

# Non-alcoholic fatty liver disease is associated with insulin resistance and lipid accumulation product in women with polycystic ovary syndrome

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**STUDY QUESTION:** What are the most relevant factors associated with non-alcoholic fatty liver disease (NAFLD) in women with polycystic ovary syndrome (PCOS)?

**SUMMARY ANSWER:** Insulin resistance (IR) and lipid accumulation product (LAP) are independently associated with NAFLD in PCOS.

**WHAT IS KNOWN ALREADY:** Obesity and IR are frequently present in both women with PCOS and subjects having NAFLD. The coexistence of PCOS and NAFLD might synergistically increase the risk for both type 2 diabetes (T2DM) and cardiovascular disease (CVD). LAP, calculated from waist circumference (WC) and triglycerides (TGs) concentrations [(WC-58) × TGs], has been shown to represent an integrated marker of cardiometabolic risk in women with PCOS.

**STUDY DESIGN, SIZE, DURATION:** This cross-sectional study included 600 Caucasian women diagnosed with PCOS by the Rotterdam criteria between May 2008 and May 2013.

**PARTICIPANTS, SETTINGS, METHODS:** The study was done at the university hospitals in Belgrade, Serbia and Thessaloniki, Greece. All subjects underwent anthropometric measurements and analyses of fasting blood glucose, insulin, lipids, total testosterone and SHBG, as well as liver tests (transaminases,  $\gamma$ -glutamyltransaminase, total bilirubin and alkaline phosphatase). Calculations for a NAFLD liver fat score (NAFLD-LFS) (with, accordingly, determination of metabolic syndrome and testing for T2DM) as well as homeostasis model assessment of IR (HOMA-IR), LAP as a marker of visceral adiposity, and free androgen index (FAI) were performed. We evaluated the prevalence of NAFLD and analyzed associations of the above variables with NAFLD.

**MAIN RESULTS AND THE ROLE OF CHANCE:** NAFLD was more prevalent in patients with PCOS than in controls (50.6 versus 34.0%, respectively). Women with PCOS had higher readings for WC, LAP, insulin and HOMA-IR, total cholesterol and TGs than controls ( $P < 0.001$ ). In PCOS women, the NAFLD-LFS significantly ( $P < 0.001$ ) correlated with WC, BMI, glucose, HOMA-IR, TGs, LAP and FAI. In multivariate logistic regression, HOMA-IR and LAP were independently associated with NAFLD ( $P \leq 0.001$ ).

**LIMITATIONS, REASONS FOR CAUTION:** A possible weakness of the study may be the absence of structural confirmation of liver status. However, liver biopsy is invasive, difficult to perform in large populations and carries some risk of complications while magnetic resonance spectroscopy does not provide any information regarding the presence of fibrosis and is not routinely available. Another possible limitation could be the measurement of total testosterone by radioimmunoassay, which can be inaccurate when determining low levels of testosterone. Finally, fewer controls than subjects in the study group could have affected the significance of the results.

**WIDER IMPLICATIONS OF THE FINDINGS:** There is a debate on the most accurate clinical method for diagnosing liver disease as an early predictor of T2DM and CVD in general population and in PCOS women. There current study provided data on this issue from a cohort of Caucasian women with PCOS.

**STUDY FUNDING/COMPETING INTEREST(S):** The study was supported by a research grant by the Serbian Ministry of Science and Education (grant nos 41009 and 175032). All authors have no competing interests.

**Keywords:** non-alcoholic fatty liver disease / insulin resistance / lipid accumulation product / polycystic ovary syndrome / metabolic syndrome

## Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women of reproductive age (Goodarzi et al., 2011). Obesity and insulin resistance (IR) are frequently present in patients with PCOS and appear to play a role in the pathogenesis of the syndrome (Goodarzi et al., 2011). Accordingly, PCOS is associated with a higher risk for type 2 diabetes mellitus (T2DM) (Boudreaux et al., 2006) and cardiovascular diseases (CVDs) (Shaw et al., 2008).

