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Metabolic and Endocrine Effects of a Polyunsaturated Fatty Acid-Rich Diet in Polycystic Ovary Syndrome

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Effects of a polyunsaturated fatty acid (PUFA)-rich diet were investigated in 17 polycystic ovary syndrome (PCOS) patients. After a 3-month habitual diet period, dietary fats were partly replaced with PUFAs for another 3 months. The PUFA-rich diet increased plasma linoleic acid from 28.36 ± 1.00% to 33.76 ± 1.08% ($P < 0.002$) and α-linolenic acid from 0.52 ± 0.03% to 1.06 ± 0.10% ($P < 0.0001$). Fasting glucose increased from 76 ± 3 to 95 ± 3 mg/dl (4.2 ± 0.2 to 5.30.2 mmol/liter; $P < 0.0001$), and the area under the curve for glucose during oral glucose tolerance test increased from 421 ± 34 to 503 ± 31 mg/dl (23.4 ± 1.9 to 27.9 ± 1.7 mmol/liter; $P < 0.001$). Plasma insulin did not change either at fasting or during oral glucose tolerance test. Fasting plasma free fatty acids decreased from 0.596 ± 0.048 to 0.445 ± 0.058 mg/dl ($P = 0.037$), and ketone bodies decreased from 9.14 ± 1.57 to 3.63 ± 0.62 mg/dl (895 ± 154 to 356 ± 61 μmol/liter; $P < 0.003$). Plasma 15-deoxyprostaglandin J_2 tended to decrease (from 239 ± 65 to 171 ± 60 ng/ml; $P = 0.053$). Plasma testosterone, free testosterone, SHBG, dehydroepiandrosterone sulfate, LH, FSH, and urinary estrogen conjugates did not change. Urinary pregnanediol 3-glucuronide increased from 18.6 ± 2.2 to 31.0 ± 5.7 μg/mg creatinine ($P = 0.038$). In conclusion, increased dietary PUFA intake can exert significant metabolic and endocrine effects in women with PCOS. (*J Clin Endocrinol Metab* 89: 615–620, 2004)

POLYCYSTIC OVARY SYNDROME (PCOS) affects 1 of 16 women. It is characterized by amenorrhea or oligomenorrhea and androgen excess (1, 2). The majority of the patients are insulin resistant and develop impaired glucose tolerance or diabetes mellitus (DM) at an early age. Treatment of insulin resistance by diet, exercise, or insulin sensitizers improves gonadal function and decreases androgen excess (3, 4).

To date, nutritional studies in PCOS patients focused on the effects of energy restriction and weight loss (5–8). The effects of dietary composition started to receive attention only recently; Moran *et al.* (9) studied the effects of the protein content of energy-restricted diets. Here, we investigated dietary polyunsaturated fatty acids (PUFAs) for the following reasons. First, PUFAs can affect glucose homeostasis both favorably and adversely. Although a recent epidemiological study showed that the essential n-6 PUFA, linoleic acid (LA; 18:2 n-6), protects against type 2 DM (10), a nutrition intervention demonstrated that an LA-rich diet increased both glucose and insulin levels in men with type 2 DM (11). We had also previously reported the adverse effects of marine oils on glycemic control in type 2 DM (12). Second, experimental evidence indicates that PUFAs improve insulin action in peripheral tissues and decrease insulin secretion from the pancreas (13–16). Third, PUFAs and their products [*i.e.* 15-deoxyprostaglandin J_2(15d-PGDJ_2)] might serve as natural ligands for peroxisomal proliferator-activated receptor γ (PPARγ) (17). As synthetic PPARγ ligands are used for treatment of insulin resistance in PCOS (3, 4, 18), it is conceivable that dietary PUFAs might also decrease insulin resistance. Changes in two adipose tissue-derived proteins that affect insulin action, adiponectin and TNFα, were also investigated because adiponectin increases, whereas TNFα decreases, insulin sensitivity (19, 20).

Walnuts were used to enrich the diet with n-6 and n-3 essential PUFAs because they are a rich source of LA and α-linolenic acid (α-LNA; 18:3 n-3). We have previously demonstrated the efficacy of this approach in patients with combined hyperlipidemia (21).

