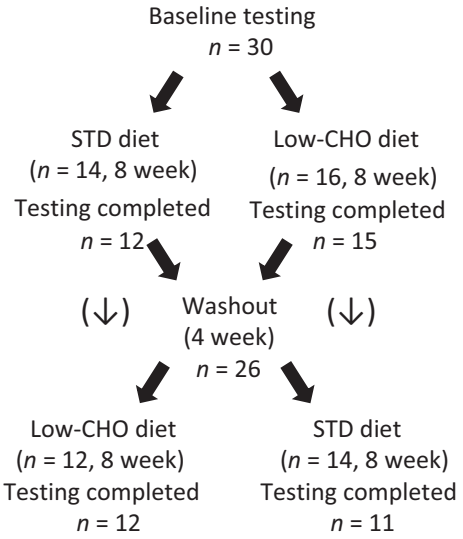

Fig. 1 Schematic diagram of crossover study design.

Details of the two diets are provided in Table 1. All food was supplied to the participants for the entirety of the two, 8-week intervention periods by the UAB CRU. The standard (STD) diet had a macronutrient composition of 55% carbohydrate/18% protein/27% fat (%energy from each), whereas the lower-CHO diet had a macronutrient composition of 41% carbohyrate/19% protein/40% fat. The glycaemic index of the standard diet was approximately 60, whereas that of the lower-CHO diet was approximately 50. The rationale for these compositions was to alter the CHO content sufficiently to see a response, but to remain within ranges that are normally consumed. Because protein stimulates insulin secretion, the protein content was intentionally calculated to be similar in both diets. The total energy content of the diet was determined using each individual's measured resting energy expenditure from indirect calorimetry with an activity factor of 1·35. After completing all testing for the first diet arm and the subsequent 4-week washout period, participants were tested again and then provided with the second diet for another 8 weeks and retested.

Liquid meal tolerance test

β-cell response to glucose was determined before and after each diet arm using glucose and C-peptide data obtained during a liquid meal tolerance test. Participants were required to fast for 12 h prior to the test. To perform the test, a flexible intravenous catheter was placed in the antecubital space of one arm. At time 'zero', a liquid meal was provided (Carnation Instant Breakfast and whole milk). The meal was calculated to provide 7 kcal/kg of body weight as 24% fat, 58·6% CHO and 17·4% protein. Participants were required to consume the meal within 5 min. Blood was drawn at −15 and −5 min before initiation of meal consumption (time 'zero'); every 5 min from time zero to 30 min; every 10 min from 30 to 180 min; and at 210 and 240 min. Sera were stored at −85 °C.

Table 1. Composition of the test diets. Data are shown for two representative energy levels (1800 and 2500 kcal/day)

kcal/day	Total CHO (g)	Total sugar (g)*	GL (total)	GL/1000 kcal	Total fiber (g)	SFA% (g)	MUFA% (g)	PUFA% (g)	ω3 (g)	Protein (g)
Standard diet (55:18:27% energy from CHO/protein/fat)										
1800	254	110	143	79·00	18	8% (17)	10% (20)	7% (13)	0·86	84
2500	350	166	192	76·60	23	8% (21)	9% (26)	8% (23)	1·30	118
Lower-CHO diet (41:19:40% energy from CHO/protein/fat)										
1800	187	79	81	45·90	22	12% (24)	14% (28)	11% (22)	1·76	86
2500	261	120	114	46·50	31	12% (34)	15% (40)	11% (29)	2·45	119

GL, glycaemic load; CHO, carbohydrate; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; ω3, omega-3 fatty acids.
*The sum of glucose, fructose, galactose, sucrose, lactose and maltose.