Non-alcoholic fatty liver disease (NAFLD) is characterized by increased accumulation of fat in the liver in the absence of increased alcohol consumption (Chalasanani et al., 2012). It includes isolated hepatic steatosis and non-alcoholic steatohepatitis (NASH), where varying degrees of inflammation and/or fibrosis are also present (Chalasanani et al., 2012). Steatosis and NASH affect ~19–34% and 10% of the general population, respectively (Browning et al., 2004; Williams et al., 2011). The pathogenesis of NAFLD is multifactorial, but both obesity and insulin IR appear to represent pivotal contributing factors (Tziomalos et al., 2012). Partly due to these associations, patients with NAFLD are at higher risk for developing both T2DM and CVD (Fraser et al., 2009; Targher et al., 2007).

Given the shared mechanisms underpinning the development of cardiometabolic complications in both PCOS and patients with NAFLD, several studies have evaluated the prevalence of NAFLD in patients with PCOS (Kauffman et al., 2010; Markou et al., 2010; Vassilatou et al., 2010; Kahal et al., 2014). However, most reports have evaluated small numbers of patients (Vassilatou et al., 2010), have not included a control group (Kauffman et al., 2010) and have assessed only overweight or obese patients (Kahal et al., 2014) or only normal-weight patients (Markou et al., 2010). The clarification of the relationship between PCOS and NAFLD is highly clinically relevant, since both disorders are common and their coexistence might synergistically increase the risk for both T2DM and CVD.

The aim of the present study was to compare the prevalence of NAFLD between patients with PCOS and body mass index (BMI)-matched controls and to identify factors associated with the presence of NAFLD in PCOS.

## Materials and Methods

### Subjects

We analyzed 600 women with PCOS (age:  $25.6 \pm 5.9$  years, BMI:  $30.6 \pm 6.9$  kg/m<sup>2</sup>) and 125 BMI-matched healthy, control women (age:  $31.4 \pm 5.3$  years, BMI:  $29.6 \pm 6.8$  kg/m<sup>2</sup>). The study included all women who were consecutively diagnosed with PCOS between May 2008 and May 2013, at the Outpatient Clinic of the Clinic of Endocrinology, Diabetes and Metabolic Diseases, University of Belgrade, Serbia and at the Gynecological Endocrinology Infirmity of the Second Department of Obstetrics and Gynecology, Aristotle University of Thessaloniki, Greece.

Subjects were recruited from the outpatient endocrine clinics, where they were referred for investigation of oligo- or amenorrhoea, fertility problems, hirsutism or acne. PCOS was defined according to the revised 2003 Rotterdam Consensus conference on diagnostic criteria for PCOS that requires the presence of at least two of the following three criteria: (i) oligomenorrhoea or anovulation; (ii) clinical and/or biochemical signs of hyperandrogenemia and (iii) polycystic ovaries on ultrasound [Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS) 2004]. In all patients, non-classical 21-hydroxylase deficiency, hyperprolactinemia, Cushing's disease, untreated hypothyroidism and androgen secreting tumors were excluded prior to examination. Women of the control group were healthy volunteers without signs of hyperandrogenism (HA) and who had normal ovulating cycles confirmed by blood progesterone during the luteal phase of  $>10$  ng/ml in two consecutive cycles, and normal ultrasound appearance of the ovaries. In both patients and controls, alcohol consumption of  $>20$  g/day and liver disease including viral, autoimmune, genetic and drug-induced were excluded. No patients or controls had received any medications and hormone treatment for at least 3 months before the study.

The study was approved by the Institutional Ethical Committees and written consent was obtained from all subjects.

### Methodology

In all subjects, anthropometric measurements [weight, height and waist circumference (WC)], and systolic and diastolic blood pressure were recorded. WC represented the smallest circumference at the level of the umbilicus. All subjects were investigated in the follicular phase of the menstrual cycle (between Days 3 and 7) after a 12 h overnight fast. In women with PCOS who did not have a spontaneous bleeding episode for  $>90$  days, 100 mg of micronized progesterone (Utrogestan, Faran Laboratories s. a., Athens, Greece) was administered to induce a bleeding episode and serum blood samples were collected afterwards. Samples for hormonal analyzes were frozen on  $-80^{\circ}\text{C}$  until measurement.

