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CLINICAL STUDY

Fatty liver index in polycystic ovary syndrome

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Abstract

Introduction: Women with polycystic ovary syndrome (PCOS) frequently suffer from metabolic disturbances and might be affected by hepatic steatosis. The fatty liver index (FLI) was developed as a simple and accurate predictor of hepatic steatosis. We aimed to analyze the association of FLI with endocrine and metabolic parameters in a cohort of PCOS and control women.

Methods: FLI was calculated using body mass index (BMI), waist circumference, triglycerides, and gamma-glutamyl transferase in 611 PCOS and 139 BMI-matched control women within the same age range. Elevated FLI was defined as >60. Metabolic, endocrine, and anthropometric measurements and oral glucose tolerance tests were performed.

Results: PCOS women had significantly higher FLI levels than control women in age-adjusted analyses (11.4 (4.3–48.8) and 8.8 (3.9–35.0), respectively, $P=0.001$), whereas fibrosis indices were similar (aspartate amino transferase-to-platelet ratio index) or lower (FIB-4) respectively. In binary logistic regression analysis adjusted for age, odds ratio (OR) for elevated FLI was 2.52 (1.31–4.85), $P=0.006$, for PCOS women when compared with controls. PCOS women with high FLI levels had an adverse anthropometric, metabolic, and endocrine risk profile. The prevalence of elevated FLI was 88.7% in PCOS women with metabolic syndrome (MS) and 11.3% in PCOS women without MS ($P<0.001$). In control women, elevated FLI was present in 66.7% of women with MS and 30.8% of women without MS.

Conclusion: High FLI levels are a common finding in obese PCOS women and are closely linked to MS. FLI calculation might be a useful tool for identifying PCOS patients at high risk for metabolic and hepatic disturbances.

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Introduction

Polycystic ovary syndrome (PCOS) is one of the most common female endocrine disorders with a prevalence of ~6.5% in women of reproductive age (1–3). PCOS is characterized by increased ovarian and adrenal androgen secretion, hyperandrogenic symptoms such as hirsutism, acne and/or alopecia, menstrual irregularity, and polycystic ovaries (4). Although the pathophysiology of PCOS is not fully understood, insulin resistance (IR) seems to play a key role in the development of PCOS (4). Moreover, obesity, central obesity in particular, is involved in the pathogenesis of this highly frequent disorder (5). Of note, a metabolic syndrome (MS) has been diagnosed in 33–47% and IR in 50–80% of PCOS women (6).

The prevalence of non-alcoholic fatty liver disease (NAFLD) is high in conditions associated with IR such as obesity, type 2 diabetes, dyslipidemia, and the MS (7). Indeed, NAFLD is considered to be one of the most common forms of chronic liver disease in the Western world. The most common explanation for the high prevalence of NAFLD is obesity, IR being the key factor linking NAFLD to MS. Thus, NAFLD is now considered the hepatic manifestation of MS (8). Considering the high prevalence of IR and MS in PCOS, a 'hepatic manifestation' of PCOS might be speculated on, as reviewed by Baranova *et al.* (9). In fact, there is evidence from small cohorts showing that PCOS women are at an increased risk of developing NAFLD (10) and conversely women with NAFLD are at an increased risk of having PCOS (11).

The fatty liver index (FLI) is an algorithm based on body mass index (BMI), waist circumference (WC), triglycerides (TG), and gamma-glutamyl transferase (GGT) and might serve as a simple and accurate predictor of hepatic steatosis in the general population (12). The prevalence of hepatic steatosis as defined by elevated FLI has not been examined in PCOS women.

The aim of this study was to analyze the association of FLI with endocrine and metabolic parameters in a cohort of PCOS and control women.

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Materials and Methods

Subjects

The study cohort consisted of 611 women with PCOS, aged 16–45 years, who were routinely referred to our outpatient clinic for PCOS evaluation between 2006 and 2010. One hundred and thirty-nine BMI-matched women within the same age range, who were routinely referred to our outpatient clinic for thyroid evaluation between 2009 and 2010, were included in the study as a control group. All control women had normal thyroid function, regular menstrual cycles, normal serum androgens, and no clinical signs of hyperandrogenism. None of the PCOS and control women reported significant alcohol abuse or a history of known liver disease of viral, genetic, autoimmune, or drug-induced origin.

PCOS women

PCOS was diagnosed using the NIH criteria (13). The two characteristics required to confirm the diagnosis are as follows: i) clinical and/or biochemical signs of hyperandrogenism and ii) oligo- and/or an-ovulation. Disorders with a similar clinical presentation, such as hyperprolactinemia, congenital adrenal hyperplasia, Cushing's syndrome, and androgen-secreting tumors, have to be excluded. Hyperandrogenism was defined by the clinical presence of hirsutism (Ferriman–Gallwey score ≥ 6), acne or alopecia and/or elevated androgen levels (normal range of testosterone: <0.77 ng/ml, normal range of free testosterone: <3.18 pg/ml). Oligo- and/or an-ovulation were defined by the presence of oligomenorrhea or amenorrhea. Hyperprolactinemia, Cushing's syndrome, congenital adrenal hyperplasia, and androgen secreting tumors were excluded by specific laboratory analysis (cortisol, ACTH, 17α-OH progesterone, and DHEAS). PCOS patients and control women did not take any medication known to affect endocrine parameters, carbohydrate metabolism, or serum lipid profile for at least 3 months before entering the study. Moreover, PCOS and control women had no history of high alcohol intake, hemochromatosis, autoimmune liver disease, or other chronic liver diseases, and none had a history of medication use causing hepatotoxicity. The study protocol was approved by the Ethics Committee of the Medical University of Graz and written informed consent was obtained from each patient.