Glucose and C-peptide values were analysed for measures of β-cell function using mathematical modelling techniques developed for data generated from an oral challenge test[16] that uses the minimal model of C-peptide secretion and kinetics originally applied to intravenous glucose-graded infusion data[17] and incorporates a commonly used model of C-peptide kinetics.[18] Both a rate of change component for glucose-stimulated insulin secretion and a delay between the glucose stimulus and the β-cell response were incorporated. The model output from this procedure includes basal (PhiB), dynamic (PhiD), static (PhiS) and total (PhiTOT) β-cell response to glucose. A detailed description of these measures has been published.[19] PhiB reflects the amount of insulin secreted for a given amount of glucose during basal (fasted) conditions. PhiD reflects the amount of insulin secreted in response to an increase in blood glucose. It is a measure of the stimulatory effect of the rate at which glucose increases upon the secretion of stored insulin. The volume of insulin secreted during the dynamic phase is also generated by the model and is termed 'X0'. PhiS reflects the amount of insulin secreted for a given amount of glucose during nonfasted (above basal) conditions. PhiTOT reflects β-cell responsiveness across the entire test. Data were modelled using *SCIENTIST* software version 2.01 (Micromath Scientific Software, Saint Louis, MO, USA).

Insulin sensitivity was calculated using a formula based on insulin and glucose values throughout the meal test:[20] Insulin sensitivity index = 10 000/√[(mean fasting insulin × mean fasting glucose) × (mean postchallenge insulin x mean postchallenge glucose)]. The HOMA-IR insulin resistance index[21] was calculated using a formula based on fasting glucose and fasting insulin: Fasting Glucose (mg/dl) × Fasting Insulin (μU/ml)/405. Badly haemolysed serum samples were not used for insulin determinations due to spurious low values. As a result, data for two fasting insulin and HOMA-IR values were not available.

Analysis of glucose, hormones and lipids

Analyses were conducted in the Core Laboratory of the Center for Clinical and Translational Science, Nutrition Obesity Research Center and Diabetes Research and Training Center. Glucose, total cholesterol, HDL-cholesterol and triglycerides were measured using a SIRRUS analyzer (Stanbio Laboratory, Boerne, TX); LDL-C was calculated using the method of Friedewald.[22] The total cholesterol-to-HDL-C ratio was calculated; a ratio of 5 to 1 or lower is the recommended target range, with an optimum ratio of 3·5 to 1. Insulin was assayed by immunofluorescence on a TOSOH AIA-II analyzer (TOSOH Corp., South San Francisco, CA); intra-assay CV of 1·5% and interassay CV of 4·4%. C-peptide was assayed by immunofluorescence using the TOSOH analyzer; intra-assay CV of 1·7% and interassay CV of 2·6%.

Follicle stimulating hormone and LH were determined by immunofluorescence using the TOSOH: intra-assay CV 3·1%, interassay CV 3·0%, minimum detectable concentration 1·0 mIU/ml; intra-assay CV 4·2%, interassay CV 2·7%, minimum detectable concentration 0·2 mIU/ml, respectively. DHEA-S was determined by direct radioimmunoassay (Siemens Corp., Los Angeles, CA, USA, intra-assay CV 7·1%, interassay CV 5·5%, minimum detectable concentration 4·47 μg/dl). Total testosterone was determined by immunofluorescence using the TOSOH (intra-assay CV 2·4%, interassay CV 2·7%, minimum detectable concentration 10 ng/dl). Sex hormone binding globulin (SHBG) was determined using an immunoradiometric assay (Siemens Corp.); intra-assay CV was 4·0%, interassay CV was 8·0%, and minimum detectable concentration was 0·68 nM. The free androgen index (FAI) was calculated as [(total testosterone in nM/SHBG in nM) × 100].

