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TOPIC HIGHLIGHT

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Nonalcoholic fatty liver disease and polycystic ovary syndrome

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Abstract

Nonalcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease in the Western world comprising a spectrum of liver damage from fatty liver infiltration to end-stage liver disease, in patients without significant alcohol consumption. Increased prevalence of NAFLD has been reported in patients with polycystic ovary syndrome (PCOS), one of the most common endocrinopathies in premenopausal women, which has been redefined as a reproductive and metabolic disorder after the recognition of the important role of insulin resistance in the pathophysiology of the syndrome. Obesity, in particular central adiposity and insulin resistance are considered as the main factors related to NAFLD in PCOS. Moreover, existing data support that androgen excess, which is the main feature of PCOS and is interrelated to insulin resistance, may be an additional contributing factor to the development of NAFLD. Although the natural history of NAFLD remains unclear and hepatic steatosis seems to be a relatively benign condition in most patients, limited data imply that advanced stage of liver disease is possibly more frequent in obese PCOS patients with NAFLD. PCOS patients, particularly obese patients with features of the metabolic syndrome, should be submitted to screening for NAFLD comprising assessment of serum aminotransferase levels and of hepatic steatosis by abdominal ultrasound. Lifestyle modifications including diet, weight loss and exercise are the most appropriate initial therapeutic interventions for PCOS patients with NAFLD. When pharmacologic therapy is considered, metformin may be used, although currently there is no medical therapy of proven benefit for NAFLD. Long-term follow up studies are needed to clarify clinical implications and guide appropriate diagnostic evaluation, follow-up protocol and optimal treatment for PCOS patients with NAFLD.

Key words: Nonalcoholic fatty liver disease; Polycystic ovary syndrome; Insulin resistance; Obesity; Hyperandrogenism; Premenopausal women

Core tip: Nonalcoholic fatty liver disease (NAFLD) is frequent in patients with polycystic ovary syndrome (PCOS). Obesity and insulin resistance are considered as the main factors related to NAFLD in PCOS. Androgen excess may be an additional contributing factor to the development of NAFLD. Limited data imply that advanced stage of liver disease is possibly more frequent in obese PCOS patients with NAFLD. PCOS patients, particularly obese patients with the metabolic syndrome, should be screened for NAFLD. Long-term follow up studies are needed to clarify clinical implications, appropriate diagnostic evaluation and optimal treatment for PCOS patients with NAFLD.

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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. For the diagnosis, exclusion of nutritional disorders, drugs and diseases known to cause secondary fatty liver disease is a prerequisite[1]. There is a wide spectrum of liver damage ranging from simple steatosis to cirrhosis[2]. The clinical relevance of NAFLD is related to its high prevalence (20%-30%)[1,3] in the general population and its possible evolution to end-stage liver disease and rarely to hepatocellular carcinoma[4]. Given that NAFLD prevalence is markedly increased in obesity, in type 2 diabetes mellitus and in dyslipidemia[1] the role of insulin resistance in the pathogenesis of this entity has been studied and a strong association has been shown between the two entities[5,6]. Existing data support that NAFLD is the hepatic component of the metabolic syndrome[7].

Polycystic ovary syndrome (PCOS) is one of the most common endocrinopathies in premenopausal women, affecting 5%-18% of this population depending on the used diagnostic criteria[8,9] and is characterised by hyperandrogenism and ovulatory dysfunction. Other diseases causing the same symptoms have to be excluded before considering the diagnosis of PCOS. Insulin resistance has been shown to be an essential feature of the syndrome in the 1980s[10,11] long after the syndrome's first description, affecting both obese and lean patients[11]. Increased prevalence of impaired glucose tolerance and diabetes mellitus[12], abdominal adiposity[13] and dyslipidemia[14] has been shown in PCOS patients implicating that these patients are at increased risk for the metabolic syndrome[15].

During the last years there is increasing evidence of an association of nonalcoholic fatty liver disease and polycystic ovary syndrome. The pathophysiologic link and the clinical significance of this association remain to be determined, in order to clarify issues concerning evaluation and management of these patients.

In this review a detailed Pubmed search of all available data concerning the association of nonalcoholic fatty liver disease and polycystic ovary syndrome was performed in order to summarize current knowledge on this topic.

NONALCOHOLIC FATTY LIVER DISEASE AND INSULIN RESISTANCE

Liver damage in the absence of significant alcohol consumption (> 20 g/d) has been reported since the 1950s[16], however it was mostly recognised after the description of histopathological findings characteristic of alcohol liver disease in patients with none or minimal alcohol consumption by Ludwig *et al*[17] in 1980, who introduced the term non-alcoholic steatohepatitis (NASH). NASH, which develops in a subset of patients with NAFLD, is an advanced stage of the disease characterised by severe steatohepatitis with lobular necroinflammation and variable degrees of fibrosis and can further progress to advanced fibrosis and cirrhosis in some cases[1,2]. Once cirrhosis is present, hepatocellular carcinoma may also develop[4]. NAFLD is considered as the most common cause of cryptogenic cirrhosis[18]. Simple steatosis is the early stage of NAFLD which does not progress to severe disease in the majority of patients and is considered as a relatively benign condition[1,2]. However, it is still not possible to predict who is at risk for advanced NAFLD.

Although the prevalence of NAFLD remains poorly defined due to variation of the characteristics of the studied populations, of the evaluating methods and of the diagnostic criteria that are used (*e.g.*, liver function tests, liver imaging studies, liver biopsy) it is estimated to affect 20%-30% of adults in the general population in developed countries[19-23]. Evaluation of fatty liver content by proton magnetic resonance spectroscopy (^{1}H-MRS), which can accurately measure hepatic triglyceride content, led to the estimation of an even higher percentage, 33.6%, in a large urban population in the United States[24]. Data showing an increased prevalence of NAFLD in obesity[22,24] reaching 90% in morbidly obese[25] and in diabetic patients up to 70%[26,27] drew attention to the possible role of insulin resistance and hyperinsulinemia in the pathophysiology of the disease.