Procedures

The FLI was calculated as: $\mathrm{FLI} = (e^{0.953 \times \log e(\text{triglycerides}) + 0.139 \times \mathrm{BMI} + 0.718 \times \log e(\text{ggt}) + 0.053 \times \text{waist circumference} - 15.745}) / (1 + e^{0.953 \times \log e(\text{triglycerides}) + 0.139 \times \mathrm{BMI} + 0.718 \times \log e(\text{ggt}) + 0.053 \times \text{waist circumference} - 15.745}) \times 100$ (12). The aspartate amino transferase (AST)-to-platelet ratio (APRI) index was calculated as: APRI = (AST/upper limit of normal)/platelets (10^9/l) $\times 100$ (14). The FIB-4-index was calculated as: FIB-4-index = age (years) $\times$ AST (U/l)/platelets (10^9/l) $\times$ (ALT (U/l)$^{0.5}$) (15).

Standard anthropometric data (height, weight, WC and hip circumference (HC), and blood pressure (BP)) were obtained from each subject. WC was measured in a standing position midway between the lower costal margin and the iliac crest. HC was measured in a standing position at the maximum circumference over the buttocks. The BMI was calculated as the weight in kilograms divided by the square of height in meters. FLI <30 rules out and FLI >60 indicates fatty liver disease (12). Hirsutism was quantified with the modified Ferriman–Gallwey score. Moreover, basal blood samples for hormonal (total testosterone, free testosterone, sex hormone-binding globulin (SHBG), androstenedione, DHEAS, free tri-iodothyronine (FT_3), free thyroxine (FT_4), TSH, 17α-OH progesterone, and cortisol) and metabolic parameters (glucose, insulin, C-peptide, total cholesterol, high-density lipoprotein (HDL)-cholesterol, low-density lipoprotein (LDL)-cholesterol, and TG) were collected between 0800 and 0900 h after an overnight fast. All participants underwent a fasting 75 g oral glucose tolerance test (OGTT). Blood samples were drawn after 30, 60, and 120 min for glucose and insulin determination. Impaired fasting glucose was determined as fasting glucose between 100 and 125 mg/dl (between 5.6 and 6.9 mmol/l) and impaired glucose tolerance (IGT) was identified by 2 h glucose levels between 140 and 199 mg/dl (between 7.8 and 11.1 mmol/l), as defined by the American Diabetes Association (ADA) (16). MS was defined by the National Cholesterol Education Program (NCEP) and the Adult Treatment Panel-III (ATP-III) in subjects presenting at least three of the following criteria: waist circumference >88 cm, HDL-cholesterol <50 mg/dl, TG level >150 mg/dl, raised BP (systolic >130 mmHg, diastolic >85 mmHg), and raised fasting glucose (>110 mg/dl) or prevalent type 2 diabetes (17). IR was estimated using the homeostatic model assessment-IR (HOMA-IR) and was assumed for levels >2. HOMA-IR was calculated as the product of fasting plasma insulin value (μU/ml) and fasting plasma glucose value (mg/dl) divided by 405 (18). Elevated GGT levels were defined as levels >38 U/l, elevated AST levels were defined as levels >30 U/l, and elevated alanine transaminase (ALT) levels were defined as levels >35 U/l. Elevated APRI index was defined as >1.50 (14). Elevated FIB-4 index was defined as ≥ 3.25 (15).

Biochemical analysis

ALT, AST, and GGT were measured by photometric assay (Roche). Fasting glucose, TG, total cholesterol, HDL-cholesterol, and LDL-cholesterol were determined using Modular Analytics SWA (Roche). Insulin and C-peptide were measured by ELISA (Siemens, Erlangen, Germany). Free testosterone was determined using a RIA (DSL, Webster, TX, USA). SHBG (Roche); ACTH and insulin-like growth factor 1 (Siemens); and

total testosterone, prolactin, cortisol, human growth hormone (HGH), and TSH (Siemens) were measured by luminescence immunoassay.

Lipometry

Measurements of subcutaneous adipose tissue (SAT) thickness were performed by means of a patented optical device (EU patent no. 0516251) on 15 anatomically well-defined body sites (19) distributed from neck to calf on the right body side. By measuring these 15 specified body sites, a detailed SAT profile of a subject is obtained. The complete measurement cycle takes 2 min and is performed with the subject in an upright standing position (20). Calibration and evaluation were done using computed tomography, dual-energy X-ray absorptiometry (DXA), and bioelectrical impedance as the reference methods (21–23). SAT mass is calculated using the 15 SAT measuring points in consideration of height; visceral adipose tissue (VAT) mass is estimated; total fat mass is calculated as the sum of VAT mass and SAT mass; and total body fat (TBF) is estimated using SAT layers of triceps, back, lower abdomen, and front thigh representing different body regions (arms, legs, upper trunk, and lower trunk) as described previously (23, 24). The lipometer system allows an excellent estimate of TBF when compared with conventional total body electrical conductivity measurements (24). Intra- and interobserver variations for SAT measurements are 1% and 2–3% respectively.