Statistical analysis

Descriptive statistics were calculated for all variables of interest. Hormone concentrations, HOMA-IR, the insulin sensitivity index and the β-cell response measures were log-10-transformed to ensure a normal distribution. Paired *t*-tests within each diet arm were used to examine changes in main outcomes of interest. Spearman's correlation analysis was used to examine the association between changes in insulin outcome measures and changes in testosterone. One outlier (studentized residual >3) was examined for its effect on results (it did not affect results) and was omitted from graphical presentation of the data. $P < 0{\cdot}05$ was considered statistically significant. Correlation analysis was performed in all women combined, as well as within subgroups

stratified by baseline FAI (above and below the median value of 3·98). Analyses were performed using SAS (version 9.3; SAS Institute, Inc., Cary, NC, USA).

Results

Baseline characteristics of the study population are shown in Table 2. Of the 30 women who were originally enrolled, 23 completed both arms of the study and 27 completed the lower-CHO arm of the study. Four women discontinued the study due to issues unrelated to the study including scheduling conflicts; one had a positive pregnancy test; and two lost interest in the study and failed to comply with diet requirements. Dropouts did not differ from participants regarding BMI ($P = 0{\cdot}353$), age ($P = 0{\cdot}942$) or ethnic distribution (both approximately 50% African American and approximately 50% European American). Although the diets were calculated to maintain energy balance, total body weight fluctuated across the intervention in some women. On average, weight change was −1·30 kg for women on the standard arm and −1·66 kg for women on the lower-CHO arm and did not differ by diet arm ($P = 0{\cdot}558$).

Table 3 and Fig. 2 show main outcome data by diet arm. Paired *t*-test indicated that the lower-CHO diet resulted in significant decreases in HOMA-IR, PhiB, fasting insulin, fasting glucose and testosterone, and significant increases in insulin sensitivity and PhiD. Despite the higher fat content of the lower-CHO diet, the lipid profile improved significantly. The STD diet was associated with a decrease in HDL-C and an increase in the cholesterol-to-HDL-C ratio. When data from both diets were combined, the change in testosterone was positively associated with the changes in fasting insulin, PhiB and insulin AUC and tended to be inversely associated with the change in insulin sensitivity index (Table 4). The change in PhiD was not associated with the change in testosterone ($r = 0{\cdot}00$, $P = 0{\cdot}995$). In preliminary analyses, weight change was not found to be associated with any of the outcomes of interest and did not substantially alter results if included as a partial variable in correlation analysis. When data were stratified by baseline FAI (high *vs* low based on the median value), the changes in fasting insulin (Fig. 3) and PhiB were associated with the change in testosterone only within the subgroup with high FAI.

Table 2. Baseline characteristics of study participants ($n = 30$)

Variable	Mean ± SD, unless indicated, SI units	Mean ± SD, unless indicated, metric units
Ethnicity: (*n*; Caucasian/African American/Hispanic)	13/16/1	–
Age (years)	31·2 ± 5·8	–
Body mass index (kg/m^2)	31·8 ± 5·7	–
Fasting glucose	5·33 ± 0·50 mM	96·0 ± 9·0 mg/dl
Fasting insulin	51·6 ± 39·6 pM	8·6 ± 6·6 μU/ml
Total testosterone	1·86 ± 0·98 nM	53·7 ± 28·3 ng/dl
Sex hormone binding globulin	49·3 ± 21·0 nM	–
Free androgen index	4·3 ± 2·8	–
DHEA-S	4·36 ± 2·49 nM	160·8 ± 91·6 μg/dl
Oestradiol	284·9 ± 199·3 pM	77·6 ± 54·3 pg/ml
FSH	6·0 ± 2·2 IU/l	6·0 ± 2·2 mIU/ml
LH	7·3 ± 4·6 IU/l	7·3 ± 4·6 mIU/ml
Triglycerides	0·87 ± 0·32 mM	77·5 ± 28·7 mg/dl
Total cholesterol	4·76 ± 0·87 mM	184·3 ± 33·8 mg/dl
HDL-cholesterol	1·40 ± 0·38 mM	54·0 ± 14·7 mg/dl
LDL-cholesterol	2·97 ± 0·84 mM	114·8 ± 32·7 mg/dl
Total cholesterol/HDL-C	3·62 ± 1·03	–

