Increased prevalence of polycystic ovary syndrome in premenopausal women with nonalcoholic fatty liver disease

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Abstract

Objective: Limited data exist concerning the presence of polycystic ovary syndrome (PCOS) in premenopausal women with nonalcoholic fatty liver disease (NAFLD). We aimed to investigate the prevalence of PCOS in overweight and obese premenopausal women with NAFLD.

Design: Prospective, observational, and cross-sectional study.

Methods: We studied 110 apparently healthy, overweight, and obese (BMI: 25.1–49.1 kg/m²) premenopausal women (age: 18–45 years) reporting no or minimal alcohol consumption for NAFLD with abdominal ultrasonography after excluding causes of secondary liver disease and for PCOS (Rotterdam criteria) with clinical examination, biochemical evaluation, and pelvic ultrasonography. Insulin resistance (IR) was assessed by homeostasis model assessment of IR (HOMA-IR), and free androgen index was calculated.

Results: NAFLD was detected in 71/110 women (64.5%). Women with NAFLD compared to women without NAFLD were more commonly diagnosed with PCOS (43.7% vs 23.1%, respectively, $P=0.04$), metabolic syndrome (30.2% vs 5.3%, respectively, $P=0.003$), and abnormal lipid profile (81.1% vs 51.3%, $P=0.002$). All women with abnormal glucose metabolism had NAFLD ($P=0.01$). Although PCOS was associated with NAFLD (OR 2.6, 95% CI: 1.1–6.2, $P=0.04$), in a multivariate analysis higher HOMA-IR values (OR 2.2, 95% CI: 1.1–4.4, $P=0.02$) and triglyceride levels (OR 1.01, 95% CI: 1.00–1.02, $P=0.04$) independently predicted NAFLD, after adjusting for age, BMI, and waist-to-hip ratio.

Conclusions: These findings indicate an increased prevalence of PCOS in overweight and obese premenopausal women with NAFLD, although it is not supported that the syndrome is primarily involved in NAFLD. Evaluation for PCOS may be considered in these women.

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Introduction

Nonalcoholic fatty liver disease (NAFLD) is an increasingly recognised chronic disorder characterised by fat accumulation in the liver, histologically identical to alcoholic liver disease, in patients with no or minimal alcohol consumption (1). The exclusion of nutritional disorders, drugs, and diseases known to cause secondary fatty liver disease is a prerequisite for the diagnosis (1). NAFLD, diagnosed by

imaging modalities, is estimated to affect 14–33% of adults in the general population (2, 3). The clinical relevance of NAFLD is related to its high prevalence and its possible evolution to end-stage liver disease and rarely to hepatocellular carcinoma (1). Existing data support that insulin resistance (IR) and compensatory hyperinsulinemia have a major role in the pathophysiology of NAFLD (4); however,

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the cause/effect relationship between NAFLD and IR still remains unclear (5). NAFLD prevalence is markedly increased in obesity (1, 6), type 2 diabetes mellitus (DM) (6, 7) and dyslipidemia (1). NAFLD is considered as the hepatic component of metabolic syndrome (8).

Epidemiologic studies have shown that NAFLD is more common in men than in women (9, 10), and there are few data from clinical series indicating a much lower prevalence in premenopausal compared to postmenopausal women (9, 11, 12). Regarding premenopausal women, NAFLD has been mostly studied in patients with polycystic ovary syndrome (PCOS), and it has been shown that it is more prevalent in these patients (13). PCOS is characterised by hyperandrogenism and ovulatory dysfunction and is one of the most common endocrinopathies in premenopausal women, affecting 6–19% of this population depending on the used diagnostic criteria (14). The important role of IR in the pathophysiology of the syndrome was recognized long after its initial description (15). An increased prevalence of IR-related morbidities in PCOS patients such as impaired glucose tolerance (IGT) and DM, abdominal adiposity, dyslipidemia, and metabolic syndrome has been reported (16). These adverse metabolic features are implicated in the pathogenesis of NAFLD. Existing data support that obesity and IR are the main factors related to NAFLD in PCOS (17, 18, 19), whereas androgen excess may be an additional contributing factor (20, 21, 22).

