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Polycystic ovary syndrome with hyperandrogenism as a risk factor for non-obese non-alcoholic fatty liver disease

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SUMMARY

Background
Non-alcoholic fatty liver disease (NAFLD) is known to be associated with polycystic ovary syndrome (PCOS). However, most studies investigated the prevalence of NAFLD in obese PCOS patients.

Aim
To compare the prevalence of non-obese NAFLD in women with or without PCOS, and to assess an independent association between PCOS and NAFLD in a non-obese Asian cohort.

Methods
This was a case–control study using a prospective PCOS cohort. After subjects with other potential causes of chronic liver disease were excluded, 275 non-obese women with PCOS and 892 non-obese controls were enrolled. NAFLD was determined by hepatic ultrasonography. Main outcomes were the prevalence of NAFLD on hepatic ultrasonography between non-obese women with or without PCOS, and an independent association between non-obese NAFLD and PCOS.

Results
Non-obese women with PCOS had a significantly higher prevalence of NAFLD than those without PCOS (5.5% vs. 2.8%, $P = 0.027$). PCOS was associated with non-obese NAFLD (odds ratio: 2.62, 95% confidence intervals: 1.25–5.48) after adjustment for age and body mass index (BMI). In women with PCOS, the level of androgenicity represented by free testosterone or free androgen index was associated with NAFLD after adjustment for age, BMI, lipid profile, insulin resistance or glycaemic status.

Conclusions
Non-obese NAFLD is more prevalent in women with PCOS than in those without. In non-obese PCOS patients, hyperandrogenemia may be an independent risk factor for non-obese NAFLD.

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) has been recognised as the most common liver disease in the world.[1, 2] NAFLD is commonly associated with metabolic comorbidities including obesity, type II diabetes, and dyslipidemia.[3] However, there is also clear epidemiologic evidence demonstrating that not all obese subjects will invariably develop NAFLD, and NAFLD can be found in non-obese adults of all ethnicities.[4–6] Therefore, additional risk factors beyond obesity may also play roles in the pathogenesis of NAFLD.

Polycystic ovary syndrome (PCOS) is a common endocrine abnormality in women of reproductive age, and is characterised by chronic anovulation and hyperandrogenism. Insulin resistance (IR) is one of the core pathophysiologies of this syndrome, and although the prevalence varies across populations with different backgrounds, obesity is also prevalent (20–70%) in women with PCOS.[7–9] Thus, IR and obesity may be the link between PCOS and NAFLD. The prevalence of NAFLD has been reported to be 30–73% in women with PCOS, whereas it has been 17–25% in unmatched or age- and body mass index (BMI)- matched controls.[10–17] In addition, previous studies reported a potential independent contribution of hyperandrogenism (HA), which is a unique feature of PCOS, to NAFLD in women with PCOS.[12, 18–20]. In a recent review study, Targher *et al.* reported that the prevalence of NAFLD is noticeably increased in young women with PCOS independent of obesity and other metabolic features. Thus, they emphasised the necessity for awareness of the presence of PCOS among female patients with NAFLD.[21]

Although NAFLD is prevalent in women with PCOS, most of the previous studies were performed in overweight and/or obese patients. PCOS is also prevalent in non-obese women; using the Rotterdam criteria, prevalence of PCOS in non-obese women (BMI < 30 kg/m^2) was 18.8% and that in obese women (BMI ≥ 30 kg/m^2) was 30.3% in a Turkish population.[22] Due to the lack of data, it is difficult to extrapolate whether NAFLD is also prevalent in non-obese PCOS patients. Only one small study reported that 17 young (mean age 25 years old), lean and insulin resistant women with PCOS did not have evidence of NAFLD, suggesting that NAFLD is mainly related to obesity.[18]

We hypothesised that PCOS is an independent risk factor for non-obese NAFLD. The aim of this study was to compare the prevalence of NAFLD in non-obese women with or without PCOS, and to determine the independent association between PCOS and NAFLD in a non-obese population.