Conclusions

In PCOS women with insulin resistance and hyperinsulinism, high concentrations of insulin are thought to stimulate androgen production and contribute to many of the associated features of the disorder. Thus, reduction in insulin through dietary means may be an attractive nonpharmacological treatment for this population. With this study, we tested the hypothesis that a reduced-CHO diet would result in lower measures of β-cell responsiveness and circulating insulin when compared with a standard higher-CHO diet. Results indicated that the lower-CHO diet intervention resulted in decreases in fasting glucose, fasting insulin, PhiB and testosterone, and a concomitant improvement in insulin sensitivity. Further, across both diets, decreases in fasting insulin, PhiB and insulin AUC were statistically associated with a decrease in circulating testosterone, particularly within women with a high baseline FAI. These data suggest that in hyperinsulinemic women with PCOS, modest reduction in dietary CHO in the context of a weight-maintaining diet may reduce fasting insulin and ultimately lead to a decrease in circulating testosterone.

We observed that PhiB, a measure of basal β-cell responsiveness that reflects basal or fasting insulin secretion, declined on the lower-CHO diet. Thus, over the 8 weeks of the diet intervention, the basal level of insulin release from the islets was downregulated. This was reflected in the lower fasting insulin also observed following the lower-CHO diet. Adequate dietary CHO is necessary to maintain β-cell function. However, the lower-CHO diet used in this study was 41% CHO, which is not extremely low. Nonetheless, even this slight decrease in dietary CHO had a significant, chronic effect on the β-cell.

Another possible explanation for the decrease in PhiB on the lower-CHO diet is a change in insulin sensitivity. We observed a significant increase in insulin sensitivity of 22% specifically on the lower-CHO diet, a change that would be expected to precipitate a decline in fasting insulin. Similarly, under weight loss conditions, a lower-CHO diet was more effective in improving insulin sensitivity than a conventional healthy diet in women with PCOS.[13] The insulin sensitivity index used in this study was a 'whole-body' index that reflects insulin action at both liver and skeletal muscle. In contrast, HOMA-IR, an index of insulin resistance, primarily reflects hepatic insulin action. In this study, the lower-CHO diet resulted in an increase in insulin sensitivity and a decrease in HOMA-IR. Future studies using clamp

Table 3. Baseline and 8-week outcomes by diet. Mean (SD)

	Standard (n = 23)		Lower CHO (n = 27)	
	Week 0	Week 8	Week 0	Week 8
PhiB (10^9/min)	8·0 (3·6)	6·8 (2·1)	9·0 (4·2)	7·3 (3·0)***
PHiD (10^9)	451·8 (99·0)	458·2 (81·7)	426·2 (87·9)	522·3 (148·2)***
X0 (pM)	1239·6 (1062·6)	1356·8 (951·0)	1141·0 (539·5)	1588·1 (817·4)***
PhiS (10^9/min)	70·6 (36·8)	62·8 (44·1)	54·6 (23·5)	62·8 (28·1)
PhiTOT (10^9/min)	70·8 (36·9)	63·0 (44·0)	54·8 (23·6)	63·0 (28·1)
Insulin sensitivity index	6·9 (4·4)	7·6 (4·8)	6·4 (4·2)	7·6 (5·0)*
HOMA-IR	1·9 (1·8)	1·5 (1·1)	2·4 (2·1)	1·7 (1·4)***
Fasting glucose (mM)	5·18 (0·55)	5·17 (0·59)	5·30 (0·47)	5·04 (0·47)**
Fasting insulin (pM)	48·0 (42·6)	37·2 (23·4)	58·8 (47·4)	43·2 (32·4)***
FSH (IU/l)	6·8 (3·5)	6·9 (3·7)	6·7 (2·5)	6·6 (5·8)
LH (IU/l)	9·0 (7·7)	14·1 (17·2)	9·7 (7·7)	8·4 (5·8)
SHBG (nM)	50·1 (23·6)	53·3 (23·9)	50·5 (22·8)	49·7 (25·6)
Testosterone (nM)	2·11 (1·11)	2·14 (1·22)	2·24 (1·71)	1·69 (0·69)*
FAI	5·2 (3·9)	4·8 (3·0)	5·2 (4·5)	4·3 (2·7)
Cholesterol (mM)	4·66 (0·82)	4·56 (0·78)	4·75 (0·84)	4·22 (0·65)***
LDL-C (mM)	2·85 (0·75)	2·84 (0·79)	2·97 (0·84)	2·56 (0·66)**
HDL-C (mM)	1·40 (0·40)	1·27 (0·39)**	1·38 (0·39)	1·27 (0·39)*
Chol/HDL-C	3·54 (0·95)	3·88 (1·16)*	3·68 (1·08)	3·58 (0·97)
Triglyceride (mM)	0·90 (0·37)	0·92 (0·36)	0·87 (0·34)	0·87 (0·37)