The reported close link between NAFLD and PCOS led to the suggestion of evaluating premenopausal women with NAFLD for the presence of PCOS (23). The hypothesis of an increased prevalence of PCOS in premenopausal women with NAFLD has been investigated so far in only one small prospective study that reported PCOS diagnosis in the majority of the participating NAFLD premenopausal patients (24). In the present study we evaluated prospectively overweight and obese premenopausal women with abdominal and pelvic ultrasonographic examination and laboratory tests for the presence of NAFLD and PCOS to test the hypothesis that overweight and obese premenopausal women with NAFLD are more likely to have PCOS than women without NAFLD.

Subjects and methods

Subjects

Premenopausal women, aged 18–45 years, with a BMI $>25.0 \text{ kg/m}^2$ were recruited, for obesity, from the outpatient endocrine clinic of our department from August

2011 to July 2014. Exclusion criteria were women who reported alcohol consumption more than 10 g alcohol/day (>1 alcoholic drink/day); history of known liver disease including viral, autoimmune, genetic, and drug induced liver disease; history of known hypertension, dyslipidemia, DM, or other systemic diseases; and use of any medication during the last 3 months preceding the entry to the study. All women were Caucasian.

PCOS diagnosis was based on the presence of at least two of the following three criteria: hyperandrogenism (clinical and/or biochemical), chronic oligo- or anovulation (clinically expressed as oligomenorrhea or amenorrhea), and polycystic ovarian morphology on ultrasound (Rotterdam criteria) (25), after excluding diseases causing androgen excess and anovulation (nonclassical adrenal 21-hydroxylase deficiency, Cushing's syndrome, hyperprolactinemia, untreated thyroid disease, and androgen secreting tumors) by appropriate tests.

Study protocol

Women were studied in the early follicular phase (days 2–6 following menstruation) of a spontaneous cycle or after progestin-induced withdrawal bleeding for those who were amenorrhoeic ($n=4$), after exclusion of any acute illness.

A detailed medical history was obtained and clinical examination was performed, during which anthropometric measurements (weight, height, and waist and hip circumference), clinical signs of hyperandrogenism (hirsutism, acne, and alopecia) and blood pressure were recorded. Hirsutism was assessed using the modified Ferriman–Gallwey (mF-G) scoring method (26) and women with scores of 8 or greater were considered hirsute. After an overnight fast, blood was drawn for biochemical evaluation followed by a 75 g oral glucose tolerance test (OGTT) with glucose and insulin measurements every hour for 2 h. The same day an abdominal and a pelvic ultrasound examination were performed. Biochemical evaluation comprised hematological profile, C-reactive protein, creatinine, fasting glucose and insulin, serum aspartate aminotransferase (AST), and alanine aminotransferase (ALT), gamma-glutamyltranspeptidase (γ GT), alkaline phosphatase (ALP), serum iron, ferritin, uric acid, total cholesterol, LDL and HDL cholesterol, triglycerides, hepatitis B surface antigen, surface and core antibodies, hepatitis C virus antibodies, antinuclear antibodies, follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E_2), total testosterone (TT), DHEA-S, and sex hormone-binding globulin (SHBG). Abnormal

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aminotransferase and γ GT levels were defined as values exceeding the upper normal level in our hospital's laboratory (ALT >33 IU/l, AST >32 IU/l, and γ GT >36 IU/l).

Women with systolic arterial blood pressure of 140 mmHg or more and/or diastolic pressure of 90 mmHg or more were defined as hypertensive (27). Women with a fasting serum glucose level >7.0 mmol/l and/or a serum glucose level >11.1 mmol/l at 2 h after glucose load were defined as diabetics (28). Women with a serum glucose level of 7.8–11.05 mmol/l at 2 h after glucose challenge were defined as having IGT and those with a fasting glucose level of 5.6–6.9 mmol/l were defined as having impaired fasting glucose (IFG) (28). Women with fasting serum triglyceride levels >1.7 mmol/l and/or serum total cholesterol levels >5.17 mmol/l and/or LDL cholesterol >3.88 mmol/l and/or HDL cholesterol <1.29 mmol/l were defined as having an abnormal lipid profile.

Metabolic syndrome was diagnosed based on the presence of three or more of the following findings: waist circumference >88 cm, serum triglycerides $\geq$ 1.7 mmol/l, HDL cholesterol <1.29 mmol/l, serum glucose levels $\geq$ 6.1 mmol/l, and blood pressure $\geq$ 130/85 mmHg according to ATP III criteria (29).