Insulin resistance assessed using the homeostasis model assessment (HOMA-IR) was demonstrated in patients with NAFLD independently of obesity and diabetes[5,28]. These findings were confirmed by studies in nonobese, nondiabetic patients with NAFLD, using the euglycemic insulin clamp technique, the gold standard method for the assessment of insulin sensitivity, which determined the sites of insulin resistance: hepatic and peripheral insulin resistance (skeletal muscle and adipose tissue)[6,7,29,30]. These data supported that insulin resistance is a primary defect in NAFLD, superimposed by obesity- and diabetes-associated insulin resistance, in obese and/or diabetic patients. Thus given that insulin resistance is the key component of the metabolic syndrome, it was suggested that NAFLD is the hepatic manifestation of the metabolic syndrome[7]. Insulin resistance in adipose tissue results in accelerated lipolysis, causing an increased flow of free fatty acids to the liver, thus favouring hepatic fat accumulation. The distribution of adipose tissue is also important since it has been shown that visceral adipose tissue is more insulin resistant than subcutaneous adipose tissue. Consequently visceral adipose tissue by increased portal free fatty acid flow to the liver seems to be an important regulator of fatty liver[31]. Insulin resistance and compensatory hyperinsulinemia are also related to increased *de novo* lipogenesis (synthesis of free fatty acids in the liver) which is another contributor to hepatic fat accumulation. Insulin has been shown to stimulate the expression of lipogenic enzymes through sterol regulatory element binding protein 1 (SREBP-1), even in the setting of insulin resistance[32]. Moreover

hyperinsulinemia contributes to a decrease in lipid oxidation as increased insulin levels have been shown to inactivate the forkhead box transcription factor Foxa2, which regulates expression of genes encoding enzymes of fatty acid oxidation[33]. In normal conditions this transcription factor is active in the fasting state, when insulin levels are low and inactive in the fed state when insulin levels are increased[33]. The enlargement of adipose tissue that characterises obesity and in particular enlargement of visceral adipose tissue with associated chronic low-grade inflammation and monocyte infiltration, leads to secretion of proinflammatory cytokines and modified release of adipose tissue hormones that modulate insulin sensitivity. Elevated levels of proinflammatory cytokines interleukin-6 and tumor necrosis factor-α, leptin resistance and decreased levels of adiponectin are considered as mediators of insulin resistance[34]. However it has been suggested as an alternative hypothesis that liver fat accumulation may develop independently of peripheral insulin resistance. Hepatic steatosis resulting in hepatic insulin resistance without changes in body weight and evidence of peripheral insulin resistance has been described in animal studies[35,36]. Data from human studies suggest that liver fat accumulation may contribute to insulin resistance and the metabolic syndrome[35,36]. The cause/effect relationship between hepatic steatosis and insulin resistance still remains unclear[37].

The association of NAFLD with insulin resistance and features of the metabolic syndrome led to the investigation of its putative role in the development and progression of cardiovascular disease. There is increasing evidence showing that NAFLD is a risk factor for cardiovascular disease. In addition existing data report that cardiovascular disease is the most common cause of death in NAFLD patients before advanced liver disease develops, as only patients with NASH have an increased liver-related mortality rate. However, additional research is needed to demonstrate that NAFLD should be considered as marker of cardiovascular disease[38].

POLYCYSTIC OVARY SYNDROME AND INSULIN RESISTANCE

PCOS was first described as a reproductive disorder comprising menstrual irregularity, infertility, hirsutism and enlarged polycystic ovaries, by Stein and Leventhal[39], in 1935. In the 1960s the term polycystic ovary syndrome was introduced and it is still considered as the most appropriate. Hyperandrogenism clinically expressed as hirsutism, acne and/or androgenic alopecia and anovulation clinically expressed as oligomenorrhea or amenorrhea and infertility are the main characteristics of the syndrome. The syndrome was classified as normogonadotropic, normoestrogenic anovulation (type 2) by World Health Organisation. Hyperandrogenism and anovulation were accepted as the required criteria for the diagnosis, after exclusion of other disorders with these manifestations, according to the National Institutes of Health Consensus Development in 1990 (NIH criteria). In 2003, in a conference in Rotterdam a third diagnostic criterion was added - the polycystic morphology of the ovaries on ultrasound, and the presence of at least two out of three criteria is required for the diagnosis (Rotterdam criteria)[40]. This definition raised controversy, because women with anovulation and polycystic ovarian morphology but without hyperandrogenism were diagnosed with the syndrome. In 2006, a panel of the Androgen Excess Society recommended that hyperandrogenism should be one of the at least two out of three criteria (hyperandrogenism, anovulation and polycystic ovarian morphology) required for the diagnosis, considering that hyperandrogenism is an essential component of PCOS[41].

The presence of hyperandrogenism in women with syndromes of extreme insulin resistance[42] was the initiative for the investigation of the possible role of insulin resistance and compensatory hyperinsulinemia in the pathophysiology of PCOS. In 1980 Burghen *et al*[10] reported the presence of hyperinsulinemia in obese PCOS patients. Following studies confirmed this finding and in addition showed that it was independent of obesity[43-45]. Studies using the euglycemic insulin clamp technique demonstrated peripheral insulin resistance, independently of obesity[11,46], although obesity and central adiposity have an important independent adverse effect on insulin sensitivity[47]. Further studies showed that patients with PCOS have an increased prevalence of impaired glucose tolerance and diabetes mellitus[12,48], abdominal adiposity[13] and dyslipidemia[14] and an evident impact of racial and ethnic differences[49]. It was also shown that metabolic syndrome is frequent in these patients, especially in obese patients. Thus it was established that PCOS is a metabolic disorder as well.