In an average diet, 6% of the energy is provided by PUFAs. Women with PCOS are frequently recommended to take PUFA-rich supplements, although metabolic and endocrine effects of PUFAs have never been investigated in this patient population. We postulated that increased PUFA intake might decrease insulin resistance, prevent excess insulin secretion, and, consequently, decrease the androgen excess and improve gonadal function in PCOS.

Subjects and Methods

Experimental subjects

Women who were previously diagnosed with PCOS were recruited from the local chapters of the PCOS Support Group and the local community after signing informed consent approved by the human subjects committee of University of California-Davis. Of 222 women who re-

Abbreviations: AUC, Area under the curve; BMI, body mass index; cr, creatinine; CV, coefficient of variation; DHEAS, dehydroepiandrosterone sulfate; DM, diabetes mellitus; E1C, estrogen conjugate; HDL, high density lipoprotein; HOMA, homeostasis model assessment for insulin resistance; ISI, insulin sensitivity index; LA, linoleic acid; α-LNA, α-linolenic acid; 15d-PGDJ_2; 15-deoxyprostaglandin J_2; OGTT, oral glucose tolerance test; PCOS, polycystic ovary syndrome; PdG, pregnanediol 3-glucuronide; PPAR, peroxisomal proliferator-activated receptor; PUFA, polyunsaturated fatty acid; T, testosterone.

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sponded by telephone or electronic mail, 53 attended the information meetings, 32 were evaluated by physical examinations and screening laboratories, 28 were invited to participate in the study, and 24 were enrolled. The diagnosis of PCOS was based on the current diagnostic criteria: amenorrhea or oligomenorrhea (fewer than 6 menstrual periods/yr); and hyperandrogenemia [elevated total or free testosterone (T)] in the absence of adult-onset 21-hydroxylase deficiency, hyperprolactinemia, or androgen-secreting tumors (1, 2). Exclusion criteria consisted of habitual dietary intake of less than 30% fat, and systemic illnesses, such as renal, hepatic, or gastrointestinal diseases; DM; hyperlipidemias; hypertension requiring medication; smoking; and alcohol intake (>2 drinks/wk). Treatment with oral contraceptives, insulin sensitizers, *d*-chiro inositol, or any other supplements affecting weight or insulin sensitivity was discontinued at least 2 months before the study.

Study design

Dietary intervention. The study comprised an initial 3-month control period, followed by a 3-month intervention period, which were not randomized. The intervention diet aimed to replace dietary fats with PUFAs without changing the intakes of total fat, total energy, carbohydrate, protein, and micronutrients. The walnut intake was calculated as 48 g walnuts/800 kcal energy intake. Forty-eight grams of walnuts contain 311 kcal (70 kcal from 30 g fat, 28 kcal from 7 g protein, and 36 kcal from 9 g carbohydrates) and provide 19 g LA and 3.3 g α-LNA. The daily supplies of walnuts were weighed and packed individually by the study personnel. One-month's supply of daily packages was distributed during each study visit.

Implementation of the diet. During the control period, the participants were instructed to follow their own habitual diets without any change. They did not receive any nutrition education to avoid influencing their usual dietary habits. At the start of the intervention, the participants received 3 h of intensive group education and 2 h of individual counseling on the fat content and composition of food items, calculation of energy intake from fat, cooking, and shopping. Once a month each participant met with the dietitian to reinforce the diet and to address individual questions.

The participants were instructed not to change their physical activity throughout the study, and physical activity was monitored by monthly questionnaires.

Data collection

Nutrition assessment. Seven-day food records were obtained once a month and analyzed using the updated version of Nutrition Data Systems 93 (University of Minnesota, Minneapolis, MN). Compliance was also assessed objectively by measuring the fatty acid composition of total plasma lipids by gas chromatography.

Anthropometric variables. Body weight and waist circumference were measured monthly. Body composition was measured at the beginning and end of the study using bioelectrical impedance (Biostat Co., Isle of Man, UK) (22).