The study protocol was approved by the ethics committee of our hospital and informed consent was obtained from all participants.

Assays and calculations

Serum glucose was measured by the oxidase method and lipid profile by an enzymatic, colorimetric method. ALT and AST were measured with an International Federation of Clinical Chemistry (IFCC) kinetic u.v. method, without pyridoxal phosphate activation (P-5'-P), and γ GT with L- γ -glutamyl-3-carboxyl-4-nitroanilide using the Cobas 8000 analyzer (Roche Diagnostics GmbH).

Serum LH, FSH, E_2 , testosterone, SHBG, DHEA-S, and insulin were measured by electrochemiluminescence immunoassays (Roche Diagnostics GmbH) using the Elecsys 411 analyzer (Roche Diagnostics GmbH). The intraassay and interassay coefficients of variation of the aforementioned assays were all <10%.

BMI was calculated as body weight (kg)/height² (m²). The waist-to-hip ratio (WHR) was calculated. Free androgen index (FAI) was calculated as TT (nmol/l) $\times$ 100/SHBG (nmol/l). IR was assessed by homeostasis model assessment of IR (HOMA-IR), which was calculated as fasting insulin (μ U/ml) $\times$ fasting glucose (mmol/l)/22.5. Insulin sensitivity was assessed with the insulin sensitivity index

(ISI 0 and 120 min) using fasting (0 min) glucose and insulin and 120 min glucose and insulin levels from OGTT, according to the formula proposed by Gutt *et al.* (30): $m/(0 \text{ min glucose} + 120 \text{ min glucose}) \times 0.5 / \log(0 \text{ min insulin} + 120 \text{ min insulin} \times 0.5)$, where m is calculated as $75\,000 \text{ mg} + (0 \text{ min glucose} - 120 \text{ min glucose}) \times 0.19 \times \text{body weight}/120 \text{ min}$.

Ultrasonography

Abdominal ultrasound ► The examination was performed by the same radiologist (H Lazaridou) who was unaware of the participants' medical histories and laboratory findings. An ATL HDI 5000 (ATL Ultrasound, Bothell, WA, USA) with a CL 4–7 MHz curvilinear transducer was used for the study.

The following ultrasonographic findings were evaluated: ultrasonographic contrast between hepatic and right renal parenchyma (hepatorenal echo contrast), abnormally intense high-level echoes arising from the hepatic parenchyma, echo penetration into the deep portion of the liver, intrahepatic vessel blurring and abnormal visualization of the diaphragm. Absence of hepatic steatosis (HS) was defined as equal echogenicity of hepatic parenchyma to that of the renal cortex with clear visualization of the intrahepatic vessels and diaphragm. Mild HS (grade 1) was defined as a slight diffuse increase in fine echoes in the hepatic parenchyma (i.e., 'bright liver') with clear visualization of the intrahepatic vessels and the diaphragm. Moderate HS (grade 2) was defined as a moderate diffuse increase in fine echoes in the hepatic parenchyma with slightly impaired visualization of the intrahepatic vessels (i.e., 'vascular blurring') and diaphragm. Severe HS (grade 3) was defined as a marked increase in fine echoes in the hepatic parenchyma with poor or absence of visualization of the intrahepatic vessels, the diaphragm, and the posterior right hepatic lobe (31).

Pelvic ultrasound ► The examination was performed by the same gynaecologist (K Salambasis) who was unaware of the subjects' medical histories and laboratory findings with an Accuvix V20, Samsung Medison, and an EC4-9IS transvaginal transducer. A transvaginal pelvic ultrasound was performed in the majority of women (women who had no sexual relations were examined transabdominally, $n=7$). Polycystic ovarian morphology was defined as the presence of 12 or more follicles measuring 2–9 mm in diameter and/or ovarian volume >10 ml of at least one ovary (32).

