Endocrine (2016) 51:211–221
DOI 10.1007/s12020-015-0640-8

REVIEW

Evidence that non-alcoholic fatty liver disease and polycystic ovary syndrome are associated by necessity rather than chance: a novel hepato-ovarian axis?

Giovanni Targher[1] · Maurizio Rossini[2] · Amedeo Lonardo[3]

Received: 16 March 2015 / Accepted: 22 May 2015 / Published online: 30 May 2015

Abstract Increasing evidence suggests that non-alcoholic fatty liver disease (NAFLD) and polycystic ovary syndrome (PCOS) are associated with obesity, insulin resistance, metabolic syndrome, cardiovascular disease, cirrhosis, and liver tumors. On these grounds, we have hypothesized that NAFLD and PCOS occur more frequently than expected by chance alone. We have tested this hypothesis by reviewing the clinical and biological evidence that supports a significant association between NAFLD and PCOS. PubMed was extensively searched for articles published through March 2015 using the keywords "nonalcoholic fatty liver disease" or "fatty liver" combined with "PCOS." Several cross-sectional and case–control studies have consistently demonstrated that the prevalence of NAFLD is remarkably increased in young women with PCOS, independent of overweight/obesity and other coexisting metabolic syndrome features, and that these women are more likely to have the more severe forms of NAFLD (non-alcoholic steatohepatitis, advanced fibrosis, and cirrhosis). Accumulating evidence suggests that NAFLD, especially its necro-inflammatory form, may exacerbate hepatic and systemic insulin resistance and releases multiple pro-inflammatory, pro-coagulant, and pro-fibrogenic mediators that may play important roles in the pathophysiology of PCOS. These findings call for more active and systematic search for NAFLD among women with PCOS. Conversely, gastroenterologists/hepatologists need to be aware of the presence of PCOS among female patients with NAFLD and compatible clinical features. Finally, all these patients should undergo regular follow-up not only for liver-related complications but also for cardiometabolic diseases.

Keywords Non-alcoholic fatty liver disease · Polycystic ovary syndrome · PCOS · Metabolic syndrome · Review

✉ Giovanni Targher
giovanni.targher@univr.it

[1] Section of Endocrinology, Diabetes and Metabolism, Department of Medicine, University and Azienda Ospedaliera Universitaria Integrata of Verona, Piazzale Stefani, 1, 37126 Verona, Italy

[2] Section of Rheumatology, Department of Medicine, University and Azienda Ospedaliera Universitaria Integrata of Verona, Verona, Italy

[3] Outpatient Liver Clinic and Division of Internal Medicine – Department of Biomedical, Metabolic and Neural Sciences, NOCSAE, Baggiovara, Azienda USL, University of Modena and Reggio Emilia, Modena, Italy

Introduction

Non-alcoholic fatty liver disease (NAFLD) is a clinicopathologic entity spanning from simple steatosis to non-alcoholic steatohepatitis (NASH) with or without cirrhosis, which may progress to liver failure and, in some cases, to hepatocellular carcinoma [1, 2]. NAFLD is the most common cause of chronic liver disease worldwide and is strongly associated with features of the metabolic syndrome (MetS), hemostatic abnormalities, and increased cardiovascular risk [3–6]. In the past, NAFLD was deemed to be simply the "hepatic manifestation" of the MetS. However, it is now becoming clear that NAFLD is also a pathogenic determinant of the MetS. With regard to this evolving concept, there is a growing body of evidence strongly supporting the notion that NAFLD precedes the development of the MetS [2, 7]. This implies that the resolution of NAFLD will prevent the development of the MetS and its cardiovascular consequences. Moreover,

NAFLD occurs more frequently in men than in women and is associated with various endocrine and metabolic diseases [8–11], including polycystic ovary syndrome (PCOS).

PCOS, which affects up to 10 % of women of reproductive age, is the most common form of anovulatory infertility and is associated with ovarian cysts, hirsutism, MetS, and hemostatic abnormalities [12]. Of concern, the epidemiological studies that have suggested an increased risk of NAFLD, liver tumors, and cardio-metabolic diseases among young women with PCOS [13–15] raise the possibility that the association between PCOS and NAFLD is not likely to be due to chance alone because, in the absence of PCOS, young women are usually spared by the above-mentioned pathologic conditions, except for some types of benign liver tumors [8, 16–19].

In this review, we discuss the current clinical evidence that supports a significant association between NAFLD and PCOS and the putative biological mechanisms underlying this association. We also briefly discuss the epidemiology, diagnosis, and treatment of NAFLD.

Pertinent studies were identified by searching PubMed for original articles published in English through March 2015 using the keywords "NAFLD" or "fatty liver" combined with "polycystic ovary syndrome" or "PCOS." Articles published in languages other than English were excluded. We only included articles in which NAFLD was diagnosed by radiological imaging or histology.

Diagnosis, epidemiology, and treatment of NAFLD

NAFLD is histologically defined by the presence of ≥5 % hepatic steatosis. The characteristic histology of NAFLD resembles that of alcohol-induced liver injury but occurs in people who do not drink or consume only a small amount of alcohol (i.e., <20 g/day for women and <30 g/day for men, respectively) and who do not have other secondary causes of chronic liver disease (e.g., virus, autoimmunity, hemochromatosis, drugs) [1, 2].