Metabolic and endocrine variables. Fasting plasma glucose, insulin, free fatty acids, triglyceride, total cholesterol, high density lipoprotein (HDL) cholesterol, ketone bodies, adiponectin, TNFα, T, free T, SHBG, dehydroepiandrosterone sulfate (DHEAS), LH, FSH, and 15d-$PGDJ_2$ were measured. Standard oral glucose tolerance tests (OGTTs; 75 g glucose) were carried out, and areas under the curve (AUCs) for glucose and insulin were calculated. Homeostasis model assessment for insulin resistance (HOMA) and insulin sensitivity index (ISI) were also calculated using the following formulas (23, 24): HOMA = $(22.5 \times 18) \div$ (fasting plasma glucose $\times$ fasting plasma insulin), and ISI = $10{,}000 \div$ square root of (mean OGTT glucose concentration $\times$ mean OGTT insulin concentration).

Samples for glucose were collected in sodium fluoride-containing tubes, and glucose was measured by the glucose oxidase method with a coefficient of variation (CV) of 1.4% (Sigma-Aldrich Corp., St. Louis, MO), insulin was measured by RIA with a CV of 8.2% (ICN Diagnostics, Costa Mesa, CA), and adiponectin was determined by RIA with a CV of 7% (Linco Research, Inc., St. Charles, MO). TNFα was measured by the Cytokine Core Laboratory of University of Maryland (Baltimore, MD) with a CV of 13.3%. Triglyceride and cholesterol were measured enzymatically using kits (Sigma-Aldrich Corp.) with CVs of 3.6% and 1.9%, respectively. HDLs were separated using dextran sulfate/magnesium chloride precipitation. The CV for HDL cholesterol was 2%. Free fatty acids and ketone bodies were measured by enzymatic methods [Wako (Richmond, VA) and Sigma-Aldrich Corp.] with CVs of 3% and 1.8%. 15d-PGJ_2 was measured using an enzyme immunoassay kit with a CV of 5.7% (Assay Designs, Inc., Ann Arbor, MI).

Sex steroid hormones and gonadotropins. Throughout the study, the daily first morning urine samples were collected, immediately frozen, stored in the participants' own freezers, and delivered to the study site once a month. For vacations and trips, special containers and dry ice were provided. In these samples, estrogen conjugates (E1C) and pregnanediol 3-glucuronide (PdG) were measured using competitive, microtiter, solid phase enzyme immunoassays (25, 26). These values correlate very closely with plasma estradiol and progesterone, with 1- to 2-d delay, and provide more detailed information. This is especially important for PCOS patients, because they do not have regular menstrual cycles. E1C and PdG concentrations were indexed to the creatinine (cr) concentration of the same sample. The sensitivity of the E1C assay was 7.8 ng/ml, and that of PdG assay was 0.15 μg/ml. The intraassay CVs for high and low internal controls were 14.7% and 13.1% for E1C, and 15.6% and 12.9% for PdG, respectively. The AUCs for E1C and PdG were calculated for each month. Plasma total and free T, SHBG, and DHEAS were measured by RIA, and LH and FSH were measured by immunoradiometric assay (Diagnostic Systems Laboratories, Webster, TX). The CVs were 8.3% for T, 7.9% for free T, 4.4% for SHBG, 9.6% for DHEAS, 7.3% for LH, and 6.2% for FSH.

To establish a reference, these hormones were also measured in a group of lean, healthy women with normal ovarian function [n = 19; age, 40 $\pm$ 1 yr; body mass index (BMI), 23.9 $\pm$ 1.5 kg/m^2] who participated in a previous study (27), and the following results were obtained: T, 0.27 $\pm$ 0.029 ng/ml; SHBG, 68.5 $\pm$ 6.6 nmol/liter, DHEAS,116 $\pm$ 24 ng/ml; E1C, 1380 $\pm$ 155 ng/mg cr; and PdG, 69.2 $\pm$ 7.0 μg/mg cr.

The fatty acid composition of plasma total lipids was measured by gas chromatography at the Clinical Nutrition Research Unit of University of California-Davis and is reported as percent weight.