Statistical analysis

Results for continuous variables are expressed as mean $\pm$ s.d. and for categorical variables as absolute numbers or percentages. Differences in continuous variables between groups were tested using the unpaired *t*-test or the Mann-Whitney *U* test, as appropriate. Differences in categorical variables between groups were tested using the χ^2 -test with the Yates correction or Fisher's exact test, as appropriate. Pearson's correlation was used to explore the association among pairs of continuous variables. Univariate logistic regression analysis was applied to evaluate the effect of age, BMI, waist circumference, HOMA-IR values, HDL cholesterol and triglyceride levels, FAI and log FAI values and PCOS diagnosis (used as independent variables) on the presence of NAFLD (used as the dependent variable) for all subjects. Multivariate regression analysis after adjusting for age, BMI, and WHR was used to control for confounding. For these analyses, variables that did not follow normal distribution were logarithmically transformed. All statistical analyses were performed using SPSS, version 22.0 (SPSS, Inc.). A $P < 0.05$ was considered statistically significant.

Results

There were 110 consecutive, eligible premenopausal women, aged 18–45 years (33.6 ± 7.6 years) with a BMI of 25.1 – 49.1 kg/m² (34.0 ± 6.1 kg/m²) (78 obese (BMI ≥ 30 kg/m²) of which 21 with a BMI > 40 kg/m² and 32 overweight (BMI 25.2 – 29.9 kg/m²)) who agreed to participate in the study. NAFLD diagnosed by abdominal ultrasound (HS) was detected in 71 of 110 women (64.5%), 51 obese and 20 overweight. HS was characterized as mild (grade 1) in the majority of women (48 of 71, 67.6%) and as moderate (grade 2) or severe (grade 3) in the rest (13 of 71 (18.3%) and ten of 71 (14.1%) respectively). Abnormal liver function tests (LFTs; ALT > 33 IU/l and/or AST > 32 IU/l and/or γ GT > 36 IU/l) were detected in 16 of 110 women (14.5%), 12 obese and four overweight. Women with abnormal LFTs had HS except one (93.7%). An abnormal lipid profile was detected in 76 of 110 women (69.1%). Metabolic syndrome was diagnosed in 21% of women. Hypertension was detected in 1.8% of women. IFG, IGT and DM were diagnosed in 1.9, 10.2, and 2.7% of women respectively (Fig. 1).

PCOS was diagnosed in 40 of 110 women (36.4%) (Fig. 1). The majority of PCOS women (30 of 40, 75%) had evidence of hyperandrogenism; biochemical hyperandrogenism (serum testosterone ≥ 2.8 nmol/l and/or FAI > 6.5)

Figure 1

Characteristics of participants (%). HS, hepatic steatosis; Ab LFTs, abnormal liver function tests; PCOS, polycystic ovary syndrome; AL, abnormal lipid profile; MetS, metabolic syndrome; HT, hypertension; IGT, impaired glucose tolerance; DM, diabetes mellitus; IFG, impaired fasting glucose.

was detected in 25 of 40 (62.5%) PCOS women while five of 40 (12.5%) PCOS women presented only clinical hyperandrogenism (mF-G ≥ 8). Ten PCOS women (25%) had no evidence of hyperandrogenism (presenting chronic anovulation and PCO morphology on ultrasound). NAFLD was diagnosed in 31 of 40 PCOS women (77.5%), being more frequent in hyperandrogenic women (21 with biochemical and five clinical hyperandrogenism, $P = 0.03$).

Women with NAFLD were more obese ($P < 0.001$), with increased waist circumference ($P < 0.001$) and WHR ($P = 0.001$) than women without NAFLD. They had higher serum levels of fasting insulin ($P < 0.001$), uric acid ($P = 0.04$), triglycerides ($P < 0.01$), ALT ($P < 0.001$), ALP ($P = 0.01$), γ GT ($P = 0.001$), and E_2 ($P = 0.04$) and lower levels of SHBG ($P = 0.01$) compared to women without NAFLD. They also had higher values of HOMA-IR ($P < 0.001$) and FAI ($P = 0.04$) and lower values of ISI 0 and 120 min ($P = 0.03$) (Table 1). Women with NAFLD had also higher levels of insulin at 120 min, which did not reach statistical significance ($P = 0.05$). Women with NAFLD compared to women without NAFLD were more commonly diagnosed with PCOS (43.7% vs 23.1%, $P = 0.04$), metabolic syndrome (30.2% vs 5.3%, $P = 0.003$) and abnormal lipid profile (81.1% vs 51.3%, $P = 0.002$). All women with abnormal glucose metabolism had NAFLD ($P = 0.01$) (Table 2).

