

MANAGEMENT OF ENDOCRINE DISEASE

Polycystic ovary syndrome and nonalcoholic fatty liver disease

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Abstract

Polycystic ovary syndrome (PCOS) is a frequent endocrine disease in women, with a number of metabolic and reproductive consequences. Obesity, insulin resistance (IR) and type 2 diabetes are prominent metabolic characteristics of PCOS and common factors affecting liver function and generating nonalcoholic fatty liver disease (NAFLD). Multiple genes involved in the synthesis of androgens, cytokines and IR, as well as acquired factors, such as endocrine disruptors, could associate the etiopathogenesis of PCOS and NAFLD. Besides the high prevalence of PCOS in general population, NAFLD was shown to be a frequent condition in transition periods, such as adolescence and menopause. Although liver biopsy is considered to be the gold standard for diagnosing liver damage, its routine use in such a prevalent condition as PCOS can be related to a higher rate of complications. Therefore, it is necessary to be able to diagnose NAFLD using simple and reliable surrogate markers. Recently, fatty liver index and NAFLD fatty liver score analyzed in large cohorts of PCOS women have been shown as accurate markers of liver damage in this metabolically vulnerable population. Lifestyle changes are still the mainstay of the management of NAFLD in PCOS, although prospective randomized controlled clinical studies remain a priority in the field. With regard to medications, metformin may be the drug of choice for treating PCOS patients with NAFLD when pharmacologic therapy is considered. Liraglutide use in obese PCOS has shown favorable effects on the predictors of liver fibrosis. In this review, we aim to summarize the influence of the common risk factors and to discuss the diagnostic approaches and management options for NAFLD in patients with PCOS.

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Invited Author's profile

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Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women in the reproductive period, with the prevalence of 6–10% (1, 2), although it is supposed to be even higher, depending on ethnic population and criteria used for diagnosing (3). The main characteristics of the syndrome are chronic anovulation with or without menstrual cycle disturbances, clinical and/or biochemical hyperandrogenism and ultrasound evidence of morphologically polycystic ovaries. With regard to reproduction, this disorder affects fertility in women of reproductive age and influences the possible neoplastic transformations in the postmenopausal age. The metabolic aspect of the syndrome significantly affects the entire life of the women in question, particularly because it can change over time, becoming worse in some cases (4, 5, 6). However, some longitudinal studies have shown stagnation or even amelioration of metabolic abnormalities in PCOS women. Such diversities in PCOS metabolic profile at different life stages could be explained by reproductive maturation and senescence influencing PCOS phenotypes (7).

Obesity appears to play an important role in the pathogenesis of PCOS and is present in 40–70% of patients with the syndrome (8, 9). Insulin resistance (IR) is another prominent characteristic of PCOS and is partly independent of obesity (10, 11, 12). The high prevalence of obesity and IR in patients with PCOS is the main driver of the increased risk of prediabetes and type 2 diabetes mellitus (T2DM) in this population (13).

Liver has an important regulatory function in various metabolic processes. This function is simultaneously modified by other organs, systems and processes, including immune, inflammatory, neuronal and endocrine ones. Therefore, hyperandrogenemia, dyslipidemia, hyperglycemia, IR and low-grade inflammation, all of which characterize PCOS, are concomitant factors that generate and aggravate nonalcoholic fatty liver disease (NAFLD) (14, 15). What is more, therapies used for PCOS, such as oral contraceptives, antiandrogens or fertility drugs might exert additional adverse effects on the already disturbed hepatic function in these women (14).

As obesity and IR are common risk factors for both PCOS and NAFLD, the association between these common disorders has been evaluated in several studies. The aim of the present review is to summarize the findings from these studies and to discuss diagnostic approaches and management options for NAFLD in patients with PCOS.

Relationship between fatty liver disease and metabolic disorders

NAFLD is the most common chronic liver disease in high-income countries (16). NAFLD is characterized by hepatic steatosis (>5%) after the exclusion of other causes, including alcohol consumption (<20 g/day for women and <30 g/day for men), other causes of chronic liver disease (viral or autoimmune hepatitis and/or hemochromatosis) and treatment with steatogenic medications (e.g. amiodarone, corticosteroids) (17). NAFLD encompasses a wide range of histologic entities, from isolated steatosis to nonalcoholic steatohepatitis (NASH) (i.e. steatosis along with varying degrees of balloon degeneration, inflammation and fibrosis) and cirrhosis (17). Approximately 34–46% of general population has hepatic steatosis and 12% has NASH (18, 19).