MATERIALS AND METHODS

Subjects

This case–control study prospectively enrolled women with or without PCOS. Eligible subjects visited a Healthcare center at Seoul National University Hospital for comprehensive health screening between 2004 and 2014. Some subjects were supported by their employer, and others voluntarily paid for a comprehensive health evaluation. According to our medical checkup programs, subjects underwent bimanual pelvic examination and/or Papanicolaou (Pap) tests and/or pelvic ultrasound examinations. Women with complete data of menstrual history, hirsutism score, and pelvic ultrasound examination were eligible for our PCOS cohort (cases and controls). After we explained our research protocols and obtained written informed consent, we finally enrolled women with or without PCOS for our studies.[23, 24]

Physicians collected medication history about combined oral contraceptives, lipid-lowering agents, insulin sensitiser, anti-androgens such as spironolactone and medications known to produce fatty liver. A total of 344 premenopausal (18–40 years of age) women with PCOS without medication history were prospectively included in the PCOS cohort (Figure 1). We excluded 31 subjects with excessive alcohol consumption ($>$ 20 g/day measured by detailed questionnaire including alcohol amount and frequency),[25] viral hepatitis (hepatitis B or C) or other chronic liver diseases. Of the 313 subjects, 38 patients who had a BMI 25 kg/m^2 and over were classified as obese subjects according to the definition of obesity for Asians.[26] Finally, 275 non-obese patients from the initial cohort were included in the final analysis.

The women without PCOS also underwent a health screening check up with the same protocol. The inclusion criteria for the control group were regular (21–35 day) menstrual cycles and a modified Ferriman and Gallwey (mF-G) score < 6. All women of the control group received a transvaginal or transrectal pelvic ultrasound examination to evaluate their ovarian morphology, and were excluded if polycystic ovary morphology existed. The exclusion criteria were also applied to the controls. Of the 941 controls, 892 subjects who had a BMI less than 25 kg/m^2 were included in the final analysis (Figure 1). The institutional review board approved this project (IRB no.0907-051-287), and written informed consent was obtained from each patient.

Clinical and biochemical measurements

Clinical variables, such as body weight, height, waist circumference and blood pressure, were assessed while the