Women with abnormal LFTs had higher serum levels of fasting glucose ($P < 0.01$) and glucose at 120 min ($P = 0.02$) than women with normal LFTs. They had also higher values of HOMA-IR ($P = 0.02$) and lower values of

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Table 1 Clinical, biochemical, and hormonal characteristics of women with and without NAFLD. Results are expressed as mean $\pm$ s.d.

Variable	HS (n=71)	Non-HS (n=39)	P value
Age (years)	32.9 $\pm$ 7.3	34.7 $\pm$ 8.1	NS
Weight (kg)	94.7 $\pm$ 16.1	80.5 $\pm$ 12.0	<0.001
BMI (kg/m ²)	36.0 $\pm$ 6.0	30.5 $\pm$ 4.5	<0.001
Waist (cm)	100.4 $\pm$ 13.7	86.9 $\pm$ 9.1	<0.001
Waist-to-hip ratio	0.84 $\pm$ 0.08	0.79 $\pm$ 0.06	0.001
Fasting glucose (mmol/l)	4.6 $\pm$ 0.8	4.6 $\pm$ 0.5	NS
Glucose 120' (mmol/l)	6.4 $\pm$ 2.0	5.6 $\pm$ 1.4	NS
Fasting insulin (pmol/l)	114.2 $\pm$ 72.5	65.1 $\pm$ 22.6	<0.001
Insulin 120 min (pmol/l)	624.5 $\pm$ 493.2	361.4 $\pm$ 194.5	0.05
HOMA-IR	3.3 $\pm$ 2.1	1.81 $\pm$ 0.67	<0.001
ISI (0 and 120 min)	79.4 $\pm$ 31.6	91.4 $\pm$ 24.4	0.03
Uric acid (μ mol/l)	284.7 $\pm$ 65.8	248.7 $\pm$ 54.4	0.04
Cholesterol (mmol/l)	4.9 $\pm$ 0.9	4.8 $\pm$ 0.9	NS
HDL (mmol/l)	1.2 $\pm$ 0.3	1.3 $\pm$ 0.3	NS
LDL (mmol/l)	3.1 $\pm$ 0.8	3.1 $\pm$ 0.9	NS
Triglycerides (μ mol/l)	1.4 $\pm$ 0.7	1.0 $\pm$ 0.4	<0.01
AST (U/l)	20.2 $\pm$ 12.1	16.0 $\pm$ 3.6	NS
ALT (U/l)	25.1 $\pm$ 17.1	16.0 $\pm$ 7.4	<0.001
ALP (U/l)	77.3 $\pm$ 41.0	62.3 $\pm$ 25.3	0.01
γ GT (U/l)	22.1 $\pm$ 15.9	13.6 $\pm$ 5.7	<0.001
Testosterone (nmol/l)	1.5 $\pm$ 0.7	1.4 $\pm$ 0.8	NS
FAI	6.8 $\pm$ 5.5	4.5 $\pm$ 3.9	0.04
SHBG (nmol/l)	30.7 $\pm$ 17.4	39.3 $\pm$ 14.3	0.01
DHEA-S (μ mol/l)	5.9 $\pm$ 2.7	7.4 $\pm$ 3.5	NS
FSH (mIU/ml)	6.6 $\pm$ 2.1	7.0 $\pm$ 2.2	NS
LH (mIU/ml)	4.6 $\pm$ 2.7	5.1 $\pm$ 1.8	NS
E ₂ (pmol/l)	177.9 $\pm$ 78.8	131.7 $\pm$ 65.5	0.04

P value <0.05 is considered statistically significant for unpaired t-test or Mann-Whitney U test. E₂, estradiol; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; FAI, free androgen index; HOMA-IR, homeostasis model assessment of insulin resistance; ISI, insulin sensitivity index; SHBG, sex hormone-binding globulin; γ GT: gamma-glutamyltranspeptidase.

ISI 0 and 120 min ($P=0.01$). Women with abnormal LFTs had also higher levels of fasting insulin, which did not reach statistical significance ($P=0.05$). All other clinical and laboratory parameters were similar between the two groups, except AST, ALT, and γ GT levels, which differed by definition between groups.