Metabolic dysfunction in PCOS patients leads to increased risk for cardiovascular disease with aging, particularly after menopause. Classic components of an adverse cardiovascular risk profile (central adiposity, impaired glucose tolerance and diabetes mellitus, dyslipidemia and hypertension) are frequently present in PCOS patients of all ages, occurring independently of obesity[50]. However, all these components are worsened when obesity is present[49,50]. In addition, increased levels of several biochemical inflammatory and thrombotic markers of cardiovascular risk are more prevalent in PCOS patients. A very recent small study compared biochemical inflammatory and thrombotic markers of cardiovascular risk between PCOS patients with and without NAFLD, without demonstrating differences between the two groups[51]. In that study, NAFLD was assessed by ultrasonography and in some patients was confirmed by biopsy[51]. In a recent meta-analysis, significantly elevated serum levels of C-reactive protein, homocysteine, plasminogen activator inhibitor-1 (PAI-1) antigen and PAI-1 activity, advanced glycation end products, lipoprotein A, asymmetric dimethylarginine and vascular endothelial growth factor were demonstrated in PCOS patients compared to controls[52]. Moreover, PCOS patients have earlier subclinical

cardiovascular disease, assessed by various methods such as coronary artery calcification, carotid intima-media thickness, pulse wave velocity, flow-mediated dilation of the branchial artery compared to controls[53-55]. However, despite the demonstrated increased cardiovascular risk markers and early subclinical atherosclerosis in PCOS patients, data from observational population studies showing an increase in cardiovascular events are controversial[56]. A 31 year follow-up study of 786 PCOS patients diagnosed by ovarian wedge resection histology, showed no increased risk of death from coronary heart disease[57]. This finding was confirmed in a subsequent retrospective study of 319 women with PCOS, although increased nonfatal cerebrovascular events were reported[58]. Similarly, there was no evidence for increased cardiovascular events in postmenopausal PCOS patients *vs* postmenopausal controls in the first long-term prospective follow-up study[59]. Conversely, the Nurses' Health Study with a participation of 82439 female nurses showed that women with a history of menstrual irregularity (as a surrogate marker for PCOS) had an increased risk of both non-fatal and fatal coronary heart disease compared to women reporting normal menses[60]. Two more studies showed that postmenopausal women with existent hyperandrogenemia and premenopausal menstrual irregularity (considered features of PCOS) had a larger number of cardiovascular events than postmenopausal controls[61,62]. Thus, large prospective long-term follow-up studies, adequately powered[56], are needed. Nevertheless, the androgen excess and polycystic ovary syndrome (AE-PCOS) Society recommends CVD risk assessment in PCOS patients at any age and appropriate interventions when needed[63].

Insulin resistance in PCOS is due to a post-receptor defect in insulin signal transduction which is present mainly in skeletal muscle and adipose tissue and concerns insulin action on glucose and lipid metabolism[49]. In the setting of this selective insulin resistance, other tissues which express insulin receptors like the ovaries are insulin sensitive and are exposed to increased circulating insulin levels (hyperinsulinemia). It has been shown that in normal women insulin acts as a co-gonadotropin to increase LH-induced androgen synthesis in theca cells and FSH-induced estrogen production in granulosa cells. This insulin action on steroidogenesis is preserved in PCOS and is "enhanced" due to hyperinsulinemia. Moreover, there is evidence that theca cells from polycystic ovaries are more responsive to androgen synthesis stimulation by insulin[64]. In addition, hyperinsulinemia decreases hepatic production of sex-hormone binding globulin (SHBG) which is the main binding protein for testosterone and prolongs its metabolic clearance, resulting in increased testosterone bioavailability. Thus insulin resistance, a prevalent finding in PCOS patients, is an important contributing factor to the ovarian androgen excess that characterizes PCOS. Androgen excess in turn may contribute to insulin resistance by modulating insulin action in muscle and adipose tissue, by increasing visceral adiposity and by reducing the secretion of adiponectin, the main insulin-sensitizing hormone of adipose tissue[49].

DIAGNOSIS AND PREVALENCE OF NAFLD IN PATIENTS WITH PCOS

Most patients with NAFLD are asymptomatic at diagnosis and symptoms, when present, are not specific, such as fatigue, malaise and right upper quadrant discomfort. Hepatomegaly may be the only physical finding, while signs of chronic liver disease are rare[1,2]. Thus, diagnosis is based on laboratory evaluation of liver function and/or liver imaging studies after the exclusion of excess alcohol consumption and secondary causes of fatty liver disease (drugs, toxins, viral infections, bariatric surgery, nutritional and metabolic factors, autoimmune liver disease, genetic causes *etc.*)[1-3]. Liver biopsy is the gold standard for diagnosis and staging of the disease[1,2], however, being an invasive method, is not routinely performed.

NAFLD was first diagnosed in a PCOS patient in 2005, as reported by Brown *et al*[65]. A 24-year-old woman with PCOS, obese, nondiabetic, with no history of alcohol consumption and no known cause of liver disease, underwent a liver biopsy because of elevated aminotransferase levels, which showed severe steatohepatitis [nonalcoholic steatohepatitis (NASH)]. It was suggested that NALFD might occur in some patients with PCOS given that insulin resistance is a common feature in both NAFLD and PCOS and both disorders are linked with metabolic syndrome. Thus a concern was raised for the frequency of this hepatic disease in these patients and the importance of screening them with liver function tests.

Laboratory evaluation of NAFLD in patients with PCOS

Elevated serum aminotransferase levels are the most common and often the only laboratory abnormality in patients with NAFLD, while elevated serum alkaline phosphatase and γ-glutalyltranferase are detected less frequently[1]. However, elevations of aminotransferase levels can be used only as a crude estimate of the presence of NAFLD given that the majority of patients with fatty liver do not have laboratory abnormalities[1,66]. In the Third National Health and Nutrition Examination Survey, 2.8% of participants had an abnormal aminotransferase level with no identifiable cause of liver disease, attributable to NAFLD[3].