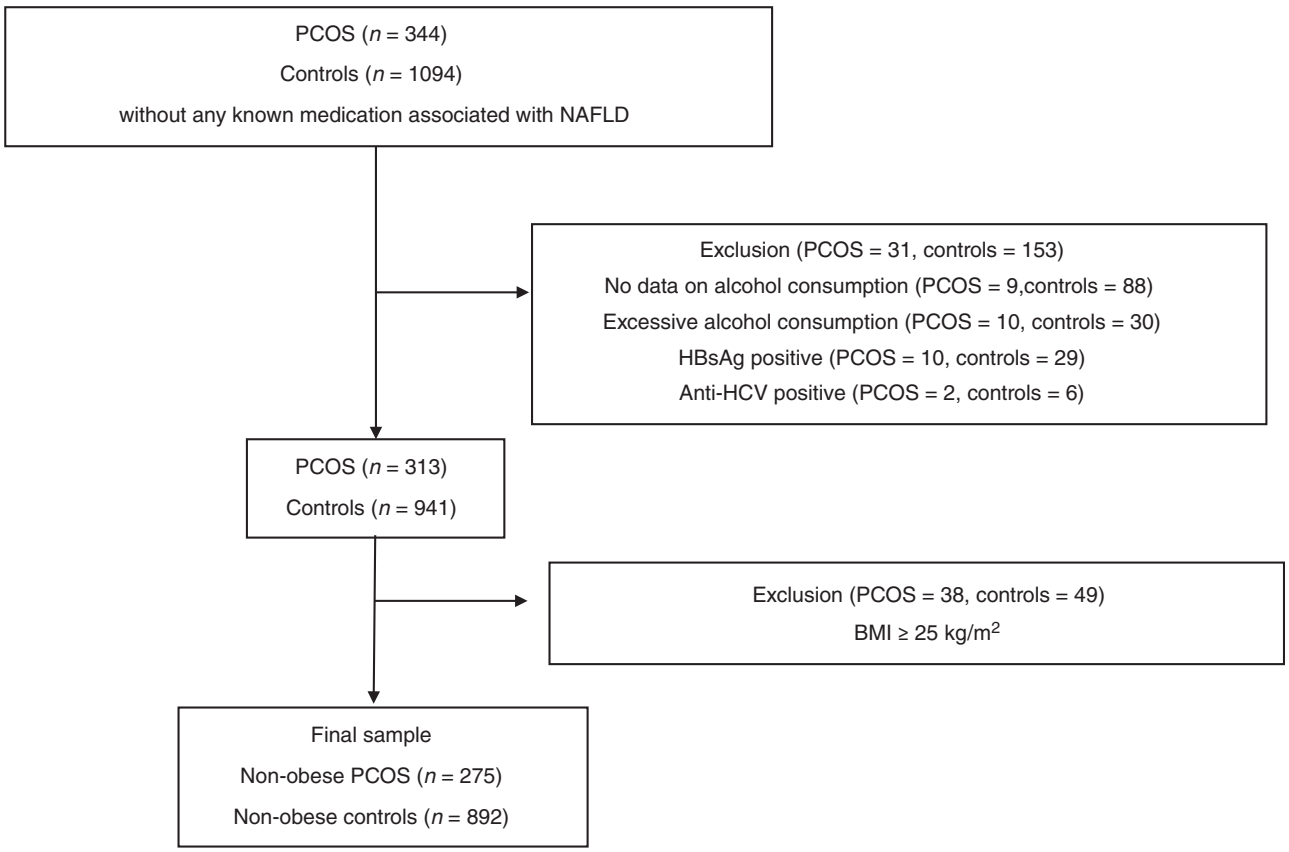

Figure 1 | Flow chart showing details of inclusion and exclusion of the subjects.

subjects wore a light gown without shoes. BMI was calculated as weight (kg) divided by the square of height (m^2). Waist circumference was measured by trained medical personnel with the tape placed horizontally at the smallest diameter between the costal margin and iliac crest while the participant gently exhaled. Systolic and diastolic blood pressures were measured twice, and the average values were used in the analysis.

After a 12-h overnight fast, we measured the fasting plasma glucose, total cholesterol, triglycerides, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, circulating highly sensitive C-reactive protein (hs-CRP) and uric acid in all subjects (Wako Pure Chemical Industries, Ltd. Osaka, Japan). Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyltranspeptidase (GGT), hepatitis B surface antigen and antibody to hepatitis C virus were evaluated in all participants (Abbott Laboratories, Abbott Park, IL, USA). Fasting insulin levels were measured using radioimmunoassay (BioSource Europe S.A., Nivelles, Belgium), and haemoglobin A1c (HbA1c) was measured with a COBAS INTEGRA 400 (Roche Diagnostics GmbH, Mannheim, Germany). Insulin resistance was determined using the homoeostasis model assessment (HOMA)–IR, and calculated according to the following equation: fasting glucose (mg/dL) x fasting insulin (μU/mL)/405. Using radioimmunoassay (Siemens, Los Angeles, CA, USA), serum levels of total testosterone (T), free T and sex hormone-binding globulin (SHBG) were measured. The intra- and inter-assay coefficients of variation were 4.0–11.0% and 5.9–12.0% for total T, respectively, and 4.0–17% and 8.0–18.3% for free T respectively. The free androgen index (FAI) was calculated as total T/SHBG × 100, and the values for T were converted from ng/mL to nmol/L using the following index proposed by the manufacturer: 1 ng/mL = 3.467 nmol/L.

Definition of PCOS

PCOS was diagnosed using the Rotterdam criteria.[27] This proposes a diagnosis of PCOS when a patient meets two of the following three criteria: (i) oligo-and/or anovulation, (ii) hyperandrogenism (clinical and/or biochemical) and (iii) polycystic ovary morphology on

ultrasonography (either 12 or more follicles measuring 2–9 mm in diameter or an increased ovarian volume >10 cm^3). Oligomenorrhea was defined as fewer than eight periods per year or cycles longer than 35 days, and amenorrhoea was defined as the absence of menstruation for more than 3 months without pregnancy. Hirsutism was assessed using the mF-G score system, and clinical HA was defined as a mF-G score of 6 or greater, which was reported in our previous study.[28] Biochemical HA was defined as follows: total T > 0.68 ng/mL, free T > 1.72 pg/mL, and FAI > 5.36.[29] All women with PCOS were screened to exclude hyperprolactinemia and thyroid dysfunction. Serum 17-hydroxyprogesterone (OHP) was also measured, and if the serum 17-OHP level was over 2 ng/mL, then a repeat test was performed in the early morning during the follicular phase. The patients who showed continuous elevation of 17-OHP were excluded from the study group. None of the women with or without PCOS had taken combined oral contraceptives, lipid-lowering agents, insulin sensitiser or anti-androgens, and had a history of diagnosed diabetes.