Subgroup analysis: Women with NAFLD and PCOS compared to women with NAFLD without PCOS

Women with NAFLD and PCOS were younger than women with NAFLD without PCOS ($P=0.001$) and had higher serum testosterone ($P<0.001$) and DHEA-S levels ($P=0.04$), higher FAI ($P<0.001$), and HOMA-IR values ($P=0.04$) and lower SHBG levels ($P=0.002$) (Table 3). All other clinical and laboratory parameters were similar between the two subgroups. HS was characterized as mild in the majority of both subgroups (19/31, 61.2% vs 29/40, 72.5% respectively), and there was no difference in the frequency of moderate (6/31, 19.3% vs 7/40, 17.5% respectively) and severe HS (6/31, 19.3% vs 4/40, 10% respectively) between subgroups.

Logistic regression analysis

Univariate regression analysis showed that increased BMI, waist circumference and WHR, higher HOMA-IR and log FAI values, higher triglyceride levels, lower SHBG levels, lower log ISI 0 and 120 min values and PCOS diagnosis

Table 2 Percentages of abnormal LFTs, metabolic abnormalities, and PCOS in women with and without NAFLD.

Variable	HS (n=71)	Non-HS (n=39)	P value
HDL <1.3 mmol/l	69.1%	34.0%	0.001
LDL >3.9 mmol/l	16.2%	13.2%	NS
Total cholesterol >5.2 mmol/l	31.9%	20.5%	NS
Triglycerides >1.7 mmol/l	25.0%	7.9%	0.03
Metabolic syndrome	30.2%	5.1%	0.002
Abnormal glucose metabolism	16.9%	0%	<0.01
Abnormal LFTs	22.0%	2.5%	0.01
PCOS	43.7%	22.5%	0.03

P value <0.05 is considered statistically significant for χ^2 test or Fisher's exact test, as appropriate. LFTs, liver function tests.

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Table 3 Clinical, biochemical, and hormonal characteristics of NAFLD women with and without PCOS. Results are expressed as mean $\pm$ s.d.

Variable	HS, PCOS (n=31)	HS, non- PCOS (n=40)	P value
Age (years)	29.6 $\pm$ 6.4	35.5 $\pm$ 6.9	0.001
Weight (kg)	97.4 $\pm$ 18.2	92.5 $\pm$ 14.1	NS
BMI (kg/m ²)	37.0 $\pm$ 6.7	35.3 $\pm$ 5.4	NS
Waist (cm)	101.9 $\pm$ 14.6	99.3 $\pm$ 13.2	NS
Waist-to-hip ratio	0.8 $\pm$ 0.1	0.8 $\pm$ 0.1	NS
Fasting glucose (mmol/l)	4.7 $\pm$ 0.9	4.6 $\pm$ 0.6	NS
Glucose 120 min (mmol/l)	6.8 $\pm$ 2.3	6.0 $\pm$ 1.5	NS
Fasting insulin (pmol/l)	136.5 $\pm$ 93.7	95.4 $\pm$ 40.9	NS
Insulin 120' (pmol/l)	747.2 $\pm$ 547.1	520.7 $\pm$ 425.9	NS
HOMA-IR	4.0 $\pm$ 2.6	2.7 $\pm$ 1.4	0.04
ISI (0 and 120 min)	74.8 $\pm$ 37.1	83.3 $\pm$ 26.1	NS
Uric acid (μ mol/l)	299.3 $\pm$ 65.0	273.6 $\pm$ 65.5	NS
Cholesterol (mmol/l)	4.9 $\pm$ 1.0	4.9 $\pm$ 0.9	NS
HDL (mmol/l)	1.2 $\pm$ 0.3	1.3 $\pm$ 0.3	NS
LDL (mmol/l)	3.2 $\pm$ 0.8	3.1 $\pm$ 0.8	NS
Triglycerides (μ mol/l)	1.6 $\pm$ 0.9	1.2 $\pm$ 0.6	NS
AST (U/l)	19.3 $\pm$ 9.9	21.0 $\pm$ 13.8	NS
ALT (U/l)	26.8 $\pm$ 18.5	23.6 $\pm$ 15.9	NS
ALP (U/l)	72.3 $\pm$ 32.0	81.5 $\pm$ 47.3	NS
γ GT (U/l)	23.4 $\pm$ 14.0	20.9 $\pm$ 15.6	NS
Testosterone (nmol/l)	2.0 $\pm$ 0.7	1.1 $\pm$ 0.5	<0.001
FAI	10.1 $\pm$ 6.1	3.8 $\pm$ 2.5	<0.001
SHBG (nmol/l)	24.6 $\pm$ 12.0	36.3 $\pm$ 19.8	0.002
DHEA-S (μ mol/l)	6.7 $\pm$ 2.8	5.2 $\pm$ 2.6	0.04
FSH (mIU/ml)	6.3 $\pm$ 1.8	7.0 $\pm$ 2.4	NS
LH (mIU/ml)	5.2 $\pm$ 3.0	4.0 $\pm$ 2.2	NS
E ₂ (pmol/l)	168.4 $\pm$ 50.8	188.2 $\pm$ 101.3	NS