Statistical analysis

Data were analyzed using SAS for TSO40 (release 6.12, mixed and correlations procedures) (SAS Institute, Inc., Cary, NC) (28). Participants served as their own controls. The significance for the effects of the PUFA diet was analyzed using a repeated measures mixed model with subject as a random effect and the PUFA diet and body mass index as fixed effects, together with an unstructured covariance matrix. Normality was checked by the Shapiro-Wilks test as well as Q-Q plots (PROC UNIVARIATE). Glucose levels before and after the PUFA diet were compared over the 2-h OGTT period using a repeated measures model, with PUFA diet and time as fixed covariates and subject as a random effect; the covariance matrix was unstructured. A Bonferroni adjustment was made for the glucose comparisons at each of the five time periods (baseline and 30, 60, 90, and 120 min).

Results

Subjects

One participant became pregnant and discontinued the study. Six others dropped out early during the control period because of the inconvenience of daily urine collections. The remaining 17 patients (age, 34 $\pm$ 5 yr; 15 Caucasians, 1 African American, and 1 Asian) completed the study and provided all of the fasting blood and daily urine samples. Twelve of these patients underwent both OGTTs.

Diet

Daily energy intakes were 1854 $\pm$ 129 kcal (7762 $\pm$ 506 kJ) during the habitual diet and 1660 $\pm$ 110 kcal (6947 $\pm$ 464 kJ)

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during the PUFA diet ($P = 0.1$; Table 1). This small decrease was due to a decrease in carbohydrate intake (from 247 ± 16 to 207 ± 14 g; $P = 0.02$). Although total fat intake did not change (71 ± 7 *vs.* 73 ± 3 g), the energy intake from fat increased relatively (from 33% to 39%; $P = 0.006$). Daily intakes of PUFA increased from 14 ± 1 to 26 ± 2 g ($P < 0.0001$), LA increased from 12 ± 1 to 21 ± 4 g ($P < 0.0001$), and α-LNA increased from 1.3 ± 0.2 to 4.0 ± 1 g ($P < 0.0001$).

TABLE 1. Changes in nutrient intake and anthropometric variables

	H-D	PUFA-D	Δ	*P*
Weight (kg)	92.8 ± 5.6	89.2 ± 5.3	−2.1 ± 0.9	0.039
BMI (kg/m²)	34.0 ± 1.9	33.2 ± 1.7	−0.8 ± 0.4	0.025
Waist (cm)	97.3 ± 4.5	96.7 ± 4.2	−1.1 ± 1.1	NS
Body fat (%)	41.3 ± 1.8	40.7 ± 1.8	−0.6 ± 0.5	NS
Energy (kcal)	1854 ± 129	1660 ± 110	−162 ± 91	0.102
Total fat				
g	71 ± 7	73 ± 3	2 ± 6	NS
%	33 ± 1	39 ± 1	6 ± 2	0.006
Saturated fat				
g	27 ± 3	20 ± 1	−7 ± 3	0.029
%	12 ± 1	11 ± 1	−1 ± 1	NS
MUFA				
g	26 ± 3	22 ± 1	−4 ± 3	NS
%	12 ± 1	12 ± 1	0 ± 1	NS
PUFA				
g	14 ± 1	26 ± 2	12 ± 2	<0.0001
%	7 ± 1	14 ± 1	7 ± 1	<0.0001
LA (g)	13 ± 1	22 ± 2	9 ± 2	0.0004
α-LNA (g)	1.4 ± 0.2	4.1 ± 0.5	2.7 ± 1.5	0.0001
CHO				
g	247 ± 16	207 ± 14	−40 ± 15	0.020
%	53 ± 2	49 ± 2	−4 ± 2	0.047
Protein				
g	69 ± 5	64 ± 3	−5 ± 4	NS
%	15 ± 1	16 ± 1	−1 ± 1	NS
Fiber (g)	16 ± 1	18 ± 2	2 ± 2	NS
Soluble fiber (g)	5 ± 1	6 ± 1	1 ± 1	NS

During the habitual (H-D) and PUFA-rich (PUFA-D) diets, nutrient intake was calculated from 7-d food records, and body composition was measured by using electrical bioimpedance. Values are the mean ± SEM (n = 17). Significance was calculated using a repeated measures mixed model, followed by Bonferroni adjustment.