Definitions of NAFLD

Hepatic ultrasonographic examinations (Acusion, Sequoia 512, Siemens, Mountain View, CA, USA) were carried out by experienced radiologists who were blinded to the subject's clinical status. Fatty liver was diagnosed if the echogenicity of the liver was higher than the echogenicity of the right kidney according to the criteria by Saadeh *et al.* in the absence of the following criteria: a positive serologic marker for the hepatitis B surface antigen or the hepatitis C virus serological marker, excessive alcohol intake > 20 g/d for women, medications known to produce fatty liver disease, and other specific hepatic diseases.[30]

Elevated liver enzyme levels were defined as an ALT level > 19 IU/L or 40 IU/L. The Hepatic Steatosis Index (HSI) was calculated as 8 * (ALT/AST ratio) + BMI (+2 if woman; +2 if diabetes).[31] We used the cut-off for NAFLD as a HSI ≥ 36 without any evidences of chronic liver disease, and used a HSI < 30 to rule out NAFLD.[31]

Metabolic syndrome was diagnosed if ≥ 3 of the following five components were met: (i) waist circumference ≥ 80 cm, (ii) triglyceride ≥ 150 mg/dL, (iii) systolic blood pressure ≥ 130 mmHg and/or diastolic blood pressure ≥ 85 mmHg, (iv) fasting plasma glucose > 100 mg/dL and (v) HDL-cholesterol < 40 mg/dL.[32] The cut-off point for IR was defined as a HOMA-IR of 2.34 from a study of Korean non-diabetic adults.[33]

Statistical analysis

Deviation of the data from a normal distribution was examined through visual inspection of the quantile-normal plots and/or Shapiro–Wilk test of normality. The data are shown as the mean ± standard deviation (s.d.) or the median value with an interquartile range (IQR). If a Gaussian distribution was achieved after a natural logarithmic or square root transformation, then the data are shown as geometric means and 95% confidence intervals (CIs). Continuous parameters were compared using the Student's t or Mann–Whitey *U*-test, and categorical variables were compared using the chi-square test. Tests for the odds ratio (OR) and significant difference among subjects with or without PCOS were performed to determine an association between PCOS and non-obese NAFLD. Logistic regression was used to analyse the adjusted OR and 95% CI for NAFLD while controlling for potential confounders. All data analyses were performed using the SPSS software (version 22.0, IBM SPSS, NY, USA), and statistical significance was set at a two-sided $P < 0.05$.

RESULTS

Clinical and biochemical characteristics of 275 non-obese women with PCOS and 892 women without PCOS are shown in Table 1. Women with PCOS were younger and had a minimally higher mean BMI than the controls. By definition, there were significant differences in the hirsutism score, serum total T, serum free T, and FAI levels between women with PCOS and those without. Diastolic blood pressure, fasting glucose, triglycerides and uric acid levels were significantly higher in women with PCOS than in those without PCOS, but other metabolic parameters and serum hepatic enzyme levels were similar between the two groups. The median HbA1c levels were 5.50% in both groups, but statistically higher in the controls.

Non-obese women with PCOS had a significantly higher prevalence of NAFLD than those without PCOS [5.5% (15/275) vs. 2.8% (25/892), $P = 0.027$] (Figure 2). There were also significant differences in the HSI distributions between patients and controls (Frequencies of HSI > 36, 30–36, < 30 were 2.6%, 30.5% and 66.9% in women with PCOS, and 1.3%, 22.9%, and 75.8% in controls, respectively, $P = 0.011$) (Table 1). However, the prevalence of abnormal ALT did not differ between the two groups.

The clinical and biochemical features of subjects with and without NAFLD are shown in Table 2. As expected, women with non-obese NAFLD had a higher BMI, waist circumference, blood pressure, serum levels of fasting glucose, total cholesterol, triglycerides, LDL-cholesterol, HbA1c, uric acid, ALT and GGT as well as lower levels

Table 1 | Clinical and biochemical features of non-obese women with or without PCOS