The first study examining the presence of elevated aminotransferase levels in PCOS patients as a surrogate marker of NAFLD, reported elevated alanine (ALT) and aspartate (AST) aminotransferase levels in 30% and 12%, respectively, of 70 patients, evaluated in an infertility clinic[67]. The used cut-off levels for elevated ALT and AST levels were > 35 U/L and > 40 U/L, respectively. In this retrospective study, patients were of several ethnicities (Hispanic 63%, White 17%, Black 10%, Asian 10%), most of them (74%) obese and there were no controls[67]. Another retrospective study with no controls too, demonstrated elevated ALT and/or AST levels in 15% of

Table 1 Studies investigating the presence of nonalcoholic fatty liver disease in polycystic ovary syndrome patients by biochemical and/or ultrasound evaluation

Ref.	Patients (*n*)	Obese patients	PCOS diagnostic criteria	NAFLD laboratory diagnosis	NAFLD ECHO diagnosis
Schwimmer *et al*[67]	70	74%	NIH	30%[1]	-
Setji *et al*[68]	200	ND	NIH	15%	100%[2]
Cerda *et al*[72]	41	58.5%	Rotterdam	39%	41.5%
Gambarin-Gelwan *et al*[83]	88	42%	NIH	15%[3]	55%
Preiss *et al*[94]	66	100%	Rotterdam	36%	-
Economou *et al*[74]	83	45%[4]	NIH	12%	-
Barfield *et al*[69]	39	100%	Rotterdam	15.4%	-
Markou *et al*[75]	17	0	Rotterdam	0%	0%
Vassilatou *et al*[73]	57	36.8%	AES	22.8%	36.8%
Tan *et al*[79]	186	53.7%	AES	28.7%	-
Chen *et al*[76]	279	ND	Rotterdam	20.9%	61.4%[5]
Lerchbaum *et al*[77]	611	24.8%	NIH	19.2%	-
Gangale *et al*[71]	140	ND	Rotterdam	57.8%	57.8%
Ma *et al*[84]	117	ND	Rotterdam	ND	39.3%
Zueff *et al*[85]	45	100%	Rotterdam	-	73.3%

[1]Elevated alanine aminotransferase (ALT) levels; [2]ECHO evaluation in a subgroup: 16/29 patients with elevated aminotransferase levels; [3]Laboratory evaluation in a subgroup: all patients with ECHO findings of hepatic steatosis; [4]Obese and overweight patients were reported in the same subgroup; [5]ECHO evaluation in patients with elevated ALT levels. ND: Not defined; NIH: National Institutes of Health; AES: Androgen Excess Society.

200 PCOS patients, evaluated in an academic endocrine clinic[68]. This cohort was also of mixed race (Caucasian 68%, Black 20%) and the used cut-off levels for elevated ALT and AST levels were > 60 U/L. Interestingly, 6 of these patients, 23-36 years-old, underwent liver biopsy because of persistently elevated aminotransferase levels which documented the presence of NASH with fibrosis[68], an unexpected finding for this age group. Another retrospective study performed in two centers, evaluated 39 adolescent, obese PCOS patients and reported elevated ALT and/or AST levels in 15.4% of patients. Patients were of several ethnicities (Hispanic 61.5%, Caucasian 10.3%, Black 12.8%, Asian 15.4%) and no controls were included[69]. In an intervention study examining the effects of metformin on NAFLD in overweight/obese PCOS patients, elevated ALT levels using a cut-off > 19 U/L[70] were shown in 57.8% of 140 patients, at baseline assessment[71].

A prospective, case-control study from Chile showed a statistically significant difference in elevated ALT levels between 41 PCOS patients compared to 31 age- and body mass index (BMI)- matched healthy women (39% *vs* 3.1%, respectively), using a cut-off > 25 U/L, according to normal values for healthy Chilean women[72]. More than half of the patients were obese. In agreement, a prospective, case-control study from Greece showed a significant difference of ALT and/or AST levels between 57 PCOS patients compared to 60 age- and BMI-matched healthy women (22.8% *vs* 3.3%, respectively), using a cut-off > 40 U/L[73]. This study comprised lean (40.3%), overweight (22.9%) and obese (36.8%) patients and only one lean patient had abnormal tests. In another case-control study from Greece although no significant difference in elevated ALT levels was reported between 83 PCOS patients compared to 64 age- and BMI-matched healthy women (12% *vs* 4.6%, respectively), using a different cut-off (> 25 U/L), when data were analysed according to BMI subgroups, the difference in abnormal tests remained non-significant for the lean subgroup of patients and controls (4.5% *vs* 5.9%, respectively), but a significant difference was demonstrated for the overweight/obese subgroup (20.5% *vs* 3%, respectively)[74]. In accordance, a case-control study with 17 PCOS patients, all lean, 20-33 years-old, and 17 age- and BMI-matched healthy women showed normal aminotransferase levels in all patients[75]. Two subsequent larger case-control studies also showed a significant difference in elevated ALT levels between patients and controls, one study from Taiwan with 279 PCOS patients and 279 age-matched healthy women[76] and one study from Austria with 611 PCOS patients and 139 BMI-matched control women[77]. Thus existing evidence clearly shows a significantly higher prevalence of elevated aminotransferase levels in PCOS patients and the reported variation (15% to 57.8%) is due to differences in laboratory diagnostic criteria (different cut-off values) and different characteristics of the studied cohorts (ethnicity, age, BMI and PCOS diagnostic criteria) (Table 1).