Saturated fat intake decreased from 27 ± 3 to 20 ± 1 g ($P = 0.029$).

Anthropometric variables

Body weight decreased from 92.8 ± 5.6 to 89.2 ± 5.3 kg ($P < 0.04$; Table 1), and BMI decreased from 34.0 ± 1.9 to 33.2 ± 1.7 kg/m² ($P < 0.03$). Body composition and waist circumference did not change.

Plasma fatty acid composition and 15d-PGJ₂

Plasma LA increased from 28.36 ± 1.00% to 33.76 ± 1.08% ($P < 0.002$), and α-LNA from 0.52 ± 0.03% to 1.06 ± 0.10% ($P < 0.0001$). γ-LNA did not change (0.42 ± 0.37% *vs.* 0.48 ± 0.04%). There were compensatory decreases in myristic, palmitic, and oleic acids. The products of LA (γ-LNA, 18:3 n-6; arachidonic acid AA, 20:4 n-6), or of α-LNA (eicosapentanoic acid, 20:5 n-3; docosahexanoic acid, 22:6 n-3) did not change. Plasma 15d-PGJ_2 tended to decrease (from 239 ± 65 to 171 ± 60 ng/ml, $P = 0.053$). When the data were analyzed using nonparametric sign and ranked sign tests, these changes were clearly significant ($P = 0.032$ and $P = 0.041$, respectively).

Glucose homeostasis and plasma lipids

Fasting plasma glucose increased from 76 ± 3 to 95 ± 3 mg/dl (4.2 ± 0.2 to 5.3 ± 0.2 mmol/liter; $P < 0.0001$; Table 2 and Fig. 1). At all time points during the OGTT, glucose values were 18 mg/dl higher on the PUFA-rich diet. However, none of the participants exhibited either impaired glucose tolerance or diabetes. AUC_{GLUOSE} increased from 421 ± 34 to 503 ± 31 mg/dl (23.4 ± 1.9 to 27.9 ± 1.7 mmol/liter; $P < 0.001$). There was no compensatory increase in either fasting insulin or $AUC_{INSULIN}$. HOMA, ISI, fasting plasma adiponectin, and TNFα did not change.

Fasting plasma free fatty acids decreased from 0.596 ± 0.048 to 0.445 ± 0.058 mg/dl ($P = 0.037$), and ketone bodies decreased from 9.14 ± 1.57 to 3.63 ± 0.62 mg/dl (895 ± 154 to 356 ± 61 μmol/liter; $P < 0.003$). Although plasma triglyceride and total cholesterol tended to decrease, and HDL

TABLE 2. Variables related to insulin resistance

	H-D	PUFA-D	Δ	*P*1	*P*2
FPG (mg/dl)	76 ± 3	95 ± 3	18 ± 4	<0.0001	<0.0001
FPI (μU/ml)	19.2 ± 2.3	19.8 ± 3.9	0.8 ± 3.6	NS	NS
$AUC_{GLUCOSE}$ (mg/dl)	421 ± 34	503 ± 31	81 ± 20	0.0012	<0.001
$AUC_{INSULIN}$ (μU/ml)	328 ± 54	304 ± 54	−24 ± 44	NS	NS
Adiponectin (μg/ml)	4.6 ± 0.5	4.5 ± 0.5	−0.1 ± 0.4	NS	NS
TNFα (pg/ml)	3.8 ± 0.6	3.4 ± 0.7	−0.3 ± 0.3	NS	NS
HOMA	5.0 ± 0.7	6.1 ± 1.2	1.1 ± 1.1	NS	NS
ISI	2.7 ± 0.3	2.7 ± 0.4	−0.0 ± 0.2	NS	NS
FFA (mg/dl)	0.596 ± 0.0	0.445 ± 0.058	−0.151 ± 0.077	0.038	0.037
Ketone bodies (mg/dl)	9.14 ± 1.57	3.63 ± 0.62	−5.52 ± 1.45	0.002	0.003
Triglycerides (mg/dl)	132 ± 16	111 ± 13	−22 ± 12	0.099	0.36
Cholesterol (mg/dl)	182 ± 7	173 ± 6	−10±	0.108	0.228
HDL cholesterol (mg/dl)	41 ± 2	44 ± 2	2 ± 1	0.063	0.149