Just like in the case of PCOS, obesity and IR are involved in the pathogenesis of NAFLD (15, 20). Similarly, the prevalence of hepatic steatosis is higher in obese patients and patients with T2DM, affecting 45% and 70% of them respectively (18, 21). Moreover, patients with NAFLD have a higher risk of developing T2DM (22).

Pathogenesis of NAFLD

The pathogenesis of NAFLD appears to involve two steps (the two-hit theory) (23). In the first step, abdominal obesity and/or IR results in reduced insulin-mediated suppression of lipolysis in the visceral adipose tissue, which leads to an increased influx of free fatty acids to the liver and steatosis (24). Both obesity and IR are independently associated with the severity of steatosis (25). Interestingly, there is an increase in *de novo* lipogenesis in the liver in patients with NAFLD, whereas the export of lipids in the form of very low-density lipoprotein cholesterol is reduced due to decreased apoB synthesis, which aggravates steatosis even further (26). The second step of the NAFLD pathogenesis involves the development of inflammation and fibrosis, i.e. the progression from isolated steatosis to NASH (23). Liver steatosis may progress to NASH in up to one-third of patients (27). Obesity and IR are independent risk factors for both inflammation and fibrosis (28). Insulin stimulates the mitosis and production of both collagen and fibrogenic cytokines from hepatic stellate cells (29). However, additional pathogenetic factors also appear to be involved in the progression to NASH. Oxidative stress is increased in patients with NAFLD, and it correlates with the severity of inflammation and fibrosis (30).

Subclinical inflammation is another feature of NAFLD, which also apparently increases the risk of developing inflammation and fibrosis (31). Additionally, patients with NAFLD exhibit increased hepatocellular apoptosis and elevated ferritin levels, which also might indicate the progression to NASH (32, 33).

Diagnostic approach to NAFLD

NAFLD is usually an asymptomatic disease with an occasional mild elevation of aminotransferases. However, serum aminotransferase levels are insensitive indicators of NAFLD presence, as they are usually in the normal reference range in most patients (17, 34). Liver biopsy is the gold standard for diagnosing NAFLD. However, there are several limitations to liver biopsy. First, performing a liver biopsy in all patients with suspected NAFLD is not feasible, given the high prevalence of the disease. Second, even though liver biopsy is relatively safe, it is still an invasive procedure and might result in life-threatening or even fatal complications (35). Finally, sampling errors have also been reported in liver biopsies in patients with NAFLD (36). Approximately 35% of patients with bridging fibrosis in one liver sample had no fibrosis in another sample obtained during the same procedure (36). This discordance is caused by an uneven distribution of histologic abnormalities in NAFLD, which might result in false negative results (36).

Given these limitations of liver biopsy, several non-invasive methods have been applied in order to diagnose NAFLD. Regarding imaging methods, ultrasound (US), computed tomography (CT) and magnetic resonance imaging (MRI) appear to have similar sensitivity and specificity for detecting steatosis (94% and 80% respectively) (37). ¹H-magnetic resonance spectroscopy has similar sensitivity compared to the three methods above, but it is more expensive and less widely available (37). However, none of these methods provides any information on the presence of fibrosis (38). Therefore, US seems to be the imaging modality of choice for detecting steatosis, since it does not expose the patient to radiation and is less expensive than CT and MRI (38). However, it shows limited sensitivity (60–90%) if steatosis is present in <30% hepatocytes (17, 34). Additionally, MRI appears to be less sensitive for detecting steatosis in patients with advanced fibrosis (39, 40). None of the diagnostic procedures above can differentiate between simple hepatic steatosis and NASH, though (17, 34). Transient elastography is a recently developed alternative method

for evaluating liver stiffness, which correlates directly with hepatic fibrosis (41, 42, 43), but it is less valid in obese patients (44). Besides transient elastography, some of the more novel methods based on the same principle include ultrasound-based acoustic radiation force impulse imaging, real-time shear wave elastography, as well as magnetic resonance elastography (45, 46, 47). The latter methods appear to have similar sensitivity and specificity compared to transient elastography. Moreover, they appear to be more reliable than transient elastography in obese patients (45, 46, 47). However, limited availability is a major obstacle for their application in diagnosing NAFLD.