The use of other laboratory markers for identifying subjects at risk for NAFLD has been also examined in PCOS patients. A case-control study showed that the caspase 3-cleaved fragment of cytokeratin 18 (CK18), an established serum marker for NASH reflecting an increased hepatic apoptosis[78], was significantly elevated, after correction for BMI, in 186 PCOS patients compared to 73 age-matched controls[79]. Moreover, 27.4% of patients had CK18 levels ≥ 395 U/L, indicating NASH, compared to 1.4% of controls[79]. Another case-control study examined the presence of hepatic steatosis by calculating the fatty liver index (FLI), an algorithm based on BMI, waist circumference, triglycerides and gamma-glutamyl transferase, considered a simple and accurate predictor of hepatic steatosis in the general population[80] and two fibrosis indices: AST-to-platelet ratio index and FIB-4-

index. Significantly higher FLI levels were detected in 611 PCOS patients than in 139 BMI-matched control women[77]. However, significantly increased prevalence of elevated FLI levels (> 60) were found only in obese patients compared to obese controls, whereas a similar prevalence of elevated FLI levels was noted in overweight patients and controls and no elevated FLI levels were shown in lean patients and controls. No elevated fibrosis indices were found in either patients or controls[77].

Imaging evaluation of NAFLD in patients with PCOS

Imaging modalities are widely used for the detection of NAFLD including ultrasonography, computerised tomography (CT), magnetic resonance imaging (MRI) and ^{1}H MRS. Ultrasonography, CT and MRI are qualitative or semiquantitative methods, whereas ^{1}H MRS is a quantitative method as it can accurately measure hepatic triglyceride content (HTGC)[24]. However none of these imaging modalities can assess inflammation and hepatic fibrosis[66]. Thus, as aforementioned, liver biopsy is the gold standard for diagnosing and staging NAFLD and for monitoring the efficacy of therapeutic interventions. However, biopsy is an invasive method, associated with potential morbidity and mortality and is prone to sampling errors[66]. Because of these limitations regarding liver biopsy, several non-invasive methods have been proposed for assessing hepatic inflammation and fibrosis, but none has been proven adequate to substitute for liver biopsy. However, non-invasive methods may be useful for selecting patients for liver biopsy. Among such methods, semiquantitative ultrasonographic scores assessing the extent of hepatic steatosis[81], including the recently described ultrasonographic fatty liver indicator (US-FLI)[82], which have been shown to correlate with histological evaluation of NAFLD may help to identify patients with increased risk for steatohepatitis needing liver biopsy.

Abdominal ultrasonography has been used extensively as a screening method for detecting fatty liver infiltration, since it has been shown to have an acceptable level of sensitivity for detecting fatty liver (sensitivity 80% in the presence of > 30% of fatty infiltration), a short examination time and a low cost[66]. Availability of ^{1}H MRS in clinical practice is limited due to high cost and long scan time, while the use of CT is limited due to the exposure of the patients to radiation.

Two studies investigated the presence of hepatic steatosis in PCOS patients by abdominal ultrasonography, without including controls. The first study, retrospective, demonstrated hepatic steatosis in 55% of 88 PCOS patients and interestingly, more than one third of hepatic steatosis patients were lean[83]. It has to be noted that patients were of several ethnicities and one third of them were on oral contraceptives when evaluated. The second study reported hepatic steatosis in 39.3% of 117 PCOS Chinese patients[84].

A prospective case-control study showed a statistically significant difference of hepatic steatosis by abdominal ultrasonography between 41 PCOS patients compared to 31 age- and BMI- matched healthy women (41.5% *vs* 19.4%, respectively)[72]. Another prospective, case-control study, using the same imaging modality, reported significant difference of hepatic steatosis between 57 PCOS patients compared to 60 age- and BMI-matched healthy women (36.8% *vs* 20.0%, respectively)[73]. Most patients with hepatic steatosis were obese and only two were lean. In agreement, a small prospective case-control study with only lean PCOS patients and age- and BMI- matched healthy women showed the absence of hepatic steatosis by abdominal ultrasonography in all study participants and by CT evaluation in PCOS patients[75]. In a case control study with only obese PCOS patients and age-matched controls, evaluated by ultrasonography, hepatic steatosis was detected in 73.3% of patients[85]. In contrast with the above data, a very recent study case-control study showed no differences in the frequency and severity of hepatic steatosis among 55 PCOS patients, 25 control women and 26 men, evaluated by ultrasonography[86].

CT evaluation of fatty liver was used in a very recent study with 30 overweight and obese adolescent girls with PCOS detecting fatty liver, as determined by a ratio of liver to spleen hounsfield attenuation units < 1, in 2 patients (6.7%)[87].

An intervention study examining the effects of omega-3 fatty acid supplementation on liver fat content in PCOS patients, assessed hepatic steatosis by ^{1}H MRS. Hepatic steatosis, defined as liver fat percentage greater than 5%, was detected in 12 out of 25 PCOS patients at baseline evaluation[88]. A case-control study showed a statistically significant difference of hepatic steatosis defined as liver fat percentage greater than 5.5%[24] assessed by ^{1}H MRS between 29 PCOS patients compared to 22 age- and BMI-matched healthy women (6.1% *vs* 1.9%, respectively)[89].

All these data provide evidence that NAFLD is more prevalent in PCOS patients including adolescent patients[69,87] (Table 1). In 2007, Carmina[90] proposed the need for liver evaluation in PCOS patients and in a prescient remark suggested that conversely women of reproductive age with NAFLD should be investigated for the presence of PCOS. Two years later, a small prospective study reported that 10 out of 14 premenopausal women with NAFLD (7 with biopsy proven NAFLD) were diagnosed with PCOS by the Rotterdam criteria and importantly all biopsied patients with PCOS (5 patients) had NASH, providing evidence of an increased prevalence of PCOS in premenopausal NAFLD patients[91].