At the end of the habitual (H-D) and PUFA-rich (PUFA-D) diets, plasma glucose and insulin were measured at fasting (FPG and FPI) and during OGTT. $AUC_{GLUCOSE}$, $AUC_{INSULIN}$, ISI, and HOMA were computed. Values are the mean ± SEM. n = 12 for $AUC_{GLUCOSE}$, $AUC_{INSULIN}$, and ISI; n = 17 for the other variables. *P*1, Significance calculated using a repeated measures mixed model, followed by Bonferroni adjustment; *P*2, significance adjusted for the changes in BMI; FPG, fasting plasma glucose; FPI, fasting plasma insulin. Conversion factors for Systeme Internationale units are: × 0.5551 for glucose, × 7.175 for insulin, × 96.05 for ketone bodies, × 0.01129 for triglycerides, and × 0.025586 for cholesterol.

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cholesterol tended to increase, these were not significant after adjusting for the decrease in BMI.

Sex steroids and gonadotropins

Compared with the reference values in lean, healthy, control women, PCOS patients had higher T (0.654 ± 0.078 *vs.* 0.27 ± 0.029 ng/ml; $P = 0.0032$; Table 3) and DHEAS (282 ± 24 *vs.*116 ± 24 μg/ml; $P = 0.0016$), lower SHBG (48.0 ± 2.9 *vs.* 68.5 ± 6.6 nmol/liter; $P = 0.053$) and AUC_{PdG} (18.6 ± 2.2 *vs.* 69.2 ± 7.0 μg/mg cr; $P < 0.0001$), and comparable E1C (1336 ± 122 *vs.*1380 ± 155 ng/mg cr). The PUFA-rich diet did not change plasma T, free T, SHBG, DHEAS, LH, FSH, or urinary AUC_{E1C}, whereas AUC_{PdG} increased from 18.6 ± 2.2 to 31.0 ± 5.7 μg/mg cr ($P = 0.038$). Although AUC_{PdG} increased in 12 of 17 subjects, only 2 exhibited patterns suggesting that ovulation had occurred.

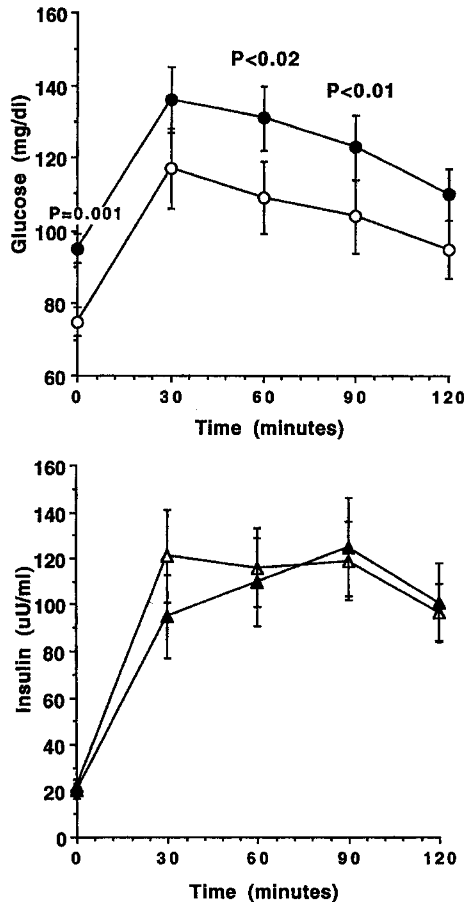

FIG. 1. Plasma glucose and insulin concentrations during the OGTT on the habitual diet (○) and the PUFA-rich diet (●) in women with PCOS (n = 12; mean ± SEM, after adjusting for multiple comparisons using the Bonferroni procedure). Conversion factors for Systeme International units are: × 0.5551 for glucose and × 7.175 for insulin.