Statistical analysis

Data are presented as median with interquartile range unless otherwise stated. Kolmogorov–Smirnov test and descriptive statistics were used to evaluate the distribution of data. All continuous parameters following a non-normal distribution were logarithmically transformed when parametric tests were performed. AN(C)OVA and χ^2 test were used to compare groups. Binary logistic regression analysis was performed to define variables predicting MS and elevated FLI levels. Receiver operating characteristic (ROC) curves analysis was performed for FLI, WC, HOMA-IR, and TG to evaluate MS. Sensitivity and specificity were calculated at different cut-off points. The highest sensitivity and specificity were obtained at the optimal cut-off value estimated by Youden index (25). A P value of 0.05 was considered statistically significant. All analyses were performed using SPSS 18.0 (SPSS, Inc., Chicago, IL, USA).

Results

Baseline characteristics

Anthropometric and biochemical characteristics of PCOS and control women are shown in Table 1. PCOS women were significantly younger, had higher diastolic BP, fasting and 2 h insulin levels, HOMA-IR, testosterone, free testosterone, AST, and ALT levels and significantly lower SHBG, HDL, and FIB-4 levels than control women. MS was more prevalent in PCOS than in control women. In age-adjusted binary logistic regression analyses, the OR for MS was 6.40 (2.22–18.50), $P<0.001$, for PCOS patients compared with control women. In binary logistic regression analysis (backward elimination), FLI ($P<0.001$) and HOMA-IR ($P=0.08$) were significant predictors of MS whereas age, testosterone, SHBG, AST, and ALT were excluded from the analysis.

The prevalence of obesity (BMI $\geq 30\,kg/m^2$) was 24.8% in PCOS and 19.7% in control women; 21.3% of PCOS and 23.8% of control women were overweight (BMI ≥ 25 and $<30\,kg/m^2$) and 53.9% of PCOS and 56.6% of control women had normal weight (BMI $<25\,kg/m^2$; $P=0.426$).

In PCOS and control women, no woman presented with elevated APRI or FIB-4 index.

Table 1 Characteristics of PCOS and control women. Data are presented as median (interquartile range). Differences between groups were tested with ANOVA.

	PCOS ($n=611$)	Control ($n=139$)	*P* value
Age (years)	27 (23–31)	30 (26–37)	<0.001
BMI (kg/m^2)	24.5 (21.4–29.4)	24.15 (20.9–28.8)	0.882
WC (cm)	82 (72–96)	81 (75–92)	0.421
WHR	0.79 (0.73–0.86)	0.82 (0.77–0.86)	0.170
SBP (mmHg)	116 (110–130)	117 (111–123)	0.468
DBP (mmHg)	80 (71–87)	76 (68–82)	0.006
FG (mg/dl)	85 (80–91)	87 (81–91)	0.228
Glucose 2 h (mg/dl)	97 (81–118)	95 (78–113)	0.370
FI (μU/ml)	6.4 (4.0–10.7)	5.0 (2.9–7.8)	0.008
Insulin 2 h (μU/ml)	35.1 (20.3–59.8)	27.9 (17.8–53.5)	0.013
HOMA-IR	1.34 (0.82–2.38)	1.04 (0.60–1.70)	0.015
Test. (ng/ml)	0.66 (0.50–0.82)	0.44 (0.32–0.55)	<0.001
FT (pg/ml)	2.66 (1.98–3.50)	1.60 (1.22–2.04)	<0.001
SHBG (nmol/l)	44.9 (28.1–61.8)	71.7 (50.0–169.5)	<0.001
TG (mg/dl)	76 (55–106)	66 (54–103)	0.161
TC (mg/dl)	179 (157–200)	183 (159–210)	0.146
HDL-C (mg/dl)	65 (54–78)	72 (62–84)	<0.001
LDL-C (mg/dl)	98 (82–117)	100 (83–122)	0.679
CRP (mg/dl)	1.1 (1.0–3.2)	1.1 (0.7–2.3)	0.959
FLI	11.4 (4.3–48.8)	8.8 (3.9–35)	0.127
APRI	0.32 (0.25–0.40)	0.28 (0.25–0.36)	0.051
FIB-4	0.56 (0.45–0.71)	0.68 (0.52–0.89)	<0.001
GGT (U/l)	15 (11–22)	12 (10–19)	0.117
AST (U/l)	24 (21–29)	23 (19–27)	0.011
ALT (U/l)	18 (15–27)	17 (14–22)	0.023
IGT (%)	10.3	8.0	0.512
MS (%)	12.1	4.9	0.025

BMI, body mass index; WC, waist circumference; WHR, waist-to-hip ratio; SBP, systolic blood pressure; DBP, Diastolic blood pressure; FG, fasting glucose; FI, fasting insulin; test., testosterone; FT, free testosterone; HOMA-IR, homeostatic model assessment-insulin resistance; SHBG, sex hormone-binding globulin; TG, triglycerides; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; CRP, C-reactive protein; FLI, fatty liver index; APRI, AST-to-platelet ratio index; GGT, gamma-glutamyl transferase; AST, aspartate amino transferase; ALT, alanine transaminase; IGT, impaired glucose tolerance; MS, metabolic syndrome.

