Polycystic Ovary Syndrome with Hyperandrogenism Is Characterized by an Increased Risk of Hepatic Steatosis Compared to Nonhyperandrogenic PCOS Phenotypes and Healthy Controls, Independent of Obesity and Insulin Resistance

Helen Jones, Victoria S. Sprung, Christopher J. A. Pugh, Christina Daousi, Andrew Irwin, Nabil Aziz, Valerie L. Adams, E. Louise Thomas, Jimmy D. Bell, Graham J. Kemp, and Daniel J. Cuthbertson

Research Institute for Sport and Exercise Sciences (H.J., V.S.S., C.J.A.P.), John Moores University, Liverpool L3 3AF, United Kingdom; Department of Obesity and Endocrinology (C.D., A.I., D.J.C.), University Hospital Aintree, Liverpool L9 7AL, United Kingdom; Department of Gynecology (N.A.), Liverpool Women's Hospital, Liverpool, Merseyside L8 7SS, United Kingdom; Magnetic Resonance and Imaging Analysis Research Centre (V.L.A., G.J.K.), University of Liverpool, Liverpool L69 3GE, United Kingdom; Metabolic and Molecular Imaging Group (E.L.T., J.D.B.), MRC Clinical Sciences Centre, Imperial College London W12 0NN, United Kingdom; and Department of Musculoskeletal Biology (G.J.K.), University of Liverpool, Liverpool L69 3GE, United Kingdom

Context: Nonalcoholic fatty liver disease may be evident in women with polycystic ovary syndrome (PCOS), both conditions being associated with obesity and insulin resistance. However, few studies have accounted for the high prevalence of obesity in PCOS.

Objective: The aim of this study was to determine whether PCOS is independently associated with hepatic steatosis, compared with healthy controls of similar age and body mass index (BMI), and whether steatosis is associated with hyperandrogenemia.

Design and Setting: We conducted a cross-sectional, case-control study at two tertiary referral centers.

Patients: Twenty-nine women with PCOS diagnosed by the Rotterdam criteria [aged 28 yr; 95% confidence interval (CI), 26–31; BMI, 33 kg/m^2; 95% CI, 31–36] and 22 healthy controls (aged 29 yr; 95% CI, 28–31; BMI, 30 kg/m^2; 95% CI, 28–33) were studied.

Methods: Proton-magnetic resonance spectroscopy quantified hepatic and skeletal muscle fat; whole body magnetic resonance imaging quantified internal, visceral, and sc adipose tissue volumes. Differences were assessed between PCOS and controls using *t* tests, and between hyperandrogenic (HA) PCOS, PCOS with normal androgens (NA), and controls using analysis of covariance.

Results: After statistical adjustment for BMI, HA-PCOS had significantly higher liver fat *vs.* NA-PCOS (3.7%; 95% CI, 0.6–13.1) and *vs.* controls (2.1%; 95% CI, 0.3–6.6). Similarly, after adjustment for homeostasis model assessment for insulin resistance, internal and visceral adipose tissue volumes, liver fat remained significantly greater in HA-PCOS compared to NA-PCOS and controls.

Conclusion: These data suggest that HA-PCOS is associated with hepatic steatosis, independent of obesity and insulin resistance. **(*J Clin Endocrinol Metab* 97: 3709–3716, 2012)**

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Abbreviations: ALT, Alanine aminotransferase; BMI, body mass index; CI, confidence interval; FAI, free androgen index; HDL, high-density lipoprotein; ^{1}H-MRS, proton magnetic resonance spectroscopy; HOMA-IR, homeostasis model assessment for insulin resistance; IAT, internal adipose tissue; IMCL, intramyocellular lipid; LDL, low-density lipoprotein; MRI, magnetic resonance imaging; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; PCOS, polycystic ovary syndrome; SAT, sc adipose tissue; VAT, visceral adipose tissue.

Polycystic ovary syndrome (PCOS) affects up to 20% of women of reproductive age and is clinically characterized by irregular menstrual cycles, hyperandrogenism, infertility, or subfertility, frequently with a characteristic ovarian morphology on ultrasonographic examination. There is no consensus as to the most appropriate diagnostic criteria, which incorporate multiple possible phenotypes, but the critical difference between the criteria is whether hyperandrogenism is a prerequisite feature (1) or not (2).

The etiology of PCOS is complex and not completely understood. Nevertheless, a central pathophysiological feature of PCOS is insulin resistance, which cannot be fully explained by the frequent association with obesity because PCOS women are more insulin resistant than healthy controls matched for body mass index (BMI) (3). Different distributions of body fat, for example increased abdominal fat in PCOS relative to controls matched for BMI, could potentially contribute to the insulin resistance in this patient group (4, 5). Visceral fat mass strongly correlates with the degree of insulin resistance and other aspects of the metabolic syndrome in women with PCOS (5, 6). Moreover, in healthy normal-weight and obese individuals, liver fat is also highly correlated with insulin resistance and other features of the metabolic syndrome (7, 8).