	With PCOS (n = 275)	Without PCOS (n = 892)	P value
Anthropometric parameters			
Age (years)*	30.4 ± 5.2	35.1 ± 4.0	<0.001
Body mass index (kg/m^2)*	20.3 ± 2.1	19.9 ± 2.0	0.001
Waist circumference (cm)*	75.0 ± 6.0	74.8 ± 5.7	0.678
Hirsutism score†	5 (1–8)	0 (0–3)	<0.001
Systolic blood pressure (mmHg)*	104.1 ± 10.6	102.7 ± 10.3	0.055
Diastolic blood pressure (mmHg)*	65.8 ± 8.7	64.4 ± 8.9	0.018
Biochemical parameters			
Total testosterone (ng/mL)‡	0.33 (0.24–0.42)§	0.25 (0.20–0.29)¶	<0.001
Free testosterone (pg/mL)‡	0.83 (0.61–1.05)§	0.54 (0.49–0.64)¶	<0.001
Sex hormone-binding globulin (nmol/L)‡	50.0 (45.3–57.7)§	61.1 (59.4–62.8)¶	<0.001
Free androgen index‡	3.7 (2.5–4.9)§	1.4 (1.1–1.7)¶	<0.001
Luteinizing hormone (IU/L)‡	7.6 (6.8–8.4)	Not checked	(–)
Follicle-stimulating hormone (IU/L)‡	4.5 (4.2–4.8)	Not checked	(–)
Estradiol (pg/mL)*	61.1 ± 10.0	Not checked	(–)
Fasting glucose (mg/dL)*	87.6 ± 7.6	85.9 ± 8.0	0.002
Fasting insulin (μU/mL)‡	5.7 (5.1–6.2)**	6.2 (5.9–7.3)	0.053
HOMA-IR‡	1.23 (1.09–1.37)**	1.33 (1.24–1.42)	0.158
Presence of insulin resistance	9.8% (17/173)	9.0% (80/892)	0.720
Total cholesterol (mg/dL)*	179.5 ± 28.7	178.2 ± 29.0	0.535
Triglyceride (mg/dL)†	66 (51–86)	62 (49–81)	0.033
High-density lipoprotein cholesterol (mg/dL)*	63.7 ± 12.4	62.4 ± 12.4	0.135
Low-density lipoprotein cholesterol (mg/dL)*	100.2 ± 25.9	102.1 ± 25.3	0.262
Haemoglobin A1$_c$ (%)‡	5.50 (5.30–5.70)	5.50 (5.40–5.70)	0.020
High sensitive C-reactive protein (mg/dL)†	0.01 (0.01–0.03)	0.02 (0.01–0.05)	0.279
Uric acid (mg/dL)*	4.42 ± 0.82	4.29 ± 0.83	0.021
Frequency of metabolic syndrome	2.9% (8/274)	1.3% (12/892)	0.079
Hepatic parameters			
Aspartate aminotransferase (IU/L)†	17 (15–20)	17 (15–20)	0.721
Alanine aminotransferase (IU/L)†	13 (10–16)	13 (10–17)	0.380
> 19 IU/L	15.1% (41/272)	15.1% (135/892)	0.989
> 40 IU/L	2.2% (6/272)	1.9% (17/892)	0.736
γ-glutamyl transpeptidase (IU/L)†	13 (11–17)	13 (11–16)	0.369
Hepatic steatosis index			
> 36	2.6% (7/272)	1.3% (12/892)	
30–36	30.5% (83/272)	22.9% (204/892)	0.011
< 30	66.9% (182/272)	75.8% (676/892)	

HOMA-IR, homoeostasis model assessment of insulin resistance; PCOS, polycystic ovary syndrome.

*Data are shown as the means ± s.d.

†Median (interquartile range).

‡Geometric mean and 95% confidence intervals.

§n = 166.

¶n = 216.

**n = 173.

P-values are indicated for the differences between groups, as analysed using the Student's t, Mann–Whitney U or chi-square test.

of HDL-cholesterol than those without non-obese NAFLD. Subjects with non-obese NAFLD were more insulin resistant and significantly higher frequencies of IR and metabolic syndrome than those without non-obese NAFLD.