Table 2 Characteristics of PCOS women with and without elevated FLI. Data are presented as median (interquartile range). Differences between groups were tested with ANOVA.

	FLI >60 (n=140)	FLI <60 (n=471)	*P* value
Age (years)	28 (24–33)	26 (22–39)	<0.001
BMI (kg/m^2)	36.9 (33.3–40.5)	22.7 (20.6–25.5)	<0.001
WC (cm)	110 (101–119)	77 (71–85)	<0.001
WHR	0.92 (0.84–0.98)	0.76 (0.72–0.82)	<0.001
SBP (mm Hg)	130 (120–140)	115 (108–125)	<0.001
DBP (mmHg)	90 (83–100)	78 (70–85)	<0.001
FG (mg/dl)	88 (82–97)	85 (79–90)	<0.001
Glucose 2 h (2 h)	129 (104–153)	92 (77–107)	<0.001
FI (μU/ml)	14.7 (10.5–20.0)	5.3 (3.4–8.0)	<0.001
Insulin 2 h (μU/ml)	81.6 (46.5–129.8)	29.3 (18.0–49.1)	<0.001
HOMA-IR	3.14 (2.28–5.09)	1.11 (0.70–1.70)	<0.001
Test. (ng/ml)	0.67 (0.52–0.85)	0.65 (0.50–0.81)	0.682
FT (pg/ml)	3.28 (2.48–4.22)	2.47 (1.88–3.23)	<0.001
SHBG (nmol/l)	24.2 (17.5–33.2)	49.9 (36.2–66.7)	<0.001
TG (mg/dl)	119 (91–166)	67 (53–91)	<0.001
TC (mg/dl)	181 (163–207)	179 (156–200)	0.162
HDL (mg/dl)	49 (42–56)	70 (60–83)	<0.001
LDL (mg/dl)	107 (95–129)	95 (80–114)	<0.001
CRP (mg/dl)	5.9 (2.6–8.7)	1.0 (0.7–1.8)	<0.001
APRI	0.34 (0.27–0.48)	0.32 (0.25–0.39)	0.004
FIB-4	0.50 (0.41–0.67)	0.58 (0.46–0.74)	0.006
GGT (U/l)	31 (20–44)	14 (11–18)	<0.001
AST (U/l)	29 (24–35)	23 (20–27)	<0.001
ALT (U/l)	33 (23–51)	17 (14–22)	<0.001
IGT (%)	33.7	4.4	<0.001
MS (%)	48.5	1.8	<0.001

FLI, fatty liver index; BMI, body mass index; WC, waist circumference; WHR, waist-to-hip ratio; SBP, systolic BP; DBP, diastolic BP; FG, fasting glucose; FI, fasting insulin; Test., testosterone; FT, free testosterone; HOMA-IR, homeostatic model assessment-insulin resistance; SHBG, sex hormone-binding globulin; TG, triglycerides; TC, total cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; CRP, C-reactive protein; APRI, AST-to-platelet ratio index; GGT, gamma-glutamyl transferase; AST, aspartate amino transferase; ALT, alanine transaminase; IGT, impaired glucose tolerance; MS, metabolic syndrome.

Fatty liver index

PCOS women had significantly higher FLI levels than control women in age-adjusted analyses (11.4 (4.3–48.8) and 8.8 (3.9–35.0), respectively, P=0.001). In binary logistic regression analysis adjusted for age, OR for elevated FLI was 2.52 (1.31–4.85), P=0.006, for PCOS women when compared with control women. Elevated FLI levels were prevalent in 22.8% and 16.5% of PCOS and control women respectively (P=0.147).

When analyses of FLI were stratified by obesity, we found an increased prevalence of elevated FLI levels in obese (BMI >30 kg/m^2) PCOS women when compared with obese control women (87.7 and 67%, respectively, P=0.024). In overweight women (BMI ≥25 and <30 kg/m^2), the prevalence of elevated FLI levels was similar in PCOS and control women (7% and 10%, respectively, P=0.457). In normal weight PCOS and control women (BMI <25 kg/m^2), no one presented with elevated FLI levels.

PCOS women with high FLI levels (>60) were significantly older; had higher BMI, WC, WHR, systolic and diastolic BP, fasting and stimulated glucose levels, fasting and stimulated insulin levels, HOMA-IR, free testosterone, TG, LDL, C-reactive protein (CRP), GGT, AST, and ALT levels; and had lower SHBG and HDL levels than PCOS women with FLI levels <60 (Table 2). Moreover, the prevalence of IGT and MS was significantly higher in PCOS women with elevated FLI levels. FLI levels were significantly higher in PCOS women with IGT and MS than in PCOS women without IGT and MS (Fig. 1).