There is some evidence of an increased prevalence of nonalcoholic fatty liver disease (NAFLD) in PCOS, using a variety of diagnostic and surrogate methods. NAFLD represents a disease spectrum, ranging from hepatic steatosis, characterized by deposition of triglycerides in the hepatocytes, through to nonalcoholic steatohepatitis (NASH), characterized by hepatocyte injury, inflammation, and fibrosis, which can in turn progress to cirrhosis (9). Using liver transaminases or ultrasonography to infer the presence of hepatic steatosis, a number of studies have demonstrated a high risk of hepatic steatosis in women with PCOS (10–13). Indirect methods have also been used to detect NASH, the intermediate stage in the NAFLD spectrum. Tan *et al.* (14) demonstrated a high prevalence of NASH in PCOS using levels of the apoptotic marker cytokeratin-18 as a surrogate index. Based on such literature, it has been suggested that women with PCOS should be screened for liver disease at an earlier age than is currently recommended for the general population (15). However, to our knowledge there have been no carefully controlled studies to address the clinically relevant question as to whether PCOS represents a specific risk factor for the development of NAFLD, or whether the increased risk of NAFLD in PCOS is mediated by the high prevalence of concomitant obesity. This latter possibility is suggested by the results of a small study in lean, insulin-resistant PCOS women demonstrating no increased risk of NAFLD (16).

Using proton magnetic resonance spectroscopy (^{1}H-MRS), a well-validated, noninvasive method for measuring liver fat content that correlates closely with liver fat determined histologically (17), it is possible to precisely determine liver fat rather than a more semiquantitative method such as ultrasonography. Thus, the aim of this study was to: 1) determine whether women with PCOS have increased liver fat, determined by ^{1}H-MRS, compared with healthy controls of similar age and BMI; and 2) examine whether a specific phenotype of PCOS is more strongly associated with NAFLD than other phenotypes. We hypothesize that hyperandrogenic PCOS women will have increased liver fat independent of body composition.

Subjects and Methods

Participants

The study population consisted of 29 PCOS patients [aged 28 yr; 95% confidence interval (CI), 26–31; BMI, 33 kg/m^2; 95% CI, 31–36], diagnosed according to the Rotterdam criteria, based on two of the following features: 1) oligo- or amenorrhea; 2) clinical or biochemical hyperandrogenism; or 3) polycystic ovary morphology on pelvic ultrasound, after exclusion of other causes of hyperandrogenism. Patients were excluded on the basis of the following: 1) other causes of irregular menstrual cycles and androgen excess or pregnancy; 2) taking the oral contraceptive pill or metformin; 3) history of known liver disease or medication known to cause elevation in liver enzymes; 4) alcohol consumption greater than 14 units per week (average alcohol consumption ranged from 0–8 units per week); and 5) smoking (nonsmoker for >5 yr). A group of 22 healthy control women, matched for age (29 yr; 95% CI, 28–31) and BMI (30 kg/m^2; 95% CI, 28–33), were recruited. Controls had a history of regular menstrual cycles and no evidence of clinical or biochemical hyperandrogenism; were not taking medications potentially affecting liver function, the oral contraceptive pill, or metformin; had alcohol consumption of less than 14 units; and did not smoke. All patients and controls were sedentary, defined as not taking part in any habitual exercise as assessed by a self-reported questionnaire. Controls were tested in the early follicular phase of the menstrual cycle (d 1–7 of cycle), determined by the first day of menstruation. This was not possible for most of the PCOS cases who had oligoamenorrhea or amenorrhea (n = 29). The study conformed to the standards set by the Declaration of Helsinki and was approved by the relevant ethics committee. All participants provided written informed consent.

Anthropometric and biochemical evaluation

After a full history, a single person recorded all anthropometric measurements (weight, height, waist and hip circumference). After an overnight fast, blood was taken for biochemical profile, including total cholesterol, triglycerides, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol, alanine aminotransferase (ALT), glucose, and insulin. Circulating reproductive hormone levels were measured, including FSH, LH, estradiol, progesterone, testosterone, and SHBG.