We tried to evaluate independent contributors to NAFLD in non-obese women. Women with PCOS had 2 (95% CI: 1.04–3.85) times higher odds for NAFLD (Table 3). Even after adjustment for age and BMI, the odds that a woman had NAFLD were 2.62 times higher (95% CI: 1.25–5.48) if she had PCOS. In non-obese women with PCOS, free T and FAI levels were significantly associated with the presence of NAFLD in both unadjusted and age- and BMI-adjusted models. In

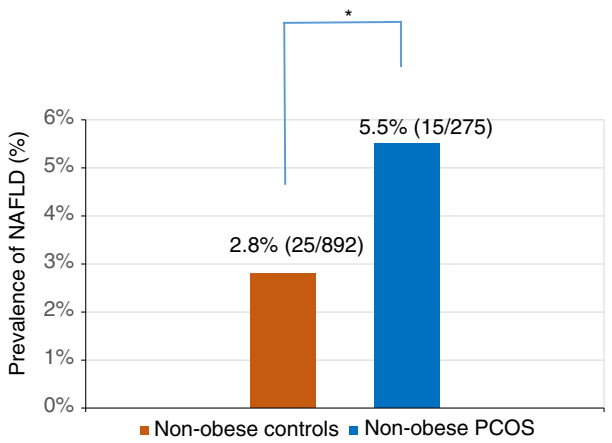

Figure 2 | The prevalence of ultrasonography diagnosed non-alcoholic fatty liver disease (NAFLD) according to the presence of polycystic ovary syndrome (PCOS). * $P = 0.027$ (a) Non-obese subjects were defined as BMI < 25 kg/m^2 according to the definition of obesity for Asians (International Association for the Study of Obesity Task Force, 2000).[26] (b) The prevalence of NAFLD was compared using the chi-square test.

subsequent logistic models, the additional adjustment for the presence of IR, lipid profiles or glycaemic status attenuated the association between NAFLD and PCOS without statistical significance. However, the associations of free T and FAI levels with NAFLD in non-obese PCOS patients remained significant.

We conducted sensitivity analysis including only the lean (BMI < 23 kg/m^2) subjects. Lean women with PCOS had a significantly higher prevalence of NAFLD than those without PCOS (5.0% (12/239) vs. 1.8% (15/822), $P = 0.006$). The association between PCOS and NAFLD was more pronounced in the lean population (OR: 2.84, 95% CI: 1.31–6.16), and this association was significant not only after adjustment for age and BMI (OR: 3.80, 95% CI: 1.60–9.04), but also lipid profile (OR: 3.25, 95% CI: 1.47–7.21) or glycaemic status (OR: 2.44, 95% CI: 1.05–5.71). In the lean women with PCOS, associations of free T and FAI levels with NAFLD remained also significant in both the unadjusted and adjusted models.

DISCUSSION

In our non-obese population, women with PCOS had a higher prevalence of NAFLD than those without. PCOS was significantly associated with non-obese NAFLD independent of age and generalised obesity, but this association became attenuated and remained insignificant after further consideration of IR, lipid profile or glycaemic status. However, in women with PCOS, the level of androgenicity represented by free T and FAI was a risk factor for NAFLD irrespective of age, obesity, lipid profile, IR or glycaemic status, suggesting an independent contribution of HA to NAFLD in non-obese women with PCOS. The majority of other studies reported an increased prevalence of NAFLD in obese PCOS patients,[10–17] and the reasons were suggested to be related to obesity, dyslipidemia, and IR in these obese patients.[34] Our current study suggests that NAFLD is not a feature confined to obese women with PCOS, and that PCOS patients with hyperandrogenemia may be more prone to developing NAFLD even though they are not obese.

One of the issues that needs to be addressed is the unique features of PCOS, such as HA, may attribute to metabolic risk; our previous studies presented a significantly increased risk of metabolic profiles in hyperandrogenic patients.[9, 29] Chen *et al.* reported that women with PCOS had a higher prevalence of elevated ALT levels, which was correlated with androgen levels independent of age, IR, obesity and dyslipidemia.[19] Therefore, the authors stated that not only metabolic variables but also HA may be considered to be possible risk factors for NAFLD in women with PCOS. Jones *et al.* compared the prevalence of NAFLD in 19 HA PCOS patients, 10 PCOS patients with normal androgens and 22 controls of similar age and BMI.[35] After adjustment for BMI or HOMA-IR, HA PCOS patients had significantly higher liver fat than non-HA PCOS patients and controls, and they concluded that HA PCOS is associated with a fatty liver. In our current study, an increased free androgen profile was associated with non-obese NAFLD even after adjustment for age, BMI or metabolic status, suggesting an independent contribution of HA to NAFLD in non-obese women with PCOS. In experimental studies, androgen levels and its receptors were shown to be critical in maintaining lipid metabolism in the liver.[36–38] McInnes *et al.* reported that dihydrotestosterone (DHT) treated ovariectomised mice exhibited increased visceral fat mass associated with triglycerides accumulation.[36] The authors stated that excess androgens can inhibit AMPK (adenosine monophosphate-activated protein kinase) activity, which is known to inhibit lipogenesis in adipose tissue. Kanaya *et al.* also reported that ovariectomised female mice treated with DHT had a significantly greater liver fat accumulation, suggesting that HA in females results in the NAFLD phenotype.[37] Considering these findings, the significance of elevated androgen in women in the pathogenesis of NAFLD needs more attention.