*$P < 0·05$, **$P < 0·01$, ***$P < 0·001$ *vs* week 0 of the same diet by paired t-test.
PhiB, basal beta-cell response to glucose; PhiD, dynamic beta-cell response to glucose; X0, volume of insulin secreted in first phase; PhiS, static beta-cell response to glucose; PhiTOT, total beta-cell response to glucose.

methodology are needed to identify the tissue location of the improvement in insulin sensitivity.

In contrast to the decrease in PhiB noted with the lower-CHO diet, PhiD (first-phase response) increased. A low or inadequate first-phase β-cell response is one of the first signs of impaired β-cell function prior to the onset of impaired glucose tolerance or type 2 diabetes.[23] It is thought that this decline in acute insulin release occurs secondary to elevated glucose [24–27] and reduction in fasting glucose is associated with increased PhiD in individuals with impaired fasting glucose.[28] In the current study, the lower-CHO diet was associated with a decrease in fasting glucose. Thus, it is possible that the increase in PhiD occurred secondary to the decrease in fasting glucose and reflects a favourable change in β-cell health that would decrease risk for type 2 diabetes. The increase in PhiD was not associated with the change in testosterone, suggesting that this acute increase in insulin following a meal may not be relevant for androgen production in women with PCOS.

Results from this study differ from those of a similar diet intervention study conducted with overweight/obese men and non-PCOS women where it was observed that the lower-CHO diet appeared to have detrimental effects on metabolic health.[28,29] It is difficult to directly compare the two studies, because the earlier study differed regarding subject population, use of a 3-day run-in period and the adjustment of food quantity to maintain body weight. Individuals who are overweight/obese (previous study) may be more sensitive to adverse effects of the elevated fat content of the lower-CHO diet. In contrast, women with PCOS are highly insulin resistant and highly sensitive to dietary glucose,[30] characteristics that may bear on their response to dietary CHO.

The ultimate goal of the diet intervention was to lower testosterone by way of lowering insulin. In this study, the lower-CHO diet was associated with a 27% reduction in fasting insulin ($P < 0·001$) and a 23% reduction in serum testosterone ($P < 0·05$). The effect of the lower-CHO diet on SHBG did not approach significance ($P = 0·768$), suggesting that the major effect of the diet was on total testosterone synthesis and not the amount bound to SHBG. When data from both arms were pooled, the changes in fasting insulin and insulin AUC were significantly correlated with the change in testosterone. This was particularly striking within women with a high baseline FAI. Thus, our data support the notion that manipulation of insulin can have favourable effects on testosterone concentration in women with PCOS. Clearly, this notion has ample support given that metformin treatment results in declines in both insulin and testosterone.[7] However, not all women may wish to adhere to a chronic drug regimen. Further, the 23% decline in testosterone observed in this study in response to diet was greater than the 7% decline observed in response to metformin in a recent large clinical trial.[6] The possible use of diet as a therapeutic option has clear advantages, but until now, little documented support.