Baseline blood samples were drawn in all subjects for determination of fasting glucose (FG) and insulin, total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides (TGs), serum aspartate (AST) and alanine aminotransferases (ALT),  $\gamma$ -glutamyltransaminase (gGT), total bilirubin (TBIL), alkaline phosphatase (ALP), total testosterone and sex-hormone-binding protein (SHBG). Abnormal aminotransferase levels were defined as values exceeding the upper normal level in our hospital's laboratory (AST and ALT  $\geq 40$  and  $41$  U/l, respectively). Levels of AST, ALT, gGT, TBIL and ALP were determined using spectrophotometric methods (Roche Diagnostics GmbH, Mannheim, Germany). TC and TG were determined using standard enzymatic methods (cholesterol oxidase and glycerol-3-phosphate oxidase, respectively; Randox, UK). HDL was determined by the direct method (Randox, UK) while LDL was calculated using the Friedewald formula. Blood glucose was measured by the glucose oxidase method (Randox, UK) and plasma insulin was determined using radioimmunoassay (RIA) [INSULIN (PEG), INEP, Belgrade, Serbia]. Serum total testosterone and SHBG were measured by RIA (Testo-CT2 and SHBG-RIACT, respectively; CIS Bio International, Gifsur-Yvette, France).

The NAFLD liver fat score (NAFLD-LFS) was calculated using the formula  $[-2.89 + 1.18 \times \text{metabolic syndrome (MetS)} (\text{yes} = 1/\text{no} = 0) + 0.45 \times$

T2DM (yes = 2/no = 0) + 0.15 × fasting serum insulin (mU/l) + 0.04 × AST (U/l) − 0.94 × AST/ALT] and a value of  $> -0.640$  was considered diagnostic of NAFLD (Kotronen et al., 2009). According to the NAFLD-LFS formula, MetS was defined according to criteria of the International Diabetes Federation (Alberti et al., 2005) while T2DM was tested according to the World Health Organization criteria (WHO.Consultation, 1999). Free androgen index (FAI) was calculated by the formula [(testosterone × 100)/SHBG] (Mathur et al., 1981). FAI  $> 8$  was considered as diagnostic of hyperandrogenemia. IR was estimated by the homeostasis model assessment of IR (HOMA-IR) method using the formula [HOMA-IR = insulin (mIU/l) × glucose (mmol/l)/22.5] (Matthews et al., 1985). Lipid accumulation product (LAP) was determined using the formula [(WC-58) × TGs] that includes the minimum WC values used to define sex-specific origin points (58 cm for women) in the Third National Health and Nutrition Examination Survey (Kahn, 2005).

## Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences software (SPSS, version 17.0; SPSS, Inc., Chicago, IL, USA). As our women with PCOS were younger than controls, differences between groups were analyzed by univariate analysis of variance with age as covariate (ANCOVA). Accordingly, results are presented as ANCOVA mean [95% confidence interval (CI)]. Associations between different variables were determined by using the Pearson correlation coefficient ( $r$ ), and significant correlations, apart from  $P$ -value, were considered those with  $r$ -value  $> 0.300$ .  $P$ -values  $< 0.05$  were considered as statistically significant.

Binary logistic regression analysis was performed in order to analyze factors associated with NAFLD. Univariate logistic regression analyses were performed for all variables that were significantly associated with NAFLD-LFS (excluding variables that are already included in the NAFLD-LFS formula). All factors significantly associated with NAFLD in univariate logistic regression analyses entered multivariate logistic regression analysis.

In order to identify factors associated with the NAFLD-LFS ( $> -0.640$ ) within our PCOS women, receiver operating characteristic (ROC) curves were generated for each continuous variable that was proved to be significantly associated with NAFLD-LFS in the multivariate logistic regression analysis. The areas under the curves (AUCs) are provided with standard error of mean (SEM), and 95% CIs. The significance of obtained cut-off values associated with NAFLD-LFS  $> -0.640$  was tested by performing both univariate and multivariate binary logistic regression analyses.

## Results

### Clinical and biochemical characteristics of PCOS and controls

Anthropometric and biochemical characteristics of women with PCOS and controls are presented in Table I. PCOS women had significantly higher WC, LAP, NAFLD-LFS, basal insulin and HOMA-IR, TC, LDL, TG, total testosterone and FAI. When adjusted for age differences, women with PCOS had the same prevalence of T2DM and MetS compared with controls (3.3 versus 3.2%,  $P = 0.973$ , and 35.7 versus 29.4%,  $P = 0.210$ , respectively). However, NAFLD assessed by NAFLD-LFS  $> -0.64$  was more prevalent in PCOS (50.6 versus 34.0%, respectively,  $P = 0.002$ ). Further, we divided the PCOS group into four phenotypes according to the Rotterdam criteria: phenotype A [clinical and/or biochemical HA + oligoamenorrhea (OA), + polycystic ovary morphology (PCOM)], phenotype B (HA + OA), phenotype C (HA + PCOM) and phenotype D (OA + PCOM). NAFLD prevalence assessed by NAFLD-LFS  $> -0.64$  in the