In addition to imaging methods, several serological markers incorporated in various algorithms have been evaluated for diagnosing NAFLD and fibrosis. Liver fat content (LF%) and NAFLD liver fat score (NAFLD-LFS) include these parameters: presence or absence of metabolic syndrome (MetS) as defined by International Diabetes Federation (IDF) criteria and/or T2DM as defined by World Health Organization criteria, fasting serum insulin, aspartate transaminase (AST), and alanine transaminase (ALT) (25). Another similar score, fatty liver index (FLI), uses body mass index (BMI), waist circumference, and levels of triglycerides and gammaGT (48). However, NAFLD-LFS has been validated against magnetic resonance spectroscopy, while FLI has been validated against ultrasound. Fibrosis index (FIB-4) includes age, AST, ALT and platelet count and appears to be the most sensitive and specific for diagnosing NASH, with an area under receiver operating characteristic curve of 0.86–0.90 (49, 50). Other frequently used scores for differentiating simple steatosis from NASH comprise the NAFLD fibrosis score, which includes age, BMI, hyperglycemia (fasting glucose levels ≥ 110 mg/dL or previously diagnosed T2DM), platelet count, albumin and AST/ALT ratio (51), and the BMI, AST/ALT ratio, and diabetes (BARD) score (52), but they appear to be less accurate than FIB-4 (49, 50). Other algorithms comprise less readily available parameters reflecting the severity of NAFLD, including markers of IR or fibrosis (e.g. hyaluronic acid, type IV collagen 7S or matrix metalloproteinase 1) (53, 54). Limited data suggest that these more expensive algorithms might have better specificity for excluding NASH than the FIB-4 score (53, 55).

Additionally, among the wide range of the biochemical markers analyzed, procollagen type III amino-terminal peptide (PIIINP) has been found to be a single marker which could adequately differentiate between simple steatosis and NASH in patients without advanced fibrosis

Table 1 Diagnostic criteria for nonalcoholic fatty liver disease (NAFLD)/nonalcoholic steatohepatitis.

Diagnostic criterion	Reference
Biochemical markers and algorithms	
Aminotransferases	15*, 17, 34, 122*
Hyaluronic acid	53, 54
Type IV collagen 7S	53, 54
Matrix metalloproteinase 1	53, 54
Caspase 3-cleaved fragment of cytokeratin 18	73*
Procollagen type III amino-terminal peptide	56
Liver fat content	25
NAFLD liver fat score	15*, 25
Fatty liver index	48*
Fibrosis index	49, 50
NAFLD fibrosis score	51
BMI, AST/ALT ratio, diabetes (BARD) score	52
Imaging modalities	
Ultrasound	37, 58*
Computed tomography	37
Magnetic resonance imaging	37
¹ H-magnetic resonance spectroscopy	37, 59*
Transient elastography	41, 42, 43, 44
Real-time shear wave elastography	45, 46
Magnetic resonance elastography	47
Histology	
Liver biopsy	35, 36, 119*, 149*

References related to patients with polycystic ovary syndrome are marked with asterisk.

ASLT, alanine transaminase; AST, aspartate transaminase; BMI, body mass index.

(56). Given diagnostic criteria for NAFLD and NASH are listed in Table 1.

PCOS and NAFLD

There is a growing body of evidence that shows that NAFLD and PCOS share the same metabolic pathway. Epidemiological studies conducted in various cohorts of adult women with PCOS showed that NAFLD is associated with indices of IR, altered lipid metabolism, and androgen levels (15, 57, 58).

Molecular basis including endocrine disruptors

As the etiopathogenesis of PCOS remains not fully understood, the exact mechanism linking PCOS and NAFLD is yet to be elucidated (60). It is likely that it involves multiple genetic and acquired factors. Among genetic factors, PCOS and NAFLD both display the disturbances in the function of some shared genes involved in the synthesis of androgens (*CYP17*, *CYP11A*,