FLI levels correlated positively and significantly with GGT, AST, ALT, age, BMI, WC, WHR, diastolic BP, systolic BP, fasting and stimulated glucose, fasting and stimulated insulin, HOMA-IR, free testosterone, TC, TG, LDL, CRP, and APRI and negatively and significantly with SHBG, HDL, and FIB-4 (Table 3).

The prevalence of elevated FLI was 88.7% in PCOS women with MS and 11.3% in PCOS women without MS (P<0.001). In control women, elevated FLI was present in 66.7% of women with MS and 30.8% of women without MS. In PCOS women with elevated HOMA-IR levels, the prevalence of elevated FLI was 56.0% compared with 7.0% in PCOS women without IR (P=0.001). In control women, high FLI levels were found in 69.2% of women with IR and 8.2% of women without IR (P<0.001).

GGT, AST, and ALT

The prevalence of elevated AST levels was higher in PCOS women than in control women (101 out of 527 PCOS women (19.2%) and 12 out of 123 control women (9.8%), P=0.017). Moreover, elevated ALT levels tended to be more common in PCOS compared to control women (75 out of 527 PCOS women (14.2%) and nine out of 123 control women (7.3%), P=0.073). The prevalence of elevated GGT levels was similar in PCOS and control women (61 out of 611 PCOS women (10.0%) and eight out of 139 control women (5.8%), P=0.190).

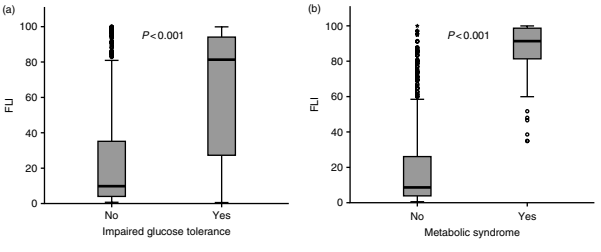

Figure 1 FLI levels in PCOS women according to the prevalence of impaired glucose tolerance and the metabolic syndrome. Data were analyzed by ANOVA. Data available in 611 PCOS women. (a) FLI levels in PCOS women according to the prevalence of impaired glucose tolerance (yes/no). (b) FLI levels in PCOS women according to the prevalence of metabolic syndrome (yes/no). Open circles represent outlier 1.5–3.0 interquarile range; *represents outlier >3.0 interquartile range.

Table 3 Correlation of FLI, GGT, AST, and ALT with anthropometric and biochemical characteristics of PCOS women (Pearson correlation analyses, $n=611$).

	FLI		GGT		AST		ALT	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
FLI	1		0.422	<0.001	0.286	<0.001	0.536	<0.001
GGT	0.422	<0.001	1		0.334	<0.001	0.504	<0.001
AST	0.286	<0.001	0.334	<0.001	1		0.659	<0.001
ALT	0.536	<0.001	0.504	<0.001	0.659	<0.001	1	
Age	0.200	<0.001	0.074	0.075	−0.018	0.676	0.039	0.367
BMI	0.917	<0.001	0.339	<0.001	0.247	<0.001	0.468	<0.001
WC	0.909	<0.001	0.342	<0.001	0.253	<0.001	0.460	<0.001
WHR	0.691	<0.001	0.269	<0.001	0.193	<0.001	0.362	<0.001
Diastolic BP	0.493	<0.001	0.142	0.003	0.154	0.002	0.254	<0.001
Systolic BP	0.489	<0.001	0.138	0.005	0.107	0.029	0.277	<0.001
Fasting glucose	0.338	<0.001	0.233	<0.001	0.222	<0.001	0.295	<0.001
Glucose 2 h	0.503	<0.001	0.297	<0.001	0.214	<0.001	0.384	<0.001
Fasting insulin	0.659	<0.001	0.381	<0.001	0.259	<0.001	0.511	<0.001
Insulin 2 h	0.516	<0.001	0.249	<0.001	0.153	0.001	0.372	<0.001
HOMA-IR	0.624	<0.001	0.427	<0.001	0.284	<0.001	0.524	<0.001
Testosterone	0.036	0.458	0.037	0.405	0.066	0.133	0.042	0.337
Free testosterone	0.358	<0.001	0.184	<0.001	0.101	0.023	0.209	<0.001
SHBG	−0.366	<0.001	−0.153	<0.001	−0.075	0.088	−0.197	<0.001
TG	0.512	<0.001	0.238	<0.001	0.139	0.001	0.261	<0.001
TC	0.119	0.013	0.098	0.027	0.068	0.121	0.074	0.091
HDL	−0.569	<0.001	−0.0178	<0.001	−0.117	0.007	−0.284	<0.001
LDL	0.297	<0.001	0.118	0.013	0.083	0.077	0.155	0.001
CRP	0.352	<0.001	0.178	<0.001	0.085	0.054	0.151	<0.001
APRI	0.105	0.029	0.213	<0.001	0.826	<0.001	0.516	<0.001
FIB-4	−0.169	<0.001	−0.047	0.290	0.375	<0.001	−0.112	0.010

FLI, fatty liver index; GGT, gamma-glutamyl transferase; AST, aspartate amino transferase; ALT, alanine transaminase; BMI, body mass index; WC, waist circumference; WHR, waist-to-hip ratio; BP, blood pressure; HOMA-IR, homeostatic model assessment-insulin resistance; SHBG, sex hormone-binding globulin; TG, triglycerides; TC, total cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; CRP, C-reactive protein; APRI, AST-to-platelet ratio index.