phenotypes were the following: 50% for A, 53% for B, 43% for C and 52% for D. There was a significant between-groups, age-adjusted difference ( $P = 0.032$ ) in the prevalence of NAFLD assessed by NAFLD-LFS  $> -0.64$ . *Post hoc* analyses revealed significant differences in NAFLD prevalence between controls and phenotypes A, B and D ( $P = 0.006$ , 0.003 and 0.042, respectively), while there were no differences between controls and phenotype C, as well as between phenotypes.

In PCOS women, there were no significant correlations between AST and other variables, while ALT significantly ( $P < 0.001$ ) correlated with WC, BMI, LAP and HOMA-IR ( $r = 0.340$ , 0.310, 0.368 and 0.300, respectively).

Also, in PCOS women, NAFLD-LFS significantly ( $P < 0.001$ ) correlated with WC and BMI ( $r = 0.585$  and 0.525, respectively), FG, HOMA-IR, TGs and LAP ( $r = 0.340$ , 0.919, 0.413 and 0.592, respectively) as well as with FAI ( $r = 0.314$ ).

### Analyses of factors associated with NAFLD in women with PCOS

Univariate binary logistic regression showed that NAFLD was significantly associated with HOMA-IR, LAP, WC, BMI, TGs, SHBG and FAI. In order to avoid collinearity effects, and after extensive collinearity diagnostics, we omitted WC, BMI, TGs and SHBG from the multivariate logistic regression analysis. Accordingly, only LAP, HOMA-IR and FAI entered the multivariate logistic regression analysis, and in the final model HOMA-IR and LAP remained significantly associated with the presence of NAFLD (Table II).

By the means of the ROC curve analyses, we evaluated the accuracy of LAP and HOMA-IR for diagnosing NAFLD in PCOS women. The identified cut-off value for LAP was 24.95 and for HOMA-IR the cut-off value was 2.55. Both LAP and HOMA-IR exhibited high diagnostic accuracy [AUC<sub>LAP</sub>: 0.82 (95% CI: 0.78–0.85), sensitivity: 82%, specificity: 61%; AUC<sub>HOMA-IR</sub>: 0.95 (95% CI: 0.95–0.97), sensitivity: 90%, specificity: 81%] (Fig. 1).

Univariate and multivariate binary logistic regression analyses (Table III) showed that both proposed HOMA-IR and LAP cut-off values were significantly associated with NAFLD assessed by NAFLD-LFS  $> -0.640$  in women with PCOS.

## Discussion

We showed that NAFLD was more prevalent in women with PCOS than in respective controls (50.6 versus 34.0%, respectively). Our group of women with PCOS had significantly higher WC, LAP, insulin and HOMA-IR, TC and TGs than controls. In PCOS women, NAFLD-LFS significantly correlated with WC, BMI, glucose, HOMA-IR, TGs, LAP and FAI, and by using the multivariate logistic regression, HOMA-IR and LAP were found to be independently associated with NAFLD. All results have been obtained after age-adjustment of analyses, because our control population was older than the study population.

The prevalence of NAFLD in our cohort of PCOS women was higher than in controls, in accordance with the findings of previous reports (Tan et al., 2010; Vassilatou et al., 2010; Lerchbaum et al., 2011; Kahal et al., 2014; Kuliczowska Plaksej et al., 2014). However, some of the latter studies were small (Kahal et al., 2014) or did not adjust for differences in BMI between patients with PCOS and controls (Tan et al., 2010). Moreover, most previous studies diagnosed steatosis using ultrasound