Biochemical assays and calculations

Samples were analyzed using the Olympus AU2700 analyzer (Beckman Coulter Ltd., High Wycombe, UK) with standard proprietary reagents as follows: glucose with hexokinase, total cholesterol, HDL with cholesterol esterase/oxidase, triglyceride with glycerol kinase, and ALT with International Federation of Clinical Chemistry and Laboratory Medicine kinetic UV (without pyridoxal phosphate activation). The intra- and interassay coefficients of variation were 10% or less. LDL was calculated according to the Friedwald formula. LH, FSH, estradiol, progesterone, total testosterone, and SHBG concentrations were measured by a chemiluminescence method (Siemens Centaur; Siemens, Munich, Germany). The intra- and interassay coefficients of variation for total testosterone and SHBG were 8% or less. The lower limits of quantitation for total testosterone and SHBG were 0.35 and 2 nmol/liter, respectively (calculated from zero standard minus 2 SD values). Free androgen index (FAI) was calculated as 100 × [testosterone concentration (nmol/liter)/SHBG concentration (nmol/liter)] (normal < 7%) (18). Insulin was measured using an ELISA kit (Invitrogen, Paisley, UK). The intra- and interassay coefficients of variation were 9% or less. Using fasting baseline glucose and insulin concentrations, insulin resistance was calculated by the homeostasis model assessment for insulin resistance (HOMA-IR) (19). All laboratory assays were performed in the clinical biochemistry laboratory at University Hospital Aintree.

Magnetic resonance methodology

All women underwent magnetic resonance imaging (MRI) scanning in a 1.5T Siemens Symphony scanner (Siemens Medical Solutions, Erlangen, Germany) in a prone position, being moved through the magnet to acquire full body coverage.

Volumetric analysis of adipose tissue content

Total internal adipose tissue (IAT) and sc adipose tissue (SAT) as well as abdominal SAT and visceral adipose tissue (VAT) were calculated from whole body axial T1-weighted fast spin echo scans (axial scans, 10-mm slice thickness followed by a 10-mm gap using the integral body coil). The abdominal region was defined as the image slices from the slice containing the femoral heads, to the slice containing the top of the liver/base of the lungs. All scans were analyzed centrally and anonymized before analysis, ensuring blindness to all clinical details.

Proton magnetic resonance spectroscopy

In liver, NAFLD was defined as intrahepatocellular lipid greater than 5.5% (20). Three voxels of interest were identified in the liver at standard sites avoiding ducts and vasculature. In skeletal muscle, a single voxel was identified in each of the tibialis anterior and soleus muscles, avoiding bone, fascia, and the neurovascular bundle. Single voxel spectroscopy was conducted at each of these five sites. Voxel size was 20 × 20 × 20 mm, TE (echo time) 135 msec, TR

TABLE 1. Overall comparison of PCOS and control women

	Control	PCOS	*P*
n	22	29	
Clinical characteristics			
Age (yr)	29 (28–31)	28 (26–31)	0.09
BMI (kg/m^2)	30 (28–33)	33 (31–36)	0.09
Weight (kg)	82 (75–92)	90 (84–97)	0.15
Waist (cm)	99 (90–107)	104 (98–110)	0.24
Biochemical and metabolic parameters			
Testosterone (nmol/liter)	1.6 (1.3–1.8)	2.7 (2.4–2.9)	**0.00**
SHBG (nmol/liter)[a]	41 (33–49)	38 (23–34)	**0.008**
FAI[a]	4 (3–5)	9 (7–12)	**0.00**
FSH (IU/liter)[a]	5.2 (4.1–6.6)	4.7 (4.1–5.4)	0.56
LH (U/liter)[a]	4.5 (3.1–6.3)	8.1 (6.1–10.8)	**0.005**
Estradiol (pmol/liter)[a]	220 (151–320)	260 (214–315)	0.47
Progesterone (nmol/liter)[a]	1.9 (1.4–2.7)	3.6 (2.6–5.0)	**0.01**
ALT (U/liter)[a]	19 (15–24) (n = 19)	24 (18–30)	0.25
Glucose (mmol/liter)	4.6 (4.4–4.8)	4.7 (4.5–4.8)	0.73
Insulin (μIU/ml)[a]	19.5 (16.3–23.5) (n = 18)	17.3 (14.1–20.4) (n = 26)	0.31
HOMA-IR[a]	3.9 (3.2–4.9) (n = 18)	3.6 (3.0–4.3) (n = 26)	0.46
Cholesterol (mmol/liter)[a]	4.7 (4.4–5.1)	5.2 (4.6–6.0)	0.22
Triglycerides (mmol/liter)[a]	2.0 (1.5–3.5)	2.2 (1.7–1.5)	0.47
HDL (mmol/liter)[a]	1.4 (1.2–1.5)	1.1 (1.1–1.5)	0.71
LDL (mmol/liter)[a]	2.8 (2.5–3.2)	2.9 (2.7–3.4)	0.46
MRI-measured adipose tissue volumes (liters)			
Total body fat	41.1 (34.4–47.8)	46.5 (41.4–51.7)	0.19
Total internal fat	5.4 (4.4–6.2)	7.0 (5.7–8.3)	**0.03**
Total sc fat	35.7 (29.6–41.8)	39.4 (34.9–43.9)	0.31
IAT:SAT ratio	0.16 (0.13–1.17)	0.28 (0.13–0.43)	0.47
Visceral fat	2.9 (2.3–3.4)	3.8 (3.0–4.6)	**0.05**
Abdominal sc fat	12.0 (9.6–14.4)	14.3 (12.3–16.3)	0.12
VAT:abdominal SAT ratio	0.27 (0.21–0.32)	0.27 (0.23–0.32)	0.99
^{1}H-MRS			
Liver fat (% CH_2/H_2O)[a]	1.9 (0.6–4.7)	6.1 (2.6–12.7)	**0.05**
Soleus IMCL (CH_2/creatine)[a]	29 (19–43)	40 (31–52)	0.14
Tibialis anterior IMCL (CH_2/creatine)[a]	32 (22–47)	42 (31–56)	0.31