We found significant positive correlations of GGT with FLI, AST, ALT, BMI, WC, WHR, diastolic BP, systolic BP, fasting and stimulated glucose, fasting and stimulated insulin, HOMA-IR, free testosterone, TC, TG, LDL, CRP, and APRI and significant negative correlations with SHBG and HDL (Table 3).

We observed significant positive correlations of AST with FLI, GGT, ALT, BMI, WC, WHR, diastolic BP, systolic BP, fasting and stimulated glucose, fasting and stimulated insulin, HOMA-IR, free testosterone, TG, APRI and FIB-4 and a significant negative correlation with HDL (Table 3).

There were significant positive correlations of ALT with FLI, GGT, AST, BMI, WC, WHR, diastolic BP, systolic BP, fasting and stimulated glucose, fasting and stimulated insulin, HOMA-IR, free testosterone, TG, LDL, and APRI and a significant negative correlation with SHBG, HDL, and FIB-4 (Table 3).

Receiver operating characteristic

In PCOS and control women, ROC curve analysis revealed that the best cut-off value for FLI to define the presence of MS was 34.6 and 25.0 respectively (sensitivity 100% and specificity 80.1%, AUC 0.96 (Fig. 2a); and sensitivity 100% and specificity 68.2%, AUC 0.87 respectively). Further, ROC curve analysis of FLI for PCOS and control women to define MS was performed with a cut-off value of 60 (sensitivity 87.0 and 50% respectively; specificity 86.7 and 84.5% respectively).

In PCOS and control women, the best cut-off value for APRI to define the presence of MS was 0.48 and 0.35 respectively (sensitivity 32.7 and specificity 90.8%, AUC 0.61 (Fig. 2b); sensitivity 60% and specificity 67.9%, AUC 0.62).

In PCOS and control women, the best cut-off value for FIB-4 to define the presence of MS was 0.71 and 0.97 respectively (sensitivity 29.1% and specificity 76.6%, AUC 0.48 (Fig. 2c); and sensitivity 40% and specificity 80.2%, AUC 0.64).

In PCOS and control women, ROC curve analysis revealed that the best cut-off value for HOMA-IR to define the presence of MS was 2.5 and 2.2 respectively (sensitivity 86.0% and specificity 84.1%, AUC 0.89 (Fig. 2d); and sensitivity 60% and specificity 90%, AUC 0.76 respectively).

In PCOS and control women, ROC curve analysis revealed that the best cut-off value for WC to define the presence of MS was 88.5 and 97.5 respectively

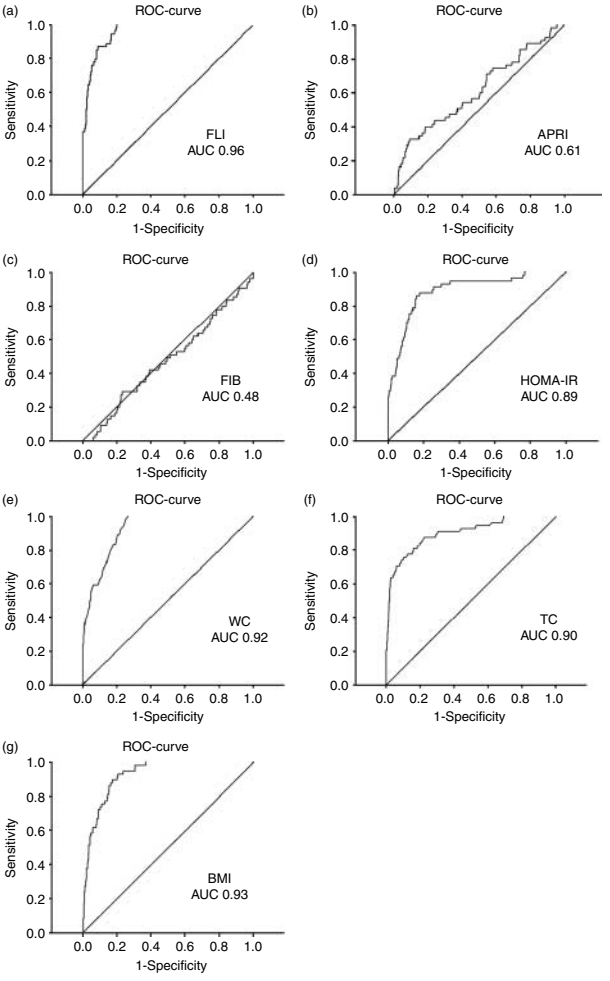

Figure 2 ROC curve analyses were performed for FLI, APRI, FIB-4, HOMA-IR, WC, TG, and BMI to define MS in PCOS women. Sensitivity and specificity were calculated at different cut-off points. Data available in 611 PCOS women. (a) ROC analyses for FLI to define MS in PCOS women. (b) ROC analyses for APRI to define MS in PCOS women. (c) ROC analyses for FIB-4 to define MS in PCOS women. (d) ROC analyses for HOMA-IR to define MS in PCOS women. (e) ROC analyses for WC to define MS in PCOS women. (f) ROC analyses for TG to define MS in PCOS women. (g) ROC analyses for BMI to define MS in PCOS women.