P value <0.05 is considered statistically significant for unpaired *t*-test or Mann–Whitney *U* test. E₂, estradiol; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; FAI, free androgen index; HOMA-IR, homeostasis model assessment of insulin resistance; ISI, insulin sensitivity index; SHBG, sex hormone-binding globulin; γ GT, gamma-glutamyltranspeptidase.

were associated with the presence of NAFLD (Table 4). Multivariate regression analysis showed that higher HOMA-IR values (OR 2.2, 95% CI: 1.1–4.4, *P*=0.02) and triglyceride levels (OR 1.01, 95% CI: 1.00–1.02, *P*=0.04) independently predicted NAFLD, after adjusting for age, BMI and WHR (Table 5).

Discussion

In the present cross-sectional prospective study, 110 apparently healthy, overweight and obese premenopausal women seeking medical advice for weight loss were evaluated for the presence of PCOS with the Rotterdam criteria and of NAFLD by ultrasonography. Women with known dyslipidemia and type 2 DM were excluded from the study because both entities are established risk factors for NAFLD, for a better assessment of the influence of

PCOS in the development of NAFLD. PCOS was diagnosed with the Rotterdam criteria in 36.4% of participating women, an increased prevalence compared to the reported overall, ~18% in premenopausal women using the same diagnostic criteria (14). This finding of increased prevalence of PCOS in overweight and obese premenopausal women is in agreement with a previous study that showed a 28.3% prevalence of PCOS with the NIH criteria in this subgroup of premenopausal women (33) compared to the reported overall 6–8% in premenopausal women with the same diagnostic criteria (14).

NAFLD by ultrasound examination was diagnosed in 64.5% of participating women in accordance with the relevant literature reporting its presence in 57.5–74% of obese individuals (1). There was no difference of age between participating women with and without NAFLD, at variance with a study reporting that age is a risk factor for NAFLD in premenopausal women (11). This discrepancy may be explained by ethnic and body weight differences of study participants, because in that study they were all Japanese and representing the whole spectrum of body weight, whereas in our study, they were all Caucasian and either overweight or obese. In our cohort, women with NAFLD were more obese and had more pronounced abdominal adiposity, were more insulin resistant and had an abnormal lipid profile compared to women without NAFLD in agreement with existing data (1, 4, 11, 12, 13). Moreover, participating women with NAFLD had decreased SHBG levels and increased FAI values compared to women without NAFLD in agreement with our previous work (20) and another study (34).

Table 4 Univariate logistic regression results for NAFLD as a dependent variable (*n*=110).

Independent variable	OR	95% CI	P value
Age	0.97	0.90–1.02	0.24
BMI	1.22	1.1–1.34	<0.001
Waist	1.11	1.06–1.16	<0.001
HOMA-IR	2.8	1.6–4.9	0.001
Log ISI (0 and 120 min)	0.02	0.001–0.680	0.03
HDL	0.3	0.09–1.2	0.08
Triglycerides	3.6	1.5–8.9	0.005
FAI	1.12	0.99–1.27	0.083
Log FAI	4.9	1.013–24.1	0.048
SHBG	0.97	0.94–0.999	0.04
PCOS	2.58	1.071–6.23	0.03

Significant *P* values <0.05 are in bold. FAI, free androgen index; HOMA-IR, homeostasis model assessment of insulin resistance; ISI, insulin sensitivity index; SHBG, sex hormone-binding globulin.

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Table 5 Multivariate logistic regression results for NAFLD as a dependent variable ($n = 110$).