Discussion

Dietary and anthropometric changes

The dietary intervention increased PUFA intake and decreased saturated fat intake without altering total fat intake. Although PUFAs might promote less weight gain than saturated fats in the animal models (29), the amount of total energy deficit (14,641 kcal) matched the amount of weight loss (2.1 kg), and no additional weight loss was observed.

Plasma fatty acid composition and 15d-PGJ$_2$

Plasma LA and α-LNA increased, consistent with the high LA and α-LNA content of the walnuts. Although these essential PUFAs can be desaturated and elongated in the human body to γ-LNA, arachidonic acid, eicosapentanoic acid, or docosahexanoic acid (the latter two are the principal PUFAs of marine oils), these did not increase, confirming our previous observations (21) and those of others (30). These findings indicate that nutrition recommendations should make distinctions between the dietary intakes of essential PUFAs from nuts and seeds and the longer chain PUFAs from marine oils.

As synthetic PPARγ ligands are used for the treatment of PCOS (3, 4), we attempted to increase the natural PPARγ ligand 15d-PGJ$_2$ by increasing dietary LA intake. Surprisingly, plasma 15d-PGJ$_2$ decreased. Because to our knowledge this is the first demonstration of dietary regulation of 15d-PGJ$_2$, we measured it in the samples from a previous study (21). In this intervention, addition of walnuts to the habitual and low fat diets tended to decrease 15d-PGJ$_2$ also (from 241 ± 38 to 180 ± 28 and from 253 ± 57 to 187 ± 44 ng/ml, respectively). The physiological significance of these changes remains to be determined.

TABLE 3. Changes in sex steroid hormones and gonadotropins

	H-D	PUFA-D	Δ	*P*1	*P*2
T (ng/ml)	0.654 ± 0.078	0.564 ± 0.056	−0.09 ± 0.05	NS	NS
Free T (pg/ml)	2.65 ± 0.34	2.40 ± 0.34	−0.25 ± 0.31	NS	NS
SHBG (nmol/liter)	48.1 ± 2.9	47.6 ± 1.8	−0.4 ± 2.9	NS	NS
DHEAS (ng/ml)	262 ± 26	243 ± 19	−19 ± 16	NS	NS
LH (IU/liter)	14.5 ± 2.9	11.9 ± 1.1	−2.7 ± 3.1	NS	NS
FSH (IU/liter)	4.3 ± 0.4	3.9 ± 0.3	−0.1 ± 0.3	NS	NS
AUC_{E1C} (ng/mg cr)	1336 ± 122	1357 ± 96	21 ± 98	NS	NS
AUC_{PdG} (μg/mg cr)	18.6 ± 2.2	31.0 ± 5.7	12.4 ± 5.2	0.027	0.038

During the habitual (H-D) and the PUFA-rich (PUFA-D) diets, total T, free T, SHBG, DHEAS, LH, and FSH were measured in the plasma. E1C and PdG were measured in daily first morning urine samples and indexed for the urine concentration. AUCs were calculated for 30-d periods. Values are the mean ± SEM (n = 17). *P*1, Significance calculated using a repeated measures mixed model, followed by Bonferroni adjustment; *P*2, significance adjusted for the changes in BMI. Conversion factors for Systeme Internationale units are: × 3.467 for T and 0.002714 for DHEAS.

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Glucose homeostasis and plasma lipids

Glucose levels increased both at fasting and during OGTT. This was unexpected because epidemiological data indicated that LA intake reduces the risk of type 2 DM (10); experimental data showed inverse correlations between the skeletal muscle PUFA and insulin resistance in humans (31), and PUFA-rich diets decreased insulin resistance induced by high fat feeding, high sucrose feeding, or TNFα administration in experimental animals (14–16). However, it was also reported that increased LA intake elevated fasting plasma glucose in men with type 2 DM (11). One explanation might be that this study compared the LA-rich diet to an oleic acid-rich diet, whereas all others compared PUFAs to saturated fats. We observed that a PUFA-rich diet decreased the plasma oleic acid concentration. Thus, the effects of dietary PUFAs on glucose homeostasis might be more favorable than those of the saturated fats, but less favorable than those of monounsaturated fats (32).