Despite the higher fat content of the lower-CHO diet, the lipid profile improved, with decreases observed in all measures of cholesterol, and no changes in triglycerides. In contrast, the STD diet induced a significant increase in the total cholesterol-to-HDL-C ratio. With the intervention, women on the

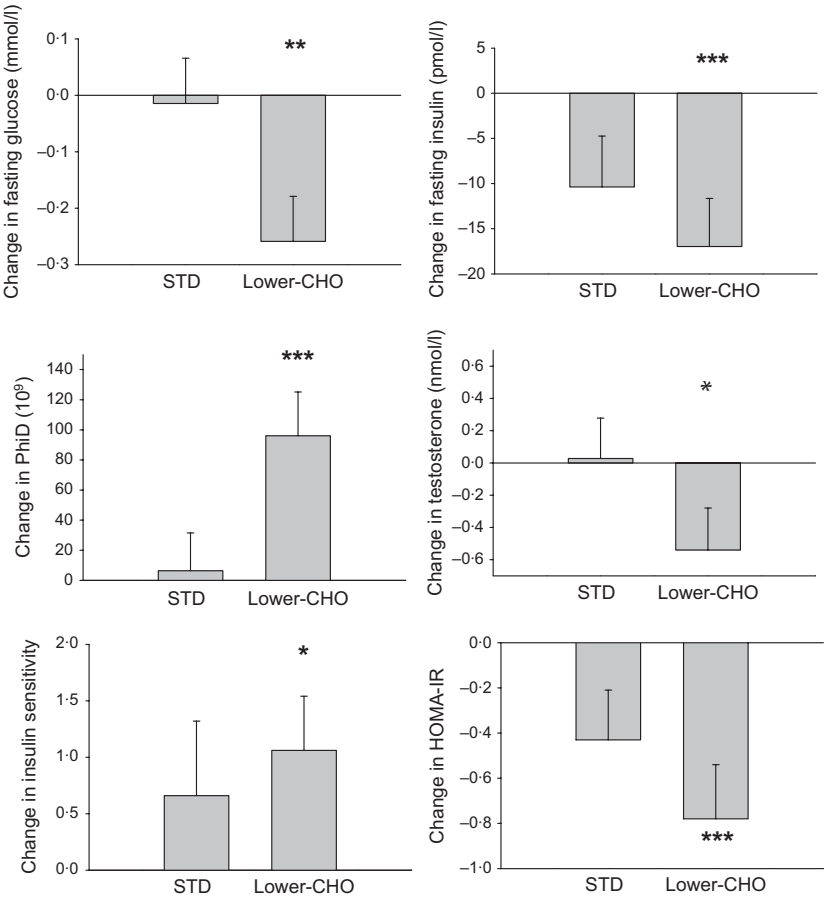

Fig. 2 Changes in fasting glucose, fasting insulin, PhiD, serum testosterone, insulin sensitivity and HOMA-IR by diet. $^{*}P < 0{\cdot}05$, $^{**}P < 0{\cdot}01$, $^{***}P < 0{\cdot}001$.

Table 4. Spearman's correlation coefficients for the change in testosterone among all women combined and stratified by high *vs* low free androgen index (FAI). Data shown are Spearman's correlation coefficient (*P* value)

Change in testosterone *vs*	All women	Low FAI	High FAI
Change in fasting insulin	0·36 (0·011)	0·15 (0·492)	0·50 (0·013)
Change in PhiB	0·33 (0·018)	0·20 (0·321)	0·50 (0·011)
Change in PhiD	0·00 (0·995)	−0·04 (0·857)	0·04 (0·815)
Change in insulin AUC	0·29 (0·041)	0·20 (0·347)	0·33 (0·112)
Change in insulin sensitivity	−0·28 (0·053)	−0·36 (0·076)	−0·17 (0·426)