Mechanisms linking NAFLD to PCOS

Given that NAFLD is rather uncommon in premenopausal women[92] the observed increased prevalence of NAFLD in PCOS patients raises the question of the factor or factors that render women with PCOS more prone to the development of NAFLD.

Clinical data

Clinical studies have shown a significant association of elevated ALT levels in PCOS patients, with age[76], obesity[67,74,76,77,93], waist circumference[67,77], serum triglycerides[67,76,77,93], HDL-cholesterol[68,76,77,93], LDL-cholesterol[76,77], sex-hormone binding globulin (SHBG) levels[74] and degree of insulin resistance assessed by indices like quantitative insulin sensitivity index (QUICKI) and HOMA-IR[67,72,76,77] and by the euglycemic insulin clamp technique[93]. Increased values of FLI, an algorithm indicating hepatic steatosis, were associated with age, obesity, waist circumference, HDL- and LDL-cholesterol and degree of insulin resistance assessed by HOMA-IR[77]. Increased levels of the apoptotic serum marker CK18 were associated with obesity, HDL- and LDL-cholesterol[79]. A significant association of hepatic steatosis in PCOS patients, detected by imaging modalities, was also shown with age[73,85,87], obesity[72,73,83-85,87-89], waist circumference[72,73,84,85], serum triglycerides[71,84,85,88], HDL-cholesterol[73,83,84], LDL-cholesterol[87], SHBG levels[71,73,88] and degree of insulin resistance assessed by QUICKI and HOMA-IR[71-73,83-85,88,89] and by the euglycemic insulin clamp technique[87].

All these data support that obesity, in particular central adiposity and insulin resistance are the main factors related to NAFLD in PCOS. This is also supported by data showing that lifestyle modifications including diet, weight loss and exercise, either alone or in combination with metformin have beneficial effects in PCOS patients with NAFLD[65,68,71,94]. Isolated features of the metabolic syndrome are also related to the presence of NAFLD in PCOS, while a significant percentage of NAFLD patients with PCOS[73,77] including adolescent patients[69,87], reaching 100% in some cohorts[71], meet the criteria for the diagnosis of metabolic syndrome.

However, a question that should be addressed is whether NAFLD is associated with PCOS as a consequence of the shared risk factors, or whether PCOS contributes to NAFLD independently of these factors. Two studies showed that PCOS diagnosis is significantly associated with NAFLD, after adjustment for age, obesity and waist circumference in the first[73] and after adjustment for age, obesity and dyslipidemia in the second study[76]. Since PCOS is a predominantly hyperandrogenic syndrome, investigation of the role of androgens as a putative contributing factor to the development of NAFLD in these patients may clarify this issue. Some of the studies that used laboratory evaluation for the diagnosis of NAFLD in PCOS patients have shown a significant association of NAFLD with androgens. One study showed a significant association of elevated ALT levels in 70 PCOS patients with the presence of hirsutism, the main clinical expression of hyperandrogenism, however serum testosterone levels were not measured[67]. A case-control study reported a positive correlation of ALT levels with total testosterone levels and free-androgen index (FAI) values only in overweight/obese PCOS patients and controls[74]. Similarly, a case-control study with the largest, up to now, number of patients demonstrated that PCOS patients with elevated FLI levels (> 60) (detected only in obese patients) indicating the presence of NAFLD, had higher free testosterone levels than PCOS patients with FLI levels < 60[77]. In addition another large case-control study showed that FAI values and total testosterone levels were positively associated with elevated ALT levels with a cut-off > 33 U/L, and this association remained after adjustment for the possible confounding effects of obesity, dyslipidemia and insulin resistance[76]. Studies that used imaging modalities for the diagnosis of NAFLD in PCOS patients have also demonstrated a significant association of NAFLD with androgens. An intervention study examining the effects of metformin on NAFLD in overweight/obese PCOS patients showed that PCOS patients with NAFLD detected by abdominal ultrasonography had significantly higher FAI values compared to PCOS patients without NAFLD, at baseline evaluation[71]. A prospective, case-control study showed that NAFLD in PCOS patients and controls, detected by abdominal ultrasonography, was significantly related to increased FAI values[73]. Similarly another case-control study with only obese PCOS patients and age-matched controls showed that NAFLD detected by abdominal ultrasonography was positively correlated with FAI values and negatively correlated with SHBG levels[85]. Moreover, a case-control study demonstrated that hyperandrogenic (defined with a FAI ⩾ 7) PCOS patients had significantly higher liver fat measured by ^{1}H MRS compared to normoandrogenic PCOS patients, which remained higher after adjustment for total adipose tissue and visceral adipose tissue volumes and insulin resistance[89]. Similarly, a very recent study which evaluated liver fat by CT in overweight/obese adolescent girls with PCOS demonstrated that age and total testosterone levels were independent contributors to fatty liver[87]. In contrast, a small, retrospective study did not find any difference in FAI values and DHEA-S levels between PCOS patients with and without NAFLD[95]. Similarly an intervention study examining the effects of omega-3 fatty acid supplementation on liver fat content in PCOS patients, did not report differences in FAI values and total testosterone levels between PCOS patients with and without NAFLD, evaluated by ^{1}H MRS, at baseline evaluation[88].