Table 2 | Clinical and biochemical features of non-obese women with and without NAFLD

	With NAFLD (n = 40)	Without NAFLD (n = 1127)	P value
Anthropometric parameters			
Age (years)*	36.3 ± 4.9	33.9 ± 4.7	0.002
Body mass index (kg/m^2)*	22.2 ± 1.6	19.9 ± 2.0	<0.001
Waist circumference (cm)*	79.9 ± 5.5	74.7 ± 5.7	0.002
Systolic blood pressure (mmHg)*	108.1 ± 9.7	102.9 ± 10.4	0.002
Diastolic blood pressure (mmHg)*	68.9 ± 9.5	64.5 ± 8.8	0.003
Biochemical parameters			
Fasting glucose (mg/dL)*	92.3 ± 9.7	86.1 ± 7.8	<0.001
Fasting insulin (μU/mL)†	8.1 (7.5–8.7)	6.1 (5.7–6.5)	0.013
HOMA-IR†	1.80 (1.65–1.95)	1.30 (1.21–1.39)	0.009
Frequency of insulin resistance	36.7% (11/30)	8.3% (86/1035)	<0.001
Total cholesterol (mg/dL)*	190.8 ± 33.2	178.1 ± 28.6	0.006
Triglyceride (mg/dL)‡	88 (50–135)	63 (50–81)	0.010
High-density lipoprotein cholesterol (mg/dL)*	54.1 ± 12.4	63.0 ± 12.3	<0.001
Low-density lipoprotein cholesterol (mg/dL)*	115.8 ± 31.0	101.2 ± 25.1	0.005
Haemoglobin A1$_c$ (%)†	5.70 (5.40–5.88)	5.50 (5.40–5.70)	0.003
hs-CRP (mg/dL)‡	0.07 (0.02–0.26)	0.02 (0.01–0.04)	0.272
Uric acid (mg/dL)*	4.63 ± 0.93	4.31 ± 0.82	0.018
Frequency of metabolic syndrome	17.5% (7/40)	1.2% (13/1126)	<0.001
Hepatic parameters			
Aspartate aminotransferase (IU/L)‡	18 (15–22)	17 (15–20)	0.172
Alanine aminotransferase (IU/L)‡	17 (14–26)	13 (10–16)	0.001
> 19 IU/L	42.5% (17/40)	14.1% (159/1124)	<0.001
> 40 IU/L	15.0% (6/40)	1.5% (17/1124)	<0.001
γ-glutamyl transpeptidase (IU/L)‡	19 (13–25)	13 (11–16)	0.013
Presence of PCOS	37.5% (15/40)	23.1% (260/1127)	0.035

HOMA-IR, homoeostasis model assessment of insulin resistance; hs-CRP, highly sensitive C-reactive protein; NAFLD, non-alcoholic fatty liver disease; PCOS, polycystic ovary syndrome.

P-values are indicated for the differences between groups, as analysed using the Student's t, Mann–Whitney U or chi-square test.

*Data are shown as the means ± s.d.

†Geometric mean and 95% confidence intervals.

‡Median (interquartile range).