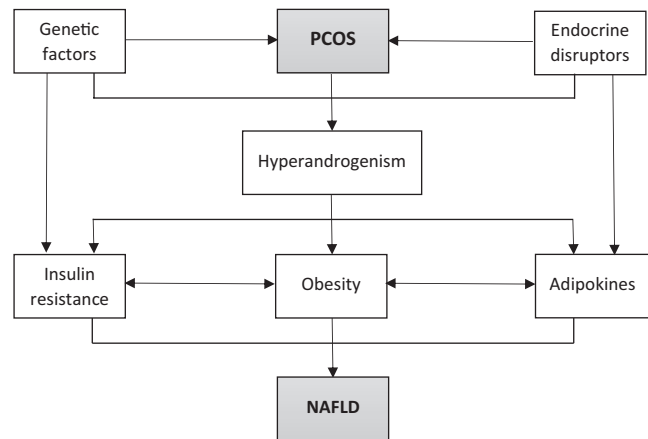


Figure 1

Factors involved in the development of nonalcoholic fatty liver disease (NAFLD) in patients with polycystic ovary syndrome (PCOS).

SHBG gene), cytokines (TNF- α , TNFR2, IL6) and IR (INS-R, insulin) (59). Multiple factors having a role in the development of NAFLD are given in Fig. 1.

Even though human studies confirming direct causality of hyperandrogenemia and the development of liver damage are lacking, it is possible to examine an animal model of prenatal androgenization of the female fetus with the consequent development of PCOS-like phenotypes (60, 61). Recently, an ovine model of prenatal androgenization suggested that maternal exposure to testosterone propionate by upregulating hepatic androgen, estrogen and glucocorticoid receptors, UDP-glucose ceramide glucosyltransferase, mitogen-activated protein kinase 4 and IGF1 leads to the development of IR and fatty liver, independently of obesity (62).

In line with these data there are clinical studies which show that IR is a common characteristic of both PCOS and NAFLD (15, 63, 64). However, there are usually no significant differences in circulating total androgens between PCOS with NAFLD and without it (65, 66, 67), but a lower SHBG independent of obesity, as well as a consequently higher free androgen index (FAI) in the former indicates a higher tissue exposure to biologically active free androgens (57, 58, 68). SHBG might be a possible mediator between IR and NAFLD, being a hormone carrier and an indicator of the metabolic and nutritional status (69) whose hepatic production is regulated by insulin. Moreover, a recent multi-ethnic study on a large population of postmenopausal women showed an association between the highest levels of bioavailable testosterone and estradiol with NAFLD, independent of age, ethnicity,

waist-to-hip ratio, lipid levels, hypertension, use of hormone replacement therapy, and IR (70). It follows that the effect of hyperandrogenemia on NAFLD development might be more profound. More specifically, androgens were shown to have a direct proapoptotic effect on hepatocytes (71). For example, in comparison to healthy controls, women with PCOS have an increased level of apoptotic marker, caspase-cleaved CK18, independently of BMI (72). Increased concentrations of caspase-cleaved CK18 in PCOS women with NAFLD could be indicative of further damage to the liver, as this marker elevation precedes progression from NAFLD to NASH (73). Additionally, androgen receptor agonists attenuate estrogen-induced upregulation of low-density lipoprotein receptor (LDLR) in hepatocytes (74), while hyperandrogenemia suppresses LDLR on adipocytes (73).

To sum up, it seems that hyperandrogenemia directly creates steatogenic and proapoptotic environment that makes women with PCOS prone to the development of NAFLD and possibly even NASH. However, the association between elevated androgen levels and NAFLD seems to be partly mediated by IR. Once developed, hepatic injury could further impact the metabolism of androgens and consequently change hormonal profile in women with PCOS.

Adipokines could be another mediator between hyperandrogenism, IR and NAFLD in PCOS women. By promoting visceral adiposity (75), androgens exert a direct and inverse effect on adipokines production (76, 77, 78). Adiponectin has a protective, antisteatotic, anti-inflammatory, antiapoptotic and consequent antifibrotic effect on hepatocytes (79, 80). In mice, administration of recombinant adiponectin prevented steatosis and suppressed hepatic inflammation (81). Clinical studies reported lower levels of adiponectin in patients with NAFLD than those in controls, and lower levels in patients with NASH than those in patients with isolated steatosis (82, 83, 84). Adiponectin levels also correlate negatively with the severity of hepatic steatosis and inflammation (82, 83, 84). Moreover, the expression of adiponectin receptors in the liver is lower in patients with NASH than in those with isolated steatosis, and it is negatively correlated with the degree of inflammation and fibrosis (85, 86). A recent study has shown that liver peroxisome proliferator-activated receptor (PPAR)- α gene expression negatively correlates to the presence of NASH while it positively correlates with adiponectin (87). Although adiponectin has a potential beneficial effect on NAFLD, the majority of other adipokines usually induce hepatic steatosis and NAFLD. A recent meta-analysis showed that