[a] Variables analyzed after logarithmic transformation.

Data are expressed as mean (95% CI). Significant differences are indicated in *bold text*.

(repetition time) 1500 msec, with 64 acquisitions. Where the muscle was too small to allow placement of a 20-mm voxel, a 15 × 15 × 20-mm voxel was placed, and the number of acquisitions was increased to 200 to maintain signal-to-noise ratio. ^{1}H-MR spectra were quantified using the AMARES algorithm in the software package jMRUI-3.0 (21). As previously described, liver fat is expressed as the percentage of CH_2 lipid signal amplitude relative to water signal amplitude after correcting for T_1 and T_2 (22), and intramyocellular lipid (IMCL) is expressed as CH_2 lipid amplitude relative to total creatine amplitude after correcting for T_1 and T_2 (23).

Reproducibility of MRI and MRS analysis

Volumetric analysis of adipose tissue content

The mean coefficients of variation were determined as total body fat, 1–2%; total sc fat, 3–4%; abdominal sc fat, 1–3%; and visceral fat, 6–8%.

Examination of liver fat (IHCL CH_2/water)

The mean interexamination coefficient of variation for using this protocol was found to be 7.0% (range, 4.0–11.7%), and the mean intra-examination coefficient of variation was significantly lower (6.0%) (22).

Statistical analysis

After analysis of distribution and appropriate transformation, differences between groups (PCOS and controls) were compared using the independent-sample *t* tests. All logarithmic-transformed data (*e.g.* liver fat) were back-transformed to the original units. Pearson's correlation coefficients were calculated to evaluate relationships between variables for PCOS and controls. Correlations demonstrating a P value < 0.05 were considered statistically significant. Subsequently, the PCOS group was subdivided based on FAI (normoandrogenic PCOS $< 7\%$, and hyperandrogenic PCOS $\geq 7\%$) and compared using general linear modeling. Statistically significant interactions were followed up with the least significant difference method for pairwise multiple comparisons (24). Subsequently, liver fat and whole body fat volumes were then analyzed while statistically controlling for BMI as a covariate, and liver fat was analyzed while controlling for IAT, VAT, and HOMR-