ALT levels have been widely used as a biochemical marker of NAFLD. The prevalence of abnormal ALT levels also has been reported to be increased in women with PCOS ranging from 15% to 30% depending on cut-off levels.[39, 40] In our current study, the frequencies of elevated ALT levels were similar between women with PCOS and without PCOS. As relatively young and non-obese women are less likely to have non-alcoholic steatohepatitis, which is the severe form of NAFLD, non-obese women with PCOS might not have an increased frequency of elevated ALT levels and advanced fibrosis by non-invasive fibrosis marker (data not shown). Meanwhile, prevalence of suspected NAFLD using HSI differed significantly between non-obese women with and without PCOS. HSI is a validated screening index for NAFLD, and uses ALT, AST, BMI, sex and the presence of diabetes as variables.[41, 42]

Strength of this study is that the sample size of the non-obese population is sufficiently large. We believe that the large number of apparently healthy control subjects is representative of the general population. In addition, our analyses on the non-obese population may shed a light on the impact of PCOS or HA on non-obese NAFLD beyond obesity or IR. However, there are also some limitations in the present study. First, although our sample size was large, our outcome variable was relatively small as expected. Therefore, we had to analyse multivariable analyses with limited but important confounders. Second, our findings were independent of factors that affect NAFLD status, including age, BMI and IR, but we cannot exclude the possibility of other potential confounding factors, such as family history, differences in diet and/or physical activity patterns. Third, women with PCOS had significantly higher fasting glucose levels than those

Table 3 | Univariate and multivariate binary analysis of the risk for non-obese NAFLD

	Odds ratio	95% confidence intervals	*P* value
Among total non-obese subjects (n = 1167)			
Presence of PCOS			
Unadjusted	2.00	1.04–3.85	0.035
Age and BMI adjusted	2.62	1.25–5.48	0.011
Multivariate model 1	1.77	0.85–3.68	0.126
Multivariate model 2	1.00	0.37–2.69	0.996
Multivariate model 3	1.92	0.96–3.83	0.065
Among PCOS (n = 275)			
Free androgen index			
Unadjusted	1.84	1.12–3.09	0.019
Age and BMI adjusted	1.11	1.01–1.22	0.029
Multivariate model 1	1.12	0.99–1.25	0.062
Multivariate model 2	1.19	1.04–1.36	0.010
Multivariate model 3	1.15	1.04–1.26	0.005
Free testosterone			
Unadjusted	1.13	1.03–1.24	0.010
Age and BMI adjusted	1.91	1.10–3.31	0.022
Multivariate model 1	2.47	1.08–5.62	0.032
Multivariate model 2	1.89	1.03–3.46	0.039
Multivariate model 3	2.02	1.15–3.53	0.014

BMI, body mass index; NAFLD, non-alcoholic fatty liver disease; PCOS, polycystic ovary syndrome.

Multivariable model 1 is adjusted for haemoglobin A1c. Multivariable model 2 is adjusted for presence of insulin resistance. Multivariable model 3 is adjusted for triglyceride and high-density lipoprotein cholesterol.

without, but lower HbA1c levels. Unfortunately, we did not have a definite explanation for higher HbA1c levels among women without PCOS, while HOMA index was not significantly different between the two groups. Effects of medication may be possible explanation, but none of the women with or without PCOS had taken any kind of insulin sensitiser or anti-diabetic medication and had diagnosed diabetes. Difference in age may be a possible explanation for this phenomenon (Mean age 35.1 ± 4.0 years in controls and 30.4 ± 5.2 years in PCOS, respectively, $P < 0.001$). Prospective study will be needed for clarification. Finally, the method that we used to assess NAFLD in this study is not ideal for individual patients. However, the advantages of ultrasonography include its non-invasiveness, repeatability, low cost, and satisfactory sensitivity and specificity for an epidemiologic study such as this one.

In summary, the prevalence of NAFLD in non-obese women with PCOS is higher than that in women without PCOS, and PCOS with hyperandrogenemia may be an emerging risk factor for non-obese NAFLD. Our results provide possible evidence about characteristics that can help identify women who are at increased risk for non-obese NAFLD. Clinician may not only focus on PCOS but also recognise the increased risk of NAFLD in non-obese women and recommend risk factor modification.

AUTHORSHIP

Guarantor of the article: Donghee Kim is a guarantor of article, developed the study concept and design, performed the statistical analysis, and critically revised the manuscript for important intellectual content.

Author contribution: Jeongyoon Yim analysed and interpreted the data. Jinhwa Kang acquired the data. Kyunghee Han acquired the data. Sunmie Kim acquired the data. Kyuri Hwang offered material support. Seungyup Ku offered material support. Changsuk Suh offered administrative support. Seokhyun Kim offered administrative support. Youngmin Choi supervised the study. Jinju Kim drafted the manuscript, obtained funding and performed the statistical analysis.

All authors approved the final version of the article, including the authorship list.

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