**Table I** Clinical and biochemical characteristics of PCOS women and controls.

	PCOS (n = 600)	Controls (n = 125)	P*
Age (years)	25.6 (25.1–26.1)	31.4 (30.4–32.4)	<0.001
BMI (kg/m <sup>2</sup> )	30.7 (30.1–31.3)	29.4 (28.1–30.7)	0.073
WC (cm)	91.8 (90.6–92.9)	88.7 (85.9–91.4)	<b>0.045</b>
SBP (mmHg)	110.9 (109.8–112.1)	111.9 (109.3–114.5)	0.518
DBP (mmHg)	71.2 (70.2–72.1)	70.0 (67.9–72.2)	0.340
AST (U/l)	19.8 (19.2–20.3)	18.5 (17.2–19.9)	0.108
ALT (U/l)	22.9 (21.8–24.1)	21.5 (18.9–24.1)	0.327
AST/ALT	1.04 (0.99–1.07)	1.00 (0.92–1.09)	0.479
gGT (U/l)	15.9 (14.3–17.6)	14.8 (10.6–18.9)	0.609
ALP (U/l)	73.1 (71.3–74.9)	73.2 (69.1–77.3)	0.962
TBIL (mmol/l)	9.3 (8.8–9.8)	9.6 (8.7–10.4)	0.607
NAFLD-LFS	−0.3 (−0.4 to −0.1)	−0.8 (−1.1 to −0.4)	<b>0.016</b>
FG (mmol/l)	5.4 (5.3–5.4)	5.4 (5.3–5.6)	0.212
Insulin (mU/l)	15.8 (15.0–16.6)	13.2 (11.4–14.9)	<b>0.009</b>
HOMA-IR	3.8 (3.6–4.0)	3.2 (2.8–3.7)	<b>0.028</b>
TC (mmol/l)	4.9 (4.9–5.0)	4.7 (4.5–4.9)	<b>0.012</b>
HDL (mmol/l)	1.3 (1.2–1.3)	1.3 (1.2–1.4)	0.158
LDL (mmol/l)	3.2 (3.1–3.2)	2.9 (2.8–3.1)	<b>0.028</b>
TG (mmol/l)	1.1 (1.1–1.2)	0.9 (0.9–1.1)	<b>0.010</b>
LAP	40.7 (38.2–43.2)	32.0 (26.3–37.7)	<b>0.007</b>
Testosterone (nmol/l)	2.6 (2.5–2.7)	1.4 (1.2–1.6)	<0.001
SHBG (nmol/l)	34.8 (33.2–36.5)	51.3 (47.6–55.1)	<0.001
FAI (%)	10.0 (9.4–10.6)	4.0 (2.6–5.3)	<0.001

BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; AST, aspartate aminotransferase; ALT, alanine aminotransferase; gGT,  $\gamma$ -glutamyltransaminase; ALP, alkaline phosphatase; TBIL, total bilirubin; NAFLD-LFS, non-alcoholic fat liver disease-liver fat score; FG, fasting glucose; HOMA-IR, homeostatic model assessment of insulin resistance; TC, total cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TGs, triglycerides; LAP, lipid accumulation product; SHBG, sex-hormone-binding protein; FAI, free androgen index.

\*P adjusted for age; results are presented as ANCOVA mean (95% CI).

**Table II** Binary logistic regression with NAFLD liver fat score (>0.640) as output variable.

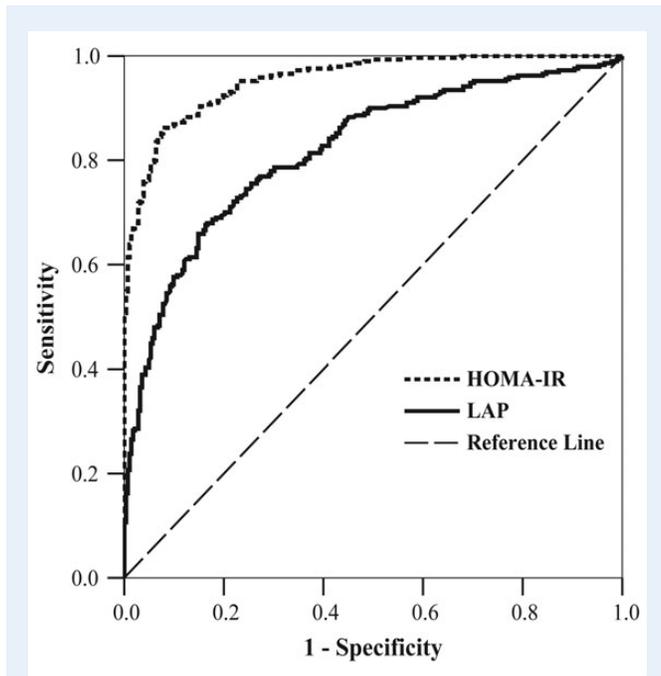
Variables	Univariate		Multivariate	
	OR (95% CI)	P	OR (95% CI)	P
HOMA-IR	9.16 (6.23–13.45)	<0.001	8.56 (5.65–12.97)	<0.001
LAP	1.06 (1.05–1.08)	<0.001	1.04 (1.03–1.06)	<0.001
FAI	1.09 (1.06–1.12)	<0.001		

HOMA-IR, homeostatic model assessment of insulin resistance; LAP, lipid accumulation product; FAI, free androgen index.