TABLE 2. Comparison of control and PCOS women subdivided by FAI

	Control	Normoandrogenic PCOS	Hyperandrogenic PCOS	*P*
n	22	10	19	
Clinical characteristics				
Age (yr)	29 (28–31)	30 (26–34)	28 (24–30)	0.11
BMI (kg/m^2)	30 (28–33)	29 (26–32)	35 (33–38)[b,c]	**0.004**
Weight (kg)	82 (75–92)	80 (71–89)	96 (87–104)[b,c]	**0.02**
Waist (cm)	99 (90–107)	92 (83–100)	111 (105–118)[b,c]	**0.005**
Biochemical and metabolic parameters				
Testosterone (nmol/liter)	1.6 (1.3–1.8)	2.1 (1.6–2.5)[d]	2.6 (2.6–3.2)[b,c]	**0.00**
SHBG (nmol/liter)[a]	41 (33–49)	44 (31–62)	22 (20–26)[b,c]	**0.00**
FAI[a]	4 (3–5)	4 (4–5)	13 (11–15)[b,c]	**0.00**
FSH (IU/liter)[a]	5.2 (4.1–6.6)	5.6 (4.6–6.9)	4.5 (3.7–5.4)	0.36
LH (IU/liter)[a]	4.5 (3.1–6.3)	9.5 (5.7–15.7)[d]	7.8 (5.4–11.1)[c]	**0.02**
Estradiol (pmol/liter)[a]	220 (151–320)	290 (102–413)	239 (191–299)	0.56
Progesterone (nmol/liter)[a]	1.9 (1.4–2.7)	2.5 (1.8–3.7)	4.1 (2.6–6.3)[c]	**0.02**
ALT (U/liter)[a]	19 (15–24) (n = 19)	16 (11–23)	28 (20–38)[b,c]	**0.03**
Glucose (mmol/liter)	4.6 (4.4–4.8)	4.6 (4.2–5.0)	4.7 (4.5–4.9)	0.71
Insulin (μIU/ml)[a]	19.5 (16.3–23.5) (n = 18)	13.1 (10.5–16.4) (n = 9)[d]	20.0 (16.3–24.0)[b]	**0.02**
HOMA-IR[a]	3.9 (3.2–4.9) (n = 18)	2.7 (2.2–3.3) (n = 9)[d]	4.2 (3.3–5.2)[b]	**0.04**
Cholesterol (mmol/liter)[a]	4.7 (4.4–5.1)	4.8 (3.6–6.4)	5.4 (4.8–6.2)	0.24
Triglycerides (mmol/liter)[a]	2.0 (1.5–3.5)	1.4 (0.8–2.3)	2.8 (2.1–3.6)[b,c]	**0.01**
HDL (mmol/liter)[a]	1.4 (1.2–1.5)	1.4 (1.3–1.6)	1.3 (1.1–1.5)	0.34
LDL (mmol/liter)[a]	2.8 (2.5–3.2)	2.4 (1.9–3.1)	3.3 (3.0–3.6)[b,c]	**0.01**
MRI-measured adipose tissue volumes (liters)				
Total body fat	41.1 (34.4–47.8)	37.1 (29.9–44.8)	51.2 (45.3–57.5)[b,c]	**0.01**
Total internal fat	5.4 (4.4–6.2)	5.1 (3.3–6.9)	8.0 (6.5–9.6)[b,c]	**0.003**
Total sc fat	35.7 (29.6–41.8)	32.2 (25.6–38.8)	43.3 (38.1–48.4)[b,c]	**0.04**
IAT:SAT ratio	0.16 (0.13–1.17)	0.32 (−0.03–0.66)	0.26 (0.09–0.41)	0.36
Visceral fat	2.9 (2.3–3.4)	2.7 (1.6–3.8)	4.4 (3.5–5.3)[b,c]	**0.004**
Abdominal sc fat	12.0 (9.6–14.4)	11.0 (8.3–13.7)	16.1 (13.7–18.4)[b,c]	**0.01**
VAT:abdominal SAT ratio	0.27 (0.21–0.32)	0.26 (0.12–0.40)	0.28 (0.23–0.33)	0.93
^{1}H-MRS				
Liver fat (% CH_2/H_2O)[a]	1.9 (0.6–4.7)	0.6 (−0.7–2.4)	12.9 (6.0–27.4)[b,c]	**0.00**
Soleus IMCL (CH_2/creatine)[a]	29 (19–43)	35 (20–62)	44 (33–59)	0.24
Tibialis anterior IMCL (CH_2/creatine)[a]	32 (22–47)	19 (7–49)	49 (36–68)[b,c]	**0.03**

[a] Variables analyzed after logarithmic transformation.

[b] Significant difference between normoandrogenic and hyperandrogenic PCOS.

[c] Significant difference between hyperandrogenic PCOS and control.

[d] Significant difference between normoandrogenic and controls.

Data are expressed as mean (95% CI). Significant differences are indicated in *bold text*.

IR. Significant differences were followed up with the least significant difference method. Data were analyzed using the SPSS 17.0 (SPSS, Chicago, IL) software.

Results

PCOS patients *vs.* healthy controls

Clinical characteristics

There was no statistical difference in BMI (3.0 kg/m^2; 95% CI, −0.5 to 6.4) or waist circumference (5.9 cm; 95% CI, −3.9 to 15.7) between PCOS and control women ($P > 0.05$; Table 1). As expected, PCOS women had significantly elevated testosterone, FAI, and LH in addition to a lower SHBG ($P < 0.05$; Table 1). Fasting glucose, insulin, HOMA-IR, triglycerides, and ALT concentrations were not different ($P < 0.05$; Table 1).