patients with NASH and simple liver steatosis have higher circulating levels of leptin than healthy controls (88). However, studies on animal model suggest that leptin could exert a dual action on NAFLD: In the initial phases of liver disease, leptin might have antisteatotic effects, while inflammation and fibrosis might develop in disease progression potentiated by leptin resistance (89). In rats, administration of leptin augments both proinflammatory and fibrogenic responses in the liver via increased expression of procollagen-I and transforming growth factor- β 1 (90). In contrast, leptin-deficient mice display decreased fibrogenesis in response to liver injury (91). Serum leptin levels are higher in patients with NASH than those in controls (88, 92). An early study also reported a positive correlation between leptin levels and the severity of steatosis (92). However, the others did not confirm this association (93, 94). What is more, leptin levels do not appear to correlate with the degree of inflammation or fibrosis (88, 93, 94).

There are heterogenous data regarding circulating levels of resistin and its relation to NAFLD (95, 96, 97, 98). Similar heterogeneity exists regarding the role of resistin in the pathogenesis of IR in PCOS (99, 100). In patients with NAFLD, serum resistin levels correlate with the severity of steatosis, inflammation and fibrosis (95, 101, 102).

Although there are some data that implicate the roles of visfatin, chemerin and retinol-binding protein 4 (RBP-4) in IR and NAFLD, definitive conclusions are lacking (103, 104). A novel circulating hormone, betatrophin, secreted both by the adipose tissue and liver, has been found to be elevated in PCOS and strongly associated with androgens, BMI and IR (105). Moreover, betatrophin was shown to be an independent predictor of fibrosis in patients with NAFLD (106).

A complex interplay between adipose tissue and immune system could influence the development of NAFLD as well. There is a positive correlation between the quantity of adipose tissue and the number of immune cells infiltrating it, thus increasing the burden of low-grade chronic inflammation (107). Consequently, cytokines produced by immune cells could affect adipocytes and their secretory profile. Among various cytokines, interleukin 6 (IL6), tumor necrosis factor alpha (TNF α), as well as visfatin, have been implicated in the pathogenesis of NAFLD (103).

To sum up, a variety of adipokines and cytokines have been implicated in the pathogenesis of IR, and consequently, their role in pathogenesis of NAFLD might be substantial. Moreover, various adipokines

(adiponectin, leptin, resistin, retinol-binding protein-4, visfatin, chemerin and novel betatrophin) have been implicated in the pathogenesis of NAFLD. However, there is not a single study in the current literature that confirms the relationship of adipokines with NAFLD independently of IR and obesity (103). This is because the methodology in such studies needs to be rather complex, so that relevant conclusions could be drawn (obese controls should not have NAFLD, and studies should be longitudinal, with multiple liver biopsies in all subjects over time). Consequently, there are insufficient data that could support the use of single adipokines or their combinations as a biochemical marker for NAFLD.

It was hypothesized that endocrine disruptors (EDs) could be an etiological trigger for the development of NAFLD and IR (108), as well as for the syndromes related to IR, such as obesity (109), T2DM (110) and PCOS (59). The main action of EDs implicated in the pathogenesis of NAFLD is initiated by altered gene transcription, protein expression and the resulting increased lipid accumulation, oxidative stress enhancement, insulin receptor downregulation leading to IR and specific changes in cytokines and adipocytokines (111). Among EDs, dioxins, phthalates and bisphenol A (BPA) are most frequently associated with IR in humans. In other words, higher serum BPA levels were observed in women with PCOS, and regarded as the ovarian manifestation of IR syndrome (112). However, the molecular evidence for the causality between EDs and NAFLD in PCOS women is still lacking.