(Vassilatou et al., 2010; Kahal et al., 2014; Kuliczowska Plaksej et al., 2014). However, the former method is operator-dependent and has reduced sensitivity in obese patients and in those with mild steatosis (Dasarathy et al., 2009; Papagianni et al., 2015). Therefore, ultrasound might underestimate the prevalence of steatosis (Dasarathy et al., 2009; Papagianni et al., 2015). The larger previous study (n = 750) used the fatty liver index, an algorithm including BMI, WC, TG and

gGT levels, to diagnose steatosis in the PCOS population (Lerchbaum et al., 2011). However, the latter algorithm was derived in a general population-based study that also used ultrasound to detect steatosis (Bedogni et al., 2006) and has not been externally validated in other populations. In contrast, we used the NAFLD-LFS to detect steatosis, an algorithm that has been validated against magnetic resonance spectroscopy, which is considered the gold-standard imaging method for assessing steatosis (Browning et al., 2004; Papagianni et al., 2015), and has been externally validated in other populations (Musso et al., 2010; Wlazlo et al., 2012).

IR, evaluated with the HOMA-IR, was independently associated with NAFLD in our population. IR is associated with impaired suppression of lipolysis in the adipose tissue leading to an increased influx of free fatty acids to the liver and steatosis (Tziomalos et al., 2012). A small study (n = 117) that used ultrasound to detect steatosis also reported an independent relationship between IR and NAFLD in patients with PCOS (Vassilatou et al., 2010). Others showed that IR is more pronounced in patients with PCOS and NAFLD than in patients with PCOS alone, but did not adjust for differences between the two groups in confounding variables, including age, obesity and androgen levels (Kauffman et al., 2010; Gangale et al., 2011; Lerchbaum et al., 2011). Transaminase



**Figure 1** ROC curve: HOMA-IR and lipid accumulation product (LAP) as predictors of the NAFLD liver fat score of > -0.640.

**Table III** Binary logistic regression with NAFLD liver fat score (> -0.640) as output variable.

Variables	Univariate		Multivariate	
	OR (95% CI)	P	OR (95% CI)	P
HOMA-IR >2.55	58.78 (30.57–113.05)	<0.001	51.42 (26.17–101.02)	<0.001
LAP >24.95	7.05 (4.81–10.35)	<0.001	5.63 (3.44–9.22)	<0.001

HOMA-IR, homeostatic model assessment of insulin resistance; LAP, lipid accumulation product.

levels are elevated in only a minority of patients with steatosis (Browning *et al.*, 2004) and this could be an explanation of similar transaminase values in our PCOS women and controls. A correlation between IR and transaminase levels has been reported in patients with PCOS in other studies (Preiss *et al.*, 2008; Economou *et al.*, 2009; Targher *et al.*, 2009; Lerchbaum *et al.*, 2011). Our finding that IR is associated with NAFLD in patients with PCOS might have therapeutic implications. Indeed, administration of metformin to patients with PCOS and NAFLD improves IR and reduces transaminase levels, despite no change in body weight (Gangale *et al.*, 2011; Tan *et al.*, 2015).

We also evaluated the association between LAP and NAFLD in patients with PCOS, revealing an independent relationship. Studies in the general population have also shown that LAP is associated with the presence of a fatty liver (Bedogni *et al.*, 2010) whereas in high cardiovascular risk patients, LAP is independently associated with all-cause mortality (Ioachimescu *et al.*, 2010). Moreover, in patients with PCOS, higher

LAP values indicate the presence of IR, MetS and impaired glucose tolerance (Wiltgen *et al.*, 2009; Wehr *et al.*, 2011; Macut *et al.*, 2015). Therefore, LAP appears to represent an inexpensive, readily available, integrated marker of cardiometabolic risk in patients with PCOS. The potential role of abdominal obesity and elevated triglyceride levels in the pathogenesis of NAFLD in patients with PCOS is also supported by the reduction in hepatic steatosis after both weight loss and TG-lowering treatment with omega-3 fatty acids (Cussons *et al.*, 2009; Gomez-Meade *et al.*, 2013). However, the cross-sectional design of our study does not allow conclusions regarding causality and mechanistic studies are required to elucidate the pathogenesis of NAFLD in patients with PCOS.