^{1}H-MRS of liver and skeletal muscle

Liver fat was greater in PCOS compared with control women (1.4% CH_2/H_2O; 95% CI, 1.0–6.0; Table 1). There was no significant difference in soleus or tibialis anterior skeletal IMCL between PCOS and control women (Table 1). In both PCOS women and healthy controls, liver fat was correlated to BMI (R = 0.60, $P = 0.001$; R = 0.41, $P = 0.06$), IAT (R = 0.59, $P = 0.001$; R = 0.61, $P = 0.00$) VAT (R = 0.66, $P = 0.001$; R = 0.65, $P = 0.001$), FAI (R = 0.66, $P = 0.000$; R = 0.31, $P = 0.19$) and HOMR-IR (R = 0.40, $P = 0.04$; R = 0.62, $P = 0.006$).

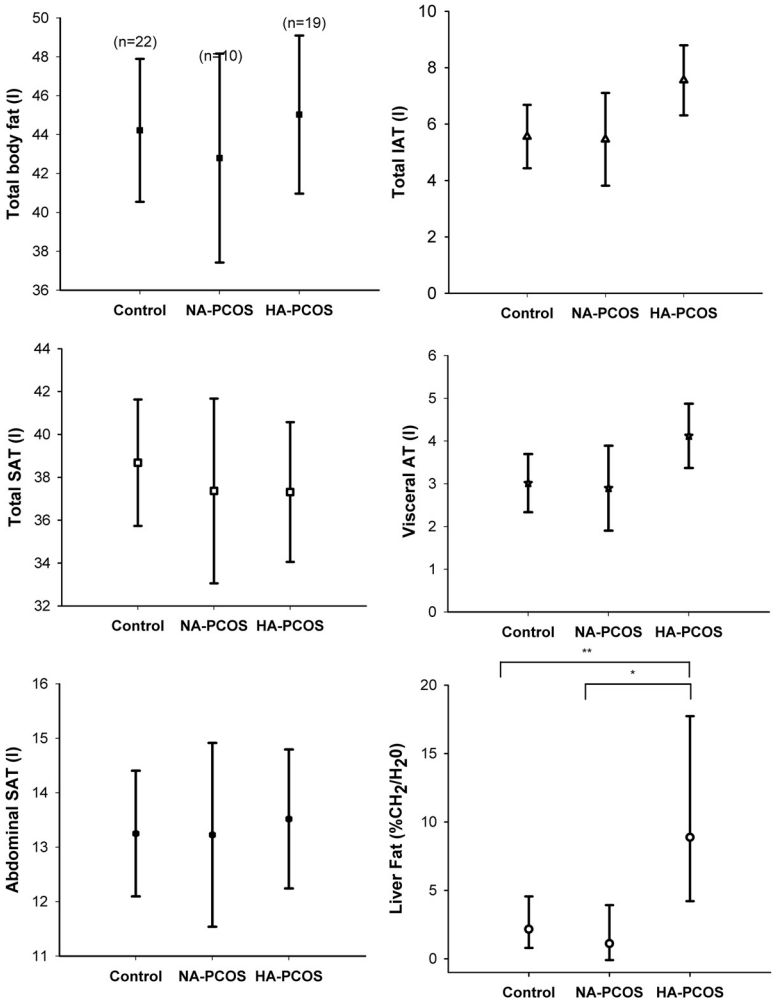

FIG. 1. MRI-derived data in controls, normoandrogenic (NA-PCOS), and hyperandrogenic (defined by FAI) PCOS (HA-PCOS), statistically covariate controlled for differences in BMI. Data are presented as means and 95% CI. *, Significant difference between normoandrogenic and hyperandrogenic PCOS. **, Significant difference between hyperandrogenic PCOS and controls.

Adipose tissue volumes

Both IAT (1.62 l 95% CI, 0.03–3.22) and VAT (0.92 l 95% CI, 0.13–1.82) were significantly higher in PCOS women compared with controls despite no significant difference in total or abdominal SAT ($P < 0.05$; Table 1).

PCOS with or without hyperandrogenism

Clinical characteristics

Hyperandrogenic PCOS women had a significantly higher BMI compared with normoandrogenic PCOS (6.5 kg/m^2; 95% CI, 2.1–10.9) and control women (5.2 kg/m^2; 95% CI, 1.7–8.8), with no significant difference between normoandrogenic PCOS and control women (1.3 kg/m^2; 95% CI, −3.0 to 5.6) (Table 2). Fasting insulin, HOMR-IR, triglycerides, LDL cholesterol, and ALT were all significantly elevated in hyperandrogenic PCOS compared with PCOS women with normal androgens ($P < 0.05$; Table 2).