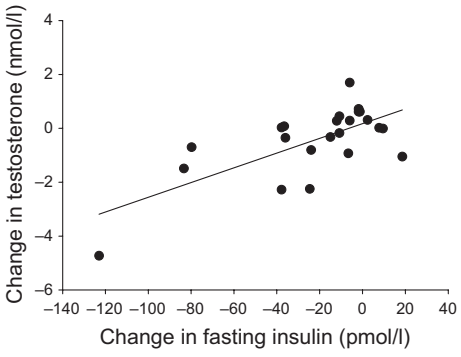

Fig. 3 The association between the change in fasting insulin and the change in serum total testosterone among women with high FAI at baseline (Spearman's $r = 0{\cdot}50$, $P = 0{\cdot}013$). Data from both intervention arms combined.

STD diet achieved a ratio of 3·88, a value that put them above the optimum of 3·5. Similarly, under weight loss conditions, a diet enriched in simple sugar had a less favourable effect on the lipid profile in women with PCOS than a diet enriched in whey protein.[14] Data suggest that high CHO diets lead to elevation in triglycerides[31] in a dose-dependent manner[32,33] and that lower-CHO diets may be beneficial for triglyceride lowering.[34] Neither of the diets used in this study had significant effects on triglycerides, possibly due to their moderate nature.

Although the goal of this study was to examine the effect of dietary CHO manipulation, it is impossible to completely match the diets for all other elements of diet quality. Thus, the lower-CHO diet was necessarily higher in fat, and it also contained a greater amount of fibre. It is possible that the greater fibre of the lower-CHO diet contributed to its observed beneficial effects.

Fibre has documented beneficial effects on, or associations with, insulin sensitivity[35] and risk for diabetes,[36] although results vary.[37]

Few data are available concerning effects of diet macronutrient composition on β-cell response in women with PCOS. One recent study examined the effect of a free-living lower-CHO/higher-protein diet *vs* standard diet matched for fat content on endocrine and body composition measures.[38] The lower-CHO/higher-protein group was reported to have lower fasting glucose, and in intention-to-treat analysis, also had lower fasting C-peptide. Several previous studies have examined the effects of manipulation of diet quality under weight loss conditions.[10–14] Results indicated either no effect of diet type or a beneficial effect of diets having lower glycaemic index, lower CHO, lower sugar or higher protein on outcomes such as fasting insulin, fasting glucose, insulin sensitivity and the lipid profile. However, results were not always independent of weight loss, which had a profoundly beneficial effect on most outcomes. Thus, the current results are unique for several reasons, including the eucaloric study design, the provision of all food, the uniform protein composition of the two test diets and the use of C-peptide-based, model-derived measures of β-cell responsiveness.

This study has several strengths. No previous study has examined the effect of diet macronutrient composition on β-cell response in women with PCOS using robust measures. Further, this study was conducted under controlled, eucaloric conditions. Limitations were the broad age range of the participants and small sample size, which did not permit subgroup analysis based on glucose tolerance status. The inclusion of only women with a BMI ≤ 45 kg/m^2 not using hormonal or drug therapy may limit the generalizability of the results. Likewise, because the NIH criteria were used for diagnosing PCOS, results may not be generalizable to PCOS as defined using other criteria. Results may have been biased by including only data from those who completed at least one arm of the intervention.

In conclusion, 8 weeks of a eucaloric 41% carbohydrate diet was associated with significant improvements in measures of carbohydrate metabolism and a 23% reduction in testosterone that may have been driven by the decrease in insulin. These results suggest that even in the absence of weight loss, dietary strategies that minimize carbohydrates may have numerous beneficial effects on the metabolic and reproductive health of hyperinsulinaemic women with polycystic ovary syndrome.

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Conflict of interest

Nothing to declare.

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