In the present study, serum androgen levels were not associated with the presence of NAFLD. This is indirectly confirmed with our result on phenotypes. Namely, there is no difference in NAFLD prevalence between hyperandrogenic, and non-hyperandrogenic or reproductive PCOS phenotypes. NAFLD is considered a hepatic component of MetS with a central pathogenic role of IR that also affects the hypothalamo-pituitary-ovarian axis in subjects with PCOS (Cicero *et al.*, 2012). Taking that into consideration, our results on PCOS phenotypes could shed light on the possible relation of NAFLD and ovarian function mediated by IR. Previous studies that have evaluated the relationship between hyperandrogenemia and NAFLD in patients with PCOS have yielded conflicting results, suggesting either no association (Economou *et al.*, 2009; Kauffman *et al.*, 2010) or an independent correlation (Vassilatou *et al.*, 2010; Jones *et al.*, 2012). In patients with PCOS, IR aggravates hyperandrogenemia by increasing ovarian androgen synthesis and by down-regulating hepatic SHBG production, resulting in increased circulating levels of free androgens (Nestler *et al.*, 1991; Nestler and Jakubowicz, 1997). In turn, hyperandrogenemia is associated with more pronounced IR in patients with PCOS (Goverde *et al.*, 2009; Panidis *et al.*, 2012). Therefore, it is possible that the association between elevated androgen levels and NAFLD is not independent but is mediated by IR.

The strength of our study is reflected in the number of well-defined women with PCOS, and the selected reliable biochemical analyses and indexes to assess the NAFLD. The possible weakness of the study may be an absence of structural confirmation of the liver status. Indeed, liver biopsy is the gold standard for the diagnosis of NAFLD (Chalasan *et al.*, 2012). However, it is difficult to perform liver biopsy in large populations given that the method is invasive and carries a small, but not negligible risk of potentially fatal complications (Cadran *et al.*, 2000; Myers *et al.*, 2008). Furthermore, given that there is no widely accepted treatment for NAFLD except lifestyle changes (Chalasan *et al.*, 2012), many patients are not willing to undergo a liver biopsy. Moreover, liver biopsy in patients with NAFLD is characterized by sampling bias, which might lead to false-negative findings (Ratz *et al.*, 2005). Regarding imaging methods, even though magnetic resonance spectroscopy is considered the gold standard for diagnosing hepatic steatosis, it does not provide any information regarding the presence of fibrosis and is available only in a few selected centers (Papagianni *et al.*, 2015). Another possible limitation of our study could be measurement of total testosterone by RIA and not with the liquid chromatography-tandem mass spectrometry (LC-MS) method. The latter is considered the golden standard while the RIA method can be inaccurate when measuring the low levels of testosterone found in women. However, LC-MS is not widely used in the clinical settings due to high costs of materials, the special skills required and the lack of practicality for large series of assays. Additionally, our

control group had fewer subjects than the study group, which could have affected the significance of the results.

In conclusion, patients with PCOS have a higher prevalence of NAFLD than BMI-matched controls. IR and LAP are independently associated with NAFLD in this population. Given the high prevalence of NAFLD in patients with PCOS and its association with increased risk for T2DM and CVD, screening of women with PCOS for the presence of NAFLD appears reasonable, particularly in patients with abdominal obesity and elevated triglyceride levels. Interventional studies are also needed to evaluate whether targeting IR, obesity and elevated TG levels will also translate into beneficial effects on hepatic steatosis and its cardiometabolic sequelae in patients with PCOS.

## Authors' roles

D.M. and D.P. contributed to the conception and design of the study, to the acquisition, analysis and interpretation of data and to drafting the article. K.T. contributed to the conception of the study, to the analysis and interpretation of data and to drafting the article. I.B.-A. contributed to the acquisition of data, to the analysis and interpretation of data and revised the article critically for important intellectual content. J.B.-M., I.K., E.P. and Z.A. contributed to the acquisition of data and revised the article critically for important intellectual content. All authors gave their final approval of the version to be published.

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## Conflict of interest

None declared.

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