Adipose tissue volumes

Unadjusted IAT was greater in hyperandrogenic PCOS compared with normoandrogenic PCOS (2.87 liters; 95% CI, 0.84–4.89) and controls (2.61 liters; 95% CI, 0.97–4.26), with no significant difference between normoandrogenic PCOS and control women (0.25 liters; 95% CI, −1.73–2.24; Table 2). Likewise, VAT was greater in hyperandrogenic PCOS compared with normoandrogenic PCOS (1.73 liters; 95% CI, 0.50–2.96) and controls (1.51 liters; 95% CI, 0.51–2.51) but was not different between normoandrogenic PCOS and control women (0.22 liters; 95% CI, −0.99 to 1.43; Table 2). Total SAT, abdominal SAT, and total body fat were also significantly elevated in hyperandrogenic PCOS compared with normoandrogenic PCOS and controls ($P < 0.05$; Table 2). After covariate adjustment for BMI, all of the adipose tissue volumes were not statistically significant ($P > 0.05$; Fig. 1).

^{1}H-MRS of liver and skeletal muscle

Unadjusted liver fat was greater in hyperandrogenic PCOS compared with normoandro-

genic PCOS (7.7% CH_2/H_2O; 95% CI, 1.0–15.2) and controls (3.6% CH_2/H_2O; 95% CI, 0.9–10.1), with no significant difference between normoandrogenic PCOS and control women (0.9% CH_2/H_2O; 95% CI, −0.7 to 4.5). After covariate adjustment for BMI, liver fat remained significantly greater in hyperandrogenic PCOS compared with normoandrogenic PCOS (3.7% CH_2/H_2O; 95% CI, 0.6–13.1) and controls (2.1% CH_2/H_2O; 95% CI, 0.3–6.6), with no significant difference between normoandrogenic PCOS and control women (0.5% CH_2/H_2O; 95% CI, −0.5 to 3.1; Fig. 1). Similarly, after adjustment for IAT, VAT, and HOMR-IR, liver fat remained significantly greater in hyperandrogenic PCOS compared with normoandrogenic PCOS and controls (Fig. 2).

Discussion

In the current study, we have shown that women with hyperandrogenic PCOS have evidence of increased liver fat, compared with PCOS women with normal androgens or with healthy controls. In contrast, the metabolic phenotype of women with PCOS with normal androgens was similar to healthy controls. This finding suggests that hyperandrogenic PCOS represents a distinct metabolic phenotype characterized by an increased risk of hepatic steatosis, part of the NAFLD spectrum. Importantly, we have also shown that the elevated liver fat within the hyperandrogenic PCOS phenotype is evident after statistical correction for differences in BMI, adipose tissue volume, and HOMA-IR, suggesting that the greater liver fat deposition is not explained by obesity or insulin resistance.

This is the first study to compare PCOS women to controls of a similar age and BMI using the most quantitative, noninvasive method for determining liver fat. Cussons *et al.* (25) have previously used MRS to investigate the effect of omega-3 acid supplementation in 25 women with PCOS with normal or high liver fat; because the aim of that study was to determine the effects of a dietary intervention, no control group was included for comparison of liver fat. We found liver fat content was greater in PCOS women diagnosed with PCOS by the Rotterdam criteria when compared with control women. Nevertheless, when we subdivided the group according to normal or elevated FAI, we observed elevated liver fat only in hyperandrogenic PCOS women, with similar levels in PCOS women with normal androgens and healthy controls. In keeping with other studies that have demonstrated an association between hyperandrogenic PCOS and NAFLD, using either elevated ALT as a surrogate marker or hepatic steatosis on ultrasound (26, 27), we also observed elevated ALT in the hyperandrogenic PCOS women. Nevertheless,