Prevalence of NAFLD in PCOS

The prevalence of NAFLD in women with PCOS was shown to be higher (35–70%) in comparison to age-, BMI- and waist circumference-matched healthy women (20–30%) (15, 57, 58, 68, 113, 114, 115). Additionally, there is a high prevalence of PCOS (50–70%) in patients with confirmed NAFLD (68, 114), while NAFLD in these patients usually presents in a more severe form. A recent meta-analysis showed that women with PCOS have a four times higher risk of NAFLD in comparison to healthy women (116). NAFLD is usually considered as a hepatic manifestation of MetS (117); however, some studies confirmed that PCOS women without concomitant MetS and obesity have a higher prevalence of NAFLD in comparison to healthy women (57, 58, 118). Taking into account the fact that women with PCOS are young and that they might already have NASH, it follows that a high risk of liver-related comorbidities and complications could extend from

reproductive to menopausal age. Accordingly, Setji *et al.* diagnosed a relatively advanced stage of NASH in women with PCOS aged between 20 and 30 years (119). Similar results were obtained in other studies, all confirming the advanced stage of NASH in women with PCOS younger than age 40 years (68, 114, 120).

Therefore, it is viable to look for NAFLD in PCOS women with severe clinical phenotypes, although recent guidelines recommended against routine screening (121). However, if screening for NAFLD is being considered in a specific case, we are in favor of simple and non-harmful biochemical assessment (15).

Predictors and diagnosis of NAFLD in PCOS

Considering the high prevalence of PCOS in general population of reproductive age women, liver biopsy, being an invasive procedure associated with potential complications, cannot be performed in every woman with PCOS. Therefore, there is a need for a quick, simple and non-invasive method for detecting NAFLD in each woman in this metabolically vulnerable population.

Just like general population, the majority of women with PCOS and confirmed NAFLD usually have normal aminotransferase levels (15, 122). Therefore, elevated liver enzymes should not be considered as a marker of more extensive liver disease. Accordingly, apoptotic cell death marker, caspase 3-cleaved fragment of cytokeratin 18, which was proved as a marker of NASH in women with PCOS, can be used as a marker of liver damage (72). NAFLD in PCOS population is usually diagnosed by ultrasound (57), although there are reports about the use of alternative methods, such as proton magnetic resonance spectroscopy (58). Two recent cross-sectional clinical studies undertaken on the largest cohorts of PCOS women confirmed the successful use of the biochemical surrogate indices in prediction of liver steatosis (15, 48). It was shown that high fatty liver index is commonly found in obese PCOS women, and that it is closely linked to MetS (48), as well as that NAFLD liver fat score is an excellent marker for NAFLD in PCOS population (15). Diagnostic criteria for NAFLD and NASH in PCOS are listed in Table 1.

PCOS and NAFLD in special groups

Most of the original studies analyzed possible association of NAFLD and PCOS in reproductive adult women, while studies on specific PCOS groups are lacking. Recently, it was shown that NAFLD was more prevalent in adolescent girls with PCOS in comparison to the girls without PCOS

(37.5 vs 15.1% respectively). Adolescent girls with NAFLD and PCOS were more obese and had higher serum leptin and HOMA-IR levels than both girls and boys without NAFLD, which suggests an independent prediction of NAFLD by PCOS (123). Similarly, 49% of obese, non-diabetic PCOS adolescents, in comparison to 14% of obese adolescents without PCOS, had hepatic steatosis. Moreover, these authors showed that hepatic steatosis was related to the visceral fat content and lipogenesis, but not to the indices of insulin sensitivity or androgen levels (124).

On the other hand, postmenopausal women without history of PCOS had similar prevalence of NAFLD as premenopausal PCOS women, and both groups had more prevalent NAFLD than premenopausal women without PCOS, which suggests possible protective effects of estrogens for the development of NAFLD in women (125). More clinical data are needed for confirming the aforementioned results from single studies.

NAFLD and metabolic syndrome in PCOS

Most studies on NAFLD in PCOS demonstrated a correlation between the presence of NAFLD with IR and MetS (57, 58, 68, 114, 125). MetS, PCOS and NAFLD share similar features, including IR and abdominal obesity. IR was proved to be an independent predictor of T2DM, hypertension, obesity, cardiovascular diseases, MetS and NAFLD (126). It is known that IR affects both lean and obese PCOS patients (127). In line with this, before T2DM is clinically manifested, the extent of IR can be different in liver and skeletal muscles (128, 129), which may be the reason for the discordance of peripheral and hepatic IR in the same subject (130). NAFLD is assumed to be directly responsible for the development of local hepatic IR (64, 131) and could aggravate IR in PCOS associated with chronic anovulation, hyperandrogenemia, atherogenic dyslipidemia and subclinical inflammation (132, 133). Accordingly, the favorable action of metformin on NAFLD can be explained by its effect on hepatic IR (66, 134). Insulin-resistant visceral adipose tissue, like the one in women with PCOS, induces lipolysis and an increased flow of free fatty acids to the liver, causing hepatic fat accumulation (135). NAFLD is considered a hepatic manifestation of MetS, and it may even precede the development of MetS (17, 136).