Independent variable	OR	95% CI	P value
HOMA-IR	2.2	1.1–4.4	0.02
Log ISI (0 and 120 min)	1.2	0.003–432.0	0.8
Triglycerides	2.80	1.04–7.50	0.04
Log FAI	1.03	0.89–1.18	0.7
SHBG	0.99	0.96–1.03	0.8
PCOS	1.8	0.5–6.5	0.4

Adjusted for age, BMI, and WHR. Significant P values <0.05 are in bold. FAI, free androgen index; HOMA-IR, homeostasis model assessment of insulin resistance; ISI, insulin sensitivity index; SHBG, sex hormone-binding globulin.

Interestingly, the association of decreased SHBG levels and increased FAI values with NAFLD has been also reported in postmenopausal women with biopsy proven NAFLD (35).

In our study an increased prevalence of PCOS was detected in participating premenopausal women with NAFLD compared to women without NAFLD (43.7% vs 23.1%), in accordance with one small prospective study (24) that has addressed this issue. In that study ten out of 14 (71%) overweight and obese premenopausal women with NAFLD, recruited from a liver clinic and diagnosed by liver ultrasound and elevated ALT levels, proven with liver biopsy in seven out of 14, were diagnosed with PCOS according to the Rotterdam criteria (24). The marked differences in percentages of prevalence between the two studies should be attributed to differences in sample size, study design and diagnostic criteria for NAFLD (24). These data suggest that overweight and obese NAFLD premenopausal women are more likely to have PCOS. Therefore, obtaining a history for the detection of menstrual disorders and fertility issues together with checking for clinical signs of hyperandrogenism are recommended in routine practice for physicians involved in the management of these women, followed by evaluation for PCOS when needed.

In our cohort, women with NAFLD and PCOS were more insulin resistant, assessed by HOMA-IR, than women with NAFLD without PCOS, despite being similarly obese. Given the crucial role of IR in the pathogenesis of NAFLD (1, 5), this finding supports the hypothesis that overweight and obese PCOS women are more susceptible than non-PCOS, apparently healthy, overweight and obese premenopausal women for the development of NAFLD. In addition, women with NAFLD and PCOS had higher levels of androgens, lower levels of SHBG and increased

bioavailable androgens, assessed by FAI, than women with NAFLD without PCOS. Moreover, PCOS women with hyperandrogenism presented an increased frequency of NAFLD, compared to PCOS women without hyperandrogenism. These findings are in agreement with our previous work (20) and other studies (21, 22), supporting the hypothesis that androgen excess, which is the main feature of PCOS and is interrelated to IR (15), is a contributing factor to the development of NAFLD in PCOS. If this is the case, it could be assumed that androgen excess is another parameter rendering PCOS women more susceptible for NAFLD. Overweight and obese women with HS and PCOS were younger than women with HS without PCOS. A plausible explanation for this finding is that PCOS women are insulin resistant and hyperandrogenic since puberty, a setting that probably favors an earlier development of NAFLD, in concert with obesity.

In this study data analysis showed an association of PCOS with NAFLD; however, this association was no longer significant after adjustment for age, BMI, and WHR. Among associated factors, only IR assessed by HOMA-IR and increased triglyceride levels were independent predictors of NAFLD in premenopausal women. Thus, although overweight and obese PCOS women are more likely to develop NAFLD than non-PCOS, age- and weight-matched apparently healthy women, PCOS diagnosis is not a major factor leading to the disease.

Our study was conducted in a clinical setting and there are selection bias concerning the prevalence of PCOS in this cohort, because overweight and obese women with hirsutism and/or menstrual disorders are more likely to seek medical care for obesity than asymptomatic overweight and obese women. Thus, results need to be validated in community-based studies that reflect characteristics of the general population. Another limitation of the study is the use of ultrasound examination for the diagnosis of NAFLD, which, despite having an acceptable level of sensitivity for detecting fatty liver (sensitivity 80% in the presence of $>30\%$ fatty infiltration), a short examination time and a low cost thus being used extensively as a screening method, is not the gold-standard diagnostic method, i.e., liver biopsy (36). The strengths of the study are the prospective collection of data, a relatively large number of participants, the exclusion of diseases and medications either affecting insulin sensitivity or favoring NAFLD and the homogenous cohort concerning race and ethnicity.

In conclusion, we report an increased prevalence of PCOS in overweight and obese premenopausal women with NAFLD. Although PCOS is associated with NAFLD,

only HOMA-IR and triglyceride levels predict independently for NAFLD. These findings have implications for PCOS screening in overweight and obese premenopausal women with NAFLD.

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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