Although the LA-rich diet was reported to also increase fasting insulin (11), suggesting increased insulin resistance, we did not observe any change in insulin, HOMA, ISI, adiponectin, or TNFα. The lack of a compensatory increase in insulin levels suggests a relative decrease in insulin secretion. The PUFAs and their metabolites can directly affect insulin secretion (13). In cultured pancreatic β-cells both LA and AA increase basal insulin secretion, but LA decreases the response to glucose. In contrast, n-3 PUFAs either inhibit or do not affect insulin secretion in response to a meal or glucose or glucagon administration (33, 34). The overall effects of our PUFA-rich diet were similar to those of the n-3 PUFAs.

Interestingly, all glucose values were 18 mg/dl higher during OGTT during the PUFA-rich diet. A similar parallel elevation in the glucose curve is seen in maturity-onset diabetes of the young type 2, where mutations in the glucokinase reset the pancreatic glucose sensor at a higher level (35). Although it is not known whether dietary PUFAs or their metabolites inhibit pancreatic glucokinase and reset the glucose threshold in humans, marine oils inhibit hepatic glucokinase activity in experimental animals (36). A rise in the pancreatic glucose threshold would have explained all of the findings of the present study: the increase in fasting glucose, the parallel rise in the glucose curve during OGTT, the lack of increase in plasma insulin, and the lack of change in insulin resistance parameters. The mechanisms underlying the effects of dietary PUFAs on glucose homeostasis are very important. A defect in pancreatic function might facilitate the onset of diabetes, whereas a rise in the pancreatic glucose threshold might prevent hyperinsulinemic response to dietary carbohydrates.

The changes in plasma triglycerides and total and HDL cholesterol were not significant after adjusting for the weight loss, although the PUFA-rich diet decreased these lipids in combined hyperlipidemia (21). Plasma free fatty acids and ketone-bodies decreased, consistent with both decreased lipolysis in the adipose tissue (37) and inhibition of hepatic glucokinase (36).

Sex steroids and gonadotropins

The PUFA-rich diet did not affect total T or free T. This might be partly due to the limited size of our study population. Although total T decreased by 14%, to detect a difference of this magnitude with 80% power and significance of $P = 0.05$, a population size of $n = 48$ is necessary. Our results are similar to those reported by Moran *et al.* (9) In this study, a 7.7-kg weight loss over 3 months was associated with a 13.7% decrease in total T, and a population size of 58 was required to detect this difference. Although Moran *et al.* (9) observed a significant decrease in SHBG, we did not find any change. This was not surprising because SHBG levels correlate inversely with plasma insulin (38), which did not change in our study. Although treatment of PCOS with insulin sensitizers decreases plasma DHEAS (39), we did not detect any change. Plasma LH or FSH did not change either. In the study by Moran *et al.* (9), DHEAS levels were not reported, and there was no change in LH or FSH.

Urinary E1C concentrations in women with PCOS were similar to those seen in lean, healthy women with normal menstrual cycles, whereas urinary PdG concentrations were 5-fold lower (27). During the PUFA-rich diet 12 of 17 women had increased PdG excretion, but only 2 of them had patterns that suggested ovulation. Another α-LNA-rich oil, flax seed oil, has been shown to increase plasma progesterone in healthy women with normal menstrual cycles (40). The mechanisms underlying this increase remain to be determined.

In conclusion, this study demonstrated that PUFA intake alters glucose homeostasis, plasma lipids, and sex steroids in women with PCOS. Despite the important health effects of PUFAs, the nutritional recommendations are not evidence-based. Generally, very little distinction is made between the essential PUFAs from nuts and seeds and the longer chain PUFAs from fish. The possible existence of a competition between dietary n-3 and n-6 PUFAs has not been addressed in humans. The effects of dietary PUFAs have not been rigorously compared with saturated and monounsaturated fats simultaneously. Further research is required to determine beneficial and harmful effects of various PUFAs, especially in insulin-resistant populations, who are at risk for DM.

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