Therapeutic options for NAFLD

Lifestyle changes are the mainstay of NAFLD management (17). It was shown that 5–7% weight loss on low-fat,

low-calorie diet combined with aerobic exercise resulted in the resolution of NASH in 26% of patients, and in the reduction of fibrosis in 18% of patients. Moreover, when the weight loss was >10% of the initial body weight, 90% experienced the resolution of NASH and 45% experienced a reduction of fibrosis (137). The optimal diet for NAFLD is yet unknown, but a small study showed a greater reduction in steatosis with Mediterranean diet than with low-fat diet, despite a similar weight loss (138). In relation to the type of training, it was shown that a modified high-intensity interval training (HIIT) not only decreased liver fat, whole-body fat mass and transaminases but it also had a beneficial effect on cardiac function by increasing early diastolic filling rate in patients with NAFLD (139). Next, a recent analysis of the studies on exercise in NAFLD patients showed that moderate-to-high volume moderate-intensity training in combination with diet improves serum levels of liver enzymes, liver fat or histology. Moreover, exercise produces beneficial effects on intrahepatic triglycerides even in the absence of weight loss (140). Regarding pharmacotherapy, vitamin E and pioglitazone are the first-line agents for patients with NAFLD (17). In a randomized, placebo-controlled trial in patients with NASH, vitamin E and pioglitazone were equally effective in reducing steatosis and inflammation, but had no effect on fibrosis (141). However, safety issues hamper the use of either agent. Indeed, vitamin E increased the risk for all-cause mortality in a meta-analysis of 46 randomized controlled trials (142), whereas pioglitazone increases the risk for heart failure, fractures and possibly bladder cancer (143, 144). More recently, obeticholic acid, a selective agonist of the farnesoid X receptors and liraglutide, a glucagon-like peptide-1 receptor agonist (GLP-1 RA), yielded promising results in patients with NAFLD, but more studies are needed to establish their benefit/harm ratio in this population (145, 146). Although metformin exerts a positive effect on the liver IR, there is not enough clinical evidence so far on its favorable effect on the liver histology, and it is not recommended as a specific therapy for NASH/NAFLD (147, 148).

Clinical studies on the effects of dietary regimens on NAFLD/NASH in PCOS women are still lacking. There is only one case-study in addition to small case-series studies that shows histology improvement in response to diet (149), or normalization of aminotransferase levels in response to diet and exercise alone, or in combination with metformin in PCOS women with biopsy-documented NASH (119). In relation to the effect of metformin on NAFLD in obese PCOS patients, there are two longitudinal studies of up to 12 months in duration

that showed a significant reduction of liver enzyme levels accompanied with the reduction of IR and androgen levels (66, 150). The rat model of T2DM showed that metformin intervention affected the expression of insulin receptors and genes associated with lipid metabolism. More specifically, metformin upregulated acyl-CoA oxidase, carnitine palmitoyl transferase-1 and PPAR- α , while it downregulated the expression levels of fetuin-A and RBP-4. Moreover, metformin administration induced regenerative changes in the hepatocyte cytoplasm and parenchyma (151). A recent animal study showed that metformin treatment resulted in the reduction of visceral fat mass independently of its anorexogenic effect and was accompanied by an upregulation of adaptive thermogenesis and fat oxidation-related enzymes in the liver (152).

It was shown that metformin and thiazolidinediones (TZDs) could ameliorate the adverse metabolic profile and restore ovulation in women with PCOS who were treated (6). Although the data on the effects of metformin in lean women with PCOS are missing, it was shown that 6–8 months of treatment with metformin could reduce transaminase levels in overweight or obese women with this syndrome (66, 150). As concerns over the cardiovascular safety of pioglitazone bring into question the clinical use of this class of insulin sensitizers, metformin could be the drug of choice for treating PCOS patients with NAFLD when pharmacologic therapy is taken into consideration.