A very recent study showed that levels of the serum apoptotic marker M30 [caspase 3-cleaved fragment of cytokeratin 18 (CK18)] were significantly elevated in 12 PCOS patients with biopsy-proven non-NASH (only steatosis) NAFLD, compared to 12 BMI-matched patients without PCOS and with biopsy-proven non-NASH NAFLD[96]. This finding, indicating a more "intense pro-apoptotic environment" in PCOS patients with NAFLD, seems to be an early feature of NAFLD since the presence of NASH was excluded by biopsy in these patients and may be attributed to hyperandrogenism. Another finding of the study was the altered expression of two genes in the adipose tissue of NAFLD patients with PCOS compared to NAFLD patients without PCOS: a

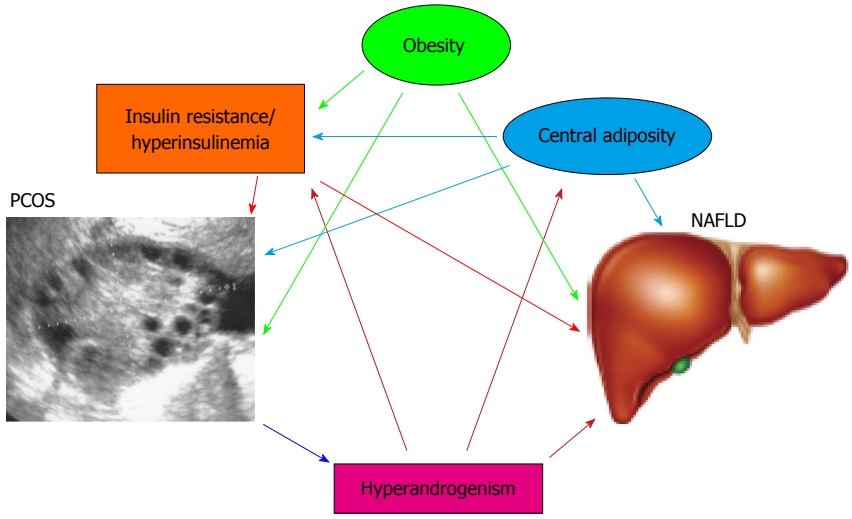

Figure 1 Mechanisms linking nonalcoholic fatty liver disease to polycystic ovary syndrome. Obesity and insulin resistance, features highly prevalent in polycystic ovary syndrome (PCOS) patients and hyperandrogenism, a predominant characteristic of PCOS, as contributing factors to the development of nonalcoholic fatty liver disease (NAFLD). Hyperandrogenism may exert direct effects on the liver and indirect effects by modulating insulin sensitivity and favoring visceral adiposity. Insulin resistance/hyperinsulinemia contributes to hyperandrogenism by affecting the production, the clearance and bioavailability of ovarian androgens.

decrease in LDL receptor mRNA expression, which may be also attributed to hyperandrogenism, and an increase in protein ninein (NIN) mRNA expression[96].

All things considered, there is increasing evidence that hyperandrogenism is related to NAFLD in PCOS. Androgen excess may contribute to NAFLD in PCOS patients by direct effects on the liver, by indirect effects through modulations of insulin sensitivity and secretion, as aforementioned or by increasing visceral adiposity and by combination of these actions (Figure 1). Insulin resistance in turn is an important contributing factor to the ovarian androgen excess by affecting the production, the clearance and bioavailability of ovarian androgens, as already mentioned (Figure 1).

Further studies are needed to assess the role of this vicious cycle of hyperandrogenism and insulin resistance in the development of NAFLD. However it can be suggested that hyperandrogenism should be considered as an additional link in synergy with obesity and insulin resistance for the development of NAFLD in PCOS.

Putative genetic links

Genetic susceptibility to PCOS has been shown by family studies and twin records estimating a heritability of approximately 70%[97]. In addition increased testosterone secretion from theca cells and defects in insulin action in skin fibroblasts from PCOS patients, persist in cultured cells over many passages suggesting they are genetically determined[49,97]. Most of the existing genetic studies on PCOS used a candidate gene approach based on hypotheses concerning the pathogenesis of the syndrome, which is complex and incompletely understood. Thus, genes related to components of PCOS pathogenesis such as genes influencing obesity and insulin resistance, β-cell dysfunction, steroid production and metabolism, androgen receptor and X-inactivation and ovarian folliculogenesis have been studied as candidate genes[97]. Several PCOS genetic susceptibility loci have been identified so far and some may be also implicated in the pathogenesis of NAFLD[98]. However, many of candidate gene studies have been limited by small sample size and small number of investigated gene variants[49,97]. It has to be mentioned that few findings have been replicated in separate cohorts. Additional limitations of these studies are the use of different diagnostic criteria for PCOS and the fact that only premenopausal women can be phenotyped for PCOS[97].

Genome-wide association studies (GWAS) have been used since 2005 to localize susceptibility genes for complex disorders in a "hypothesis-free" concept. The understanding of the pathogenesis of a disorder is not a prerequisite for GWAS and study results may reveal unknown or unexpected pathogenetic pathways. The first GWAS in PCOS was conducted in Chinese PCOS patients diagnosed by the Rotterdam criteria, and identified susceptibility loci for PCOS on chromosome 2p16.3, 2p21 and 9q33.3[99]. Subsequent studies in European PCOS cohorts have replicated some of the Chinese PCOS GWAS signals[100,101]. Interestingly, the finding that the same susceptibility loci contribute to disease risk in Chinese and European PCOS cohorts suggests that PCOS is an ancient trait present in ancestral populations[49].

The role of genetic factors in the development and progression of NAFLD has been suggested by family and twin studies and inter-ethnic variation studies[102]. Most of the studies investigating the genetic basis of NAFLD used the hypothesis driven candidate gene approach. Thus, numerous genes involved in lipid metabolism, insulin signaling, inflammation, fibrotic mediators and oxidative stress have been studied and multiple genetic susceptibility loci have been identified[102]. However, many of these studies are underpowered resulting in inconsistencies and their findings were not replicated in larger cohorts[102]. The first GWAS in NAFLD identified

a missense mutation (I148M) in patatin-like phospholipase domain-containing (*PNPLA*) 3 gene (adiponutrin gene) which was strongly associated with increased hepatic fat, independently of visceral adiposity and insulin resistance[103]. Subsequent studies conducted in different ethnicities confirmed that the I148M variant of *PNPLA3* gene is not only a major determinant of liver fat content, but a predisposing factor to the full spectrum of liver damage in NAFLD - steatohepatitis and progressive fibrosis - as well[104]. Moreover, it was shown that this variant is a major determinant of liver disease progression in other liver diseases such as alcoholic liver disease and chronic hepatitis C[104].