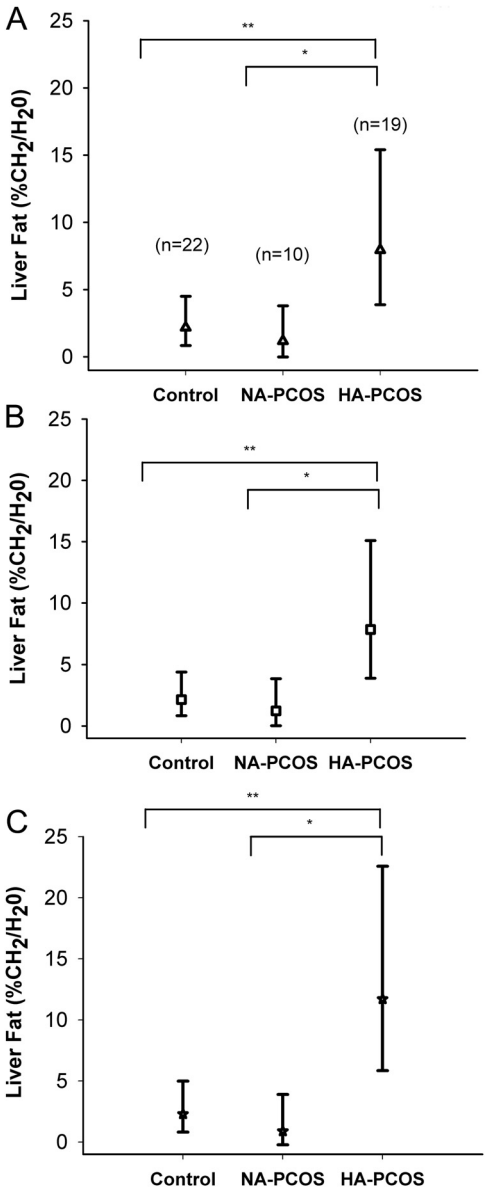

FIG. 2. Liver fat in controls, normoandrogenic (NA-PCOS) and hyperandrogenic (defined by FAI) PCOS (HA-PCOS), statistically covariate controlled for differences in IAT (A), VAT (B), and HOMA-IR (C). Data are presented as means and 95% CI. *, Significant difference between normoandrogenic and hyperandrogenic PCOS. **, Significant difference between hyperandrogenic PCOS and controls.

the novel finding of the current study is that these differences in liver fat remained apparent after adjusting for differences in BMI, IAT and VAT volume, and HOMA-IR across the control and patient groups, suggesting that it is the phenotype of PCOS, rather than obesity or insulin resistance, that explains the elevated liver fat.

We also observed evidence of increased internal, visceral, and sc fat deposition in women with hyperandrogenic PCOS relative to PCOS women with normal androgens or with healthy controls. This is a notable finding, given the recent suggestion of differential gene expression in SAT in obese PCOS women (28), a mechanism that

could contribute to insulin resistance. Yet, after statistical adjustment for differences in BMI, these differences were no longer evident. Importantly, we also observed that the group with hyperandrogenic PCOS had significantly higher fasting insulin concentrations and HOMA-IR index. Taken together, these findings support a previous suggestion that the phenotypic subgroup of PCOS, which includes hyperandrogenism, influences the metabolic features of PCOS. Barber *et al.* (29) previously demonstrated that metabolic dysfunction in PCOS was restricted to the subgroup with all three of the Rotterdam criteria and that hyperandrogenism was fundamental; women who were normoandrogenic had a metabolic profile (presence of metabolic syndrome and/or insulin resistance) indistinguishable from control women without PCOS. The correlation between both insulin resistance and FAI with liver fat in PCOS women further supports this suggestion of high risk of metabolic and hepatic complications associated with this specific phenotype, *i.e.* hyperandrogenic PCOS.

One limitation of this study was that we were unable to ascertain the phase of the menstrual cycle for the women with PCOS. This is highlighted in Tables 1 and 2 because differences in reproductive hormones LH and progesterone are evident. Although differences in LH between women with PCOS and controls are expected, the progesterone data are intriguing and could be related to menstrual cycle phase. Alternatively, it could be possible that women with hyperandrogenic PCOS may have elevated progesterone levels. A further limitation was the method to measure insulin resistance; the use of an oral glucose tolerance test (30) or a two-stage hyperinsulinemic-euglycemic clamp, with infusion of deuterated glucose (31), would have facilitated an assessment of hepatic and peripheral (skeletal muscle) insulin sensitivity. However, given that it is well known that insulin resistance is highly correlated with liver and internal/visceral fat deposition (5–7), as we have shown, we are confident that the HOMR-IR data are sensitive enough to detect differences in insulin resistance for the purposes required in the current study. Finally, we were not able to provide data on the association between PCOS and other components of the NAFLD spectrum (NASH or cirrhosis) because ^{1}H-MRS does not provide information on hepatic inflammation (NASH) or fibrosis. Similarly, our blood sampling did not permit estimation of other noninvasive scores of fibrosis such as the NAFLD fibrosis score or Fib-4 scores, which have been validated in NAFLD using liver biopsy specimens (32, 33). However, there are noteworthy strengths to the design and methodology of the current study. The application of ^{1}H-MRS is considered to be the most sensitive, noninvasive method to quantitate liver fat content, and we employed this method along with whole body MRI. We also included a suitable control group, which was similar in age and BMI, and also statistically controlled for BMI as a confounding variable.

In summary, our data suggest that hyperandrogenic PCOS, evidenced via elevated FAI, may represent a distinct risk factor for the development of NAFLD in PCOS, independent of obesity or insulin resistance.

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Address all correspondence and requests for reprints to: Daniel J. Cuthbertson, Clinical Sciences Centre, University Hospital Aintree, Liverpool L9 7AL, United Kingdom. E-mail: daniel.cuthbertson@liverpool.ac.uk.

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