Another therapeutic attempt at ameliorating NAFLD was supplementation of omega-3 fatty acid. There was an attempt to evaluate the effects of omega-3 fatty acids on liver fat content in a clinical trial. Magnetic resonance spectroscopy used in this short-lasting trial showed a reduction in fat content mainly in the NAFLD subgroup of PCOS patients. This was attributed to the fact that omega-3 fatty acids modulate intrahepatic lipid metabolism through activation of PPAR- α (113).

A recent study on the treatment of obese PCOS women with liraglutide, showed an effect on the reduction of body weight and the procollagen type 3 amino-terminal peptide, an independent predictor of liver fibrosis (120). This small pharmacological study recognized the need for treating NAFLD in PCOS, thus preventing the development of more deleterious liver fibrosis states, as well as worsening of the metabolic profile leading to the development of T2DM. Phase II of another ongoing clinical study on the effect of semaglutide in subjects with NASH (clinicaltrials.gov, NCT02970942) is looking into the effect of this GLP-1 RA on the NASH resolution

Table 2 Therapeutic options for nonalcoholic fatty liver disease/nonalcoholic steatohepatitis.

Therapeutic modality	Reference
Lifestyle modification	
Diet	138
Exercise	139
Pharmacotherapy	
Vitamin E	17, 141
Pioglitazone	6*, 17, 141
Metformin	6*, 67*, 150*, 151, 152
Liraglutide	120*, 146
Semaglutide	NCT02970942 (clinicaltrials.gov)
Omega-3 fatty acids	113*
Obeticholic acid	145
Ipragliflozin	153
3-iodothyronamine	154*
Combination regimens	
Diet and exercise	17, 137, 140, 149*
Diet, exercise and/or metformin	119*

References related to patients with polycystic ovary syndrome are marked with asterisk.

without fibrosis worsening. Recently, ipragliflozin, a sodium-glucose co-transporter 2 (SGLT-2) inhibitor, was suggested as a second-line treatment for NAFLD patients with T2DM who do not respond to incretin-based therapy. It was shown that apart from achieving improvement in glycemic control, this class of drugs had beneficial effects on body weight reduction, normalization of ALT levels, and the reduction of the FIB-4 index even in patients who did not respond to incretin-based therapy (153).

In addition to the standardized therapeutic approach, a recent study on the animal model of PCOS showed a favorable effect of 3-iodothyronamine, an analog of thyroid hormone, on IR and consequently on NAFLD/NASH. This novel compound induced a profound tissue-specific antilipogenic effect in liver by lowering the expression of key lipid metabolism genes, such as PTP1B and PLIN2 (154). We believe that there is a need for more randomized, placebo-controlled and longitudinal clinical studies with the primary goal to analyze the effects of the available compounds on the amelioration of NAFLD and NASH, central metabolic diseases leading to IR and consequently to type 2 diabetes. Therapeutic modalities for NAFLD and NASH are listed in Table 2.

Conclusions

PCOS is a hyperandrogenic state sharing the main characteristics of the IR syndrome with the MetS including dyslipidemia, hyperglycemia, IR and consequent low-grade

inflammation. These factors simultaneously generate and aggravate NAFLD. Consequently, women with both PCOS and NAFLD have a higher risk of both metabolic and cardiovascular complications, which should be carefully looked for. Currently, there is no specific screening, nor are there treatment recommendations for NAFLD in women with PCOS. Given that transaminases are usually in the reference range in NAFLD, we propose the use of liver ultrasonography in combination with non-invasive surrogate markers of NAFLD in this population of relatively young women. Also, women with a suspected progression to NASH should undergo liver biopsy for confirmation. There are no studies on NAFLD in specific PCOS phenotypes. However, it has been suggested that hyperandrogenic phenotypes display an increased risk of developing NAFLD in comparison to normoandrogenic PCOS phenotypes (58). Regarding treatment, moderate weight reduction of 5–10% was shown to reduce hepatic steatosis (34). Metformin might ameliorate the adverse metabolic profile and restore ovulation in women with PCOS (6, 66, 150), therefore, being the drug of choice in comparison to other insulin sensitizers used for treating PCOS patients with NAFLD. Clinical trials on the effects of metformin in lean women with PCOS are still lacking, though.

Declaration of interest

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

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