(sensitivity 100% and specificity 73.1%, AUC 0.92 (Fig. 2e); and sensitivity 100% and specificity 80.9%, AUC 0.93 respectively).

In PCOS and control women, ROC curve analysis revealed that the best cut-off value for TG to define the presence of MS was 121.5 and 98.5 respectively (sensitivity 75.9% and specificity 89.4%, AUC 0.90 (Fig. 2f); and sensitivity 83.3% and specificity 71.8%, AUC 0.76 respectively).

In PCOS and control women, ROC curve analysis revealed that the best cut-off value for BMI to define the presence of MS was 28.1 and 31.5 respectively (sensitivity 93% and specificity 79.6%, AUC 0.93 (Fig. 2g); and sensitivity 80% and specificity 88.6%, AUC 0.92 respectively).

Lipometry

Lipometry was available in 175 PCOS women. Correlations between FLI and body fat parameters are shown in Table 4. We found a significant positive correlation of FLI levels with TBF, fat mass, SAT mass, VAT mass, and fat layers located on the trunk (neck, triceps, biceps, front chest, lateral chest, upper abdomen, and upper back) as well as a negative correlation of FLI with leg SAT layers (front thigh, lateral thigh, rear thigh, inner thigh, and calf). PCOS women with elevated FLI levels had significantly higher TBF (data not shown), fat mass (Fig. 3a), SAT mass (data not shown), and VAT mass (Fig. 3b).

Discussion

Our data from a large cohort of PCOS and control women indicate that FLI levels are higher in PCOS than in BMI-matched control women. NAFLD, as defined by high FLI levels, is a common finding in obese PCOS women, whereas no evidence for elevated FLI levels was found in normal weight PCOS women. Moreover, NAFLD, as defined by elevated FLI levels, was evident in almost all PCOS patients affected by MS. FLI might thus be the best predictor of MS in PCOS women. FLI as well as GGT, AST, and ALT levels were associated with an adverse metabolic profile including obesity, higher BP, IR, and dyslipidemia in PCOS women. We found no evidence of elevated fibrosis indices such as FIB-4 or APRI in PCOS as well as in control women.

In age-adjusted analyses, we found higher FLI levels in PCOS than in BMI-matched control women as well as

Table 4 Correlation of FLI with body fat measures in PCOS women (Pearson correlation analyses). Data available in 175 PCOS women.

	Correlation	*P* value
TBF	0.270	<0.001
Fat mass	0.813	<0.001
SAT mass	0.684	<0.001
VAT mass	0.914	<0.001
SAT layers		
Neck	0.761	<0.001
Triceps	0.348	<0.001
Biceps	0.580	<0.001
Front chest	0.504	<0.001
Lateral chest	0.470	<0.001
Upper abdomen	0.133	0.038
Lower abdomen	0.048	0.457
Upper back	0.367	<0.001
Lower back	−0.025	0.700
Hip	0.050	0.436
Front thigh	−0.245	<0.001
Lateral thigh	−0.232	0.002
Rear thigh	−0.395	<0.001
Inner thigh	−0.170	0.025
Calf	−0.262	<0.001

FLI, fatty liver index; TBF, total body fat; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue.

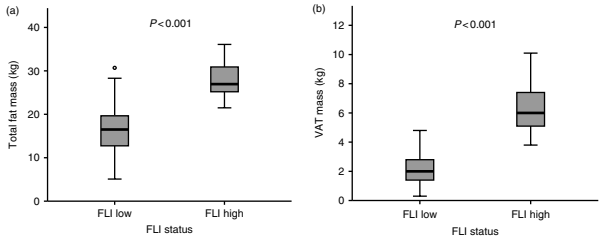

Figure 3 Total fat mass and visceral adipose tissue (VAT) mass in PCOS women according to fatty liver index (FLI) levels. High FLI level indicates FLI >60 and low FLI level indicates FLI <60. Data were analyzed by ANOVA. Data available in 175 PCOS women. (a) Total fat mass in PCOS women according to FLI levels. (b) VAT mass in PCOS women according to FLI levels. Open circle represents outlier 1.5–3.0 interquartile range.

a tight association of high FLI levels with an adverse metabolic profile. Of note, FLI has been associated with coronary heart disease and early atherosclerosis in a cross-sectional study (26). Moreover, high FLI levels are associated not only with increased hepatic and cardiovascular mortality but also with a higher prevalence of cancer as well as all-cause mortality (27). There is accumulating evidence linking NAFLD with IR and MS (28); NAFLD represented by elevated FLI levels is recognized to be an important manifestation of IR. Whether IR leads to hepatic steatosis or whether fat accumulation in the liver is the primary event leading to hepatic and consequently peripheral IR is not entirely clear (7). It has been proposed that a fatty liver and hepatic IR are key factors in and initiators of a twin vicious cycle underlying the pathophysiology of type 2 diabetes (29). NAFLD, as manifested by elevated ALT levels, predicts the future development of diabetes (30).