Future genetic studies are expected to elucidate the genetics of NAFLD and PCOS and the possible genetic links between the two entities.

Implications and management

Although it is debated whether simple hepatic steatosis truly represents a disease, it is known that a yet-to-be determined percentage of patients will develop NASH, which may in turn putatively progress to end-stage liver disease. The natural history of NAFLD remains poorly understood, mainly due to the lack of prospective long-term follow-up studies. Focusing on NAFLD patients with PCOS there are scarce data of patients with biopsy proven NASH[51,65,68,91,105]. However the fact that 6 obese, young PCOS patients in a cohort of 200 PCOS patients, had biopsy documented NASH with fibrosis[68] raises concern for a possibly more frequent advanced stage of liver disease in PCOS patients. Benign hepatocellular tumors in obese patients with nonalcoholic steatohepatitis have been reported. Recently, a 50-year-old, obese PCOS patient with metabolic syndrome and focal liver lesions on imaging evaluation was diagnosed with inflammatory hepatocellular adenomatosis and biopsy proven NASH[106].

Early detection of NAFLD in PCOS patients is important because appropriate intervention at the stage of simple steatosis or steatohepatitis may decrease or even eliminate the possibility of disease progression. In this context, PCOS patients should have a liver evaluation comprising assessment of aminotransferase levels and of hepatic steatosis by ultrasound, especially those with metabolic syndrome.

Lifestyle modifications including diet, weight loss and exercise, either alone or in combination with pharmacologic therapy have been shown to have beneficial effects in patients with simple steatosis or NASH. However there are no long-term data confirming a better prognosis[1]. Therapeutic interventions primarily target the risk factors for NAFLD - obesity and adverse metabolic profile (insulin resistance, dyslipidemia, hyperglycemia). Thus diet, weight loss and exercise are the cornerstone of treatment and may be combined with insulin-sensitizers (metformin or pioglitazone), hypolipidemic drugs and hepatoprotective agents like antioxidants and anti-inflammatory agents.

Few interventional data exist for NAFLD in PCOS patients. There is one case with response to diet, moderate weight loss and exercise with an improvement in her histology findings at a post-treatment liver biopsy[65]. Four out of the 6 aforementioned PCOS patients with biopsy documented NASH, demonstrated normalization of aminotransferase levels in response to diet and exercise alone or in combination with metformin[68]. In addition, 9 PCOS patients with elevated aminotransferases from the same cohort responded to interventions with normalization of aminotransferases - 6 patients underwent diet, weight loss and exercise, 2 were treated with metformin and 1 underwent bariatric surgery[68]. It has to be mentioned that 7 PCOS patients with elevated aminotransferases demonstrated a spontaneous normalization at repeated evaluation[68]. A randomized, crossover study examined the effects of omega-3 fatty acid supplementation on liver fat content in obese PCOS patients, assessed by ^{1}H MRS and showed a reduction in fat content mainly in the NAFLD subgroup of patients, after a 8 wk treatment[88]. This beneficial effect was attributed to omega-3 fatty acids modulation of intrahepatic lipid metabolism, probably through activation of peroxisome proliferator-activated receptor-α[88]. The effect of metformin on NAFLD in obese PCOS patients has been assessed in two prospective studies, after 8 and 12 mo of treatment respectively[71,94]. In both studies a significant reduction of liver enzyme levels was shown in patients who completed the treatment. In addition a reduction of insulin resistance assessed by HOMA-IR and in FAI values together with an increase of SHBG and HDL levels were observed in the second study[71].

Metformin, a drug that improves the sensitivity of peripheral tissues to insulin, resulting in a decrease of circulating insulin levels, has been used in PCOS since 1994. Many studies have shown that metformin not only has a beneficial effect on glucose metabolism but also has an effect on lipid metabolism and on androgen excess by increasing SHBG levels and decreasing androgen levels[107]. These actions result in amelioration of the adverse metabolic profile and in restoration of ovulatory menstrual cycles in treated PCOS patients. Similar actions have been reported in PCOS patients with thiazolidinediones, however pioglitazone is not recommended for use in non-diabetic PCOS because of concerns about cardiovascular safety[107]. Thus metformin is recommended in combination with lifestyle modifications in overweight and obese women with PCOS who have impaired glucose tolerance and other features of metabolic syndrome[107]. In that context, metformin might be the drug of choice for treating PCOS patients with NAFLD when pharmacologic therapy is considered. However randomised control studies are needed, as there is still no proven effective medication for NAFLD.

CONCLUSION

NAFLD is more prevalent in PCOS, which has been

redefined as a reproductive and metabolic disorder after the recognition of the important role of insulin resistance in the pathophysiology of the syndrome. Obesity and insulin resistance are the main factors related to NAFLD in PCOS. Androgen excess which is the main feature of PCOS and is interrelated to insulin resistance may be an additional contributing factor to the development of NAFLD in PCOS. Limited data imply that advanced stage of liver disease is possibly more frequent in obese PCOS patients with NAFLD. PCOS patients, in particular obese patients with features of the metabolic syndrome, should be submitted to liver evaluation comprising assessment of aminotransferase levels and abdominal ultrasound. Lifestyle modifications including diet, weight loss and exercise are the most appropriate initial therapeutic interventions for PCOS patients with NAFLD and when pharmacologic therapy is considered, metformin may be used, although there is still no proven effective medication for NAFLD.

Long-term follow up studies would help to clarify clinical implications and guide appropriate diagnostic evaluation, follow-up protocol and optimal treatment for PCOS patients with NAFLD.

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