Although overall obesity is clearly associated with NAFLD, body fat distribution appears to play a key role in the development of NAFLD. Expanded and inflamed VAT releases a wide array of molecules potentially involved in the development of IR and atherosclerosis. The resulting adipose tissue inflammation is one of the earliest steps in the chain of events leading to IR (31). Thus, excess intra-abdominal fat in particular may be an important determinant in the pathogenesis of NAFLD, both via its strong association with IR and possibly as a source of free fatty acids (7). This notion is supported by our finding showing a strong correlation of VAT and trunk located fat layers with FLI, whereas leg fat layers were inversely correlated with FLI. Of note, high levels of lipid accumulation product reflecting increased abdominal fat deposition are higher in PCOS than in BMI-matched control women and have been linked to IGT in PCOS women (32). Interestingly, PCOS women with a fatty liver have increased preperitoneal, mesenteric, and subcutaneous fat as well as an increased intima-media thickness (33), which is in line with our data showing a correlation of FLI with an adverse metabolic profile. Thus, vice versa, one might speculate on an important role of fatty liver and hepatic IR in the development of PCOS.

NAFLD is considered the hepatic manifestation of MS, which is consistent with our data showing that 88.7% of women with MS have elevated FLI levels indicating NAFLD. Moreover, ROC curve analyses revealed that FLI might be the best predictor to define MS in PCOS women. In our study, the prevalence of MS was 12.1% in PCOS women, in accordance with PCOS cohorts from Southern Europe showing a prevalence of MS between 8 and 25%. These results are, however, much lower than the ones from the United States showing a MS prevalence of up to 47% (6). In our cohort, PCOS women had a 6.4-fold higher risk for MS than BMI-matched controls.

PCOS women had higher FLI levels than BMI-matched control women. This is in line with previous smaller studies, showing a higher prevalence of hepatic steatosis in PCOS women (10). Elevated ALT levels are a common finding in PCOS (10, 34–36). Similarly, we found a trend toward higher ALT levels in our PCOS cohort when compared with control women. Cerda *et al.* (37) demonstrated that 41% of PCOS women had concomitant NAFLD as diagnosed by hepatic steatosis and abnormal ALT levels, whereas the incidence was 19% in a group of age- and weight-matched controls. In our study, 23% of PCOS women had increased FLI and having PCOS results in a 2.5-fold increased risk of high FLI levels in age-adjusted analyses. Likewise, Tan *et al.* (38) demonstrated increased caspase-cleaved cytokeratin (CK) 18 levels indicating non-alcoholic steatohepatitis (NASH) in 27% of PCOS women (mean age 28.4 ± 6.7, mean BMI 31.5 ± 8.3). Gambarin-Gelwan *et al.* (39) found a prevalence of 55% of NAFLD defined by abdominal ultrasound in PCOS women (median age 31.4 years, 39% normal weight).

Interestingly, in obese PCOS women (BMI $>30\ kg/m^2$), the prevalence of high FLI was 88% whereas only 7% of overweight and none of the lean PCOS patients had elevated FLI levels. In contrast, Gambarin-Gelwan *et al.* (39) found that 39% of PCOS women with ultrasonographic evidence of hepatic steatosis were lean.

NAFLD is considered a non-specific term encompassing several clinicopathological entities including steatosis alone, steatohepatitis, and histologically alcoholic-like steatohepatitis. These entities are similar to alcoholic liver diseases in the absence of significant alcohol abuse (40). Simple hepatic steatosis and hepatic steatosis with nonspecific inflammation are believed to be generally benign conditions, whereas NASH can progress to cirrhosis, leading to liver failure as well as to hepatocellular carcinoma (41, 42). FLI was developed as an accurate index for defining NAFLD and was validated in a large cohort of subjects with and without suspected liver disease (12). This index is easy to employ as BMI, WC, TG, and GGT are routine measurements in clinical practice. Potential clinical uses of FLI include

the selection of subjects to be referred to ultrasonography and the identification of patients for intensified lifestyle counseling, which might be highly important in PCOS women. Considering the high prevalence of PCOS (43), liver biopsy cannot be performed in every patient due to serious procedural risks. Therefore, new, easily determined, and noninvasive surrogates such as FLI are warranted. Routine liver screening should be performed in all obese women with PCOS as well as in those affected by MS.

Our study has several limitations that should be noted. First, we did not perform ultrasound of the liver and therefore cannot comment on morphological liver aspects. Moreover, we did not test for hepatitis B or C. The strengths of our study are the large sample size of our PCOS cohort, the exact metabolic characterization with respect to MS including OGTT, as well as the availability of fat measure in a subgroup of PCOS patients.

In conclusion, we present evidence that high FLI levels are a common finding in obese PCOS women and are closely linked to MS. FLI might be a useful index to identify PCOS women at high metabolic and hepatic risk in whom a very careful surveillance is needed and who might benefit from intensified lifestyle counseling. Further studies evaluating the impact of FLI levels in combination with ultrasonography of the liver are warranted.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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