NAFLD has reached epidemic proportions and is the most common cause of chronic liver disease in many parts of the world. NAFLD is predicted to become also the most frequent indication for liver transplantation within the next decade. Approximately, 20–30 % of adults in the general population in Western countries have NAFLD, and its prevalence increases to 70–90 % among persons who are obese or have diabetes. Major risk factors for the development and progression of NAFLD include older age, male sex, ethnicity, some genetic polymorphisms, MetS, obesity, and type 2 diabetes [1–3, 6, 7].

NAFLD is typically asymptomatic. Hepatomegaly may be the only physical finding in most patients. The presence of mildly to moderately elevated serum aminotransferases are the most common and often the only laboratory abnormality found in NAFLD patients. However, serum aminotransferase levels are in the normal range in most cases and thus are insensitive markers for the detection of NAFLD [2, 20].

At present, liver biopsy is the gold standard investigation for diagnosing and assessing the severity of disease progression in NAFLD. However, this is an invasive and not invariably safe procedure. Moreover, sampling variability and incomplete reproducibility in the interpretation of histological findings are major issues with liver biopsy [2, 20]. Ultrasonography remains the recommended first-line imaging modality for diagnosing hepatic steatosis in clinical practice. It provides a subjective and qualitative assessment of hepatic fat content, generally believed to be of limited sensitivity (60–90 %) if <30 % of hepatocytes are steatotic. By contrast, magnetic resonance imaging and proton magnetic resonance spectroscopy are the best available diagnostic techniques for assessing quantitatively the severity of liver fat accumulation. However, both techniques are resource intensive and cannot reliably discriminate simple steatosis from NASH [1, 2, 20]. Over the last decade, researchers have attempted to develop accurate and reproducible simple tests for diagnosing and monitoring NAFLD, in order to replace the need for liver biopsy or other imaging modalities for detecting liver fat. There are now a few histologically validated algorithmically derived hepatic fibrosis scores (e.g., the NAFLD fibrosis score and the Fib4 score) as well as the liver stiffness measurement by transient elastography for diagnosing hepatic fibrosis in NAFLD [1, 2, 20]. However, further work is needed to examine whether these non-invasive methods are able to replace liver biopsy for diagnosing and monitoring progression (or resolution) of the different stages of NAFLD.

Detailed discussion of the interventions that have been tested for NAFLD is beyond the scope of this review and have been discussed elsewhere [1–6, 20]. Presently, there is no licensed treatment for NAFLD. Current recommendations for NAFLD/NASH therapy are limited to weight reduction through diet and physical exercise (a 5–10 % weight loss reduces hepatic steatosis, whereas up to a 10 % weight loss is needed to improve hepatic necro-inflammation) and the treatment of individual components of the MetS, possibly with the use of therapies that may have potential beneficial liver effects, including bariatric surgery for severe obesity, insulin-sensitizing agents for type 2 diabetes (especially pioglitazone), and drugs directed at the renin–angiotensin–aldosterone system to control hypertension [1–6, 20]. Metformin has beneficial effects on serum aminotransferases and hepatic insulin resistance but has no effect on liver histology and is not recommended as a specific treatment for liver disease in patients with NAFLD/NASH. Pioglitazone is the most effective drug in patients with histologically proven NASH in the short term.

However, its long-term cardiovascular and non-cardiovascular adverse effects are a serious concern. Preliminary evidence suggests some benefit of vitamin E, obeticholic acid, high-dose omega-3 fatty acids, and other hepatoprotectants, but to date, there are insufficient data to advocate the use of any of these agents in patients with non-cirrhotic NAFLD [1–6, 20]. Future therapeutic research in NAFLD/NASH should focus on exploring innovative molecules that have better efficacy and a better safety profile.

Epidemiological evidence linking NAFLD to PCOS

PCOS is a common endocrine disorder of premenopausal women, characterized by chronic anovulation, polycystic ovaries, and hyperandrogenism, along with obesity and insulin resistance as frequent metabolic disorders [21].

Table 1 summarizes the principal cross-sectional and case–control studies that have assessed the relationship between PCOS and NAFLD [22–38]. In most of these studies, PCOS was diagnosed using the Rotterdam criteria, except for two studies, which used the National Institutes of Health criteria [22, 23].

Over a dozen studies showed that the prevalence of NAFLD is increased in young women with PCOS [22–28, 30–34, 36–38], independent of coexisting MetS features. Overall, the prevalence of NAFLD in women with PCOS ranged from approximately 35 to 70 % compared with approximately 20 to 30 % in healthy controls, who were matched for age, BMI, and waist circumference. Only two small case–control studies did not reveal any significant differences in NAFLD prevalence between PCOS women and healthy controls [29, 35].

Two studies performed at tertiary gastroenterology centres demonstrated that PCOS is also a very common condition among patients with biopsy-confirmed NAFLD [27, 30]. Among these patients, the prevalence of PCOS ranged from approximately 50 to 70 %, and these women were also more likely to develop the more severe forms of NAFLD. With regard to the relationship between PCOS and the severity of NAFLD histology, it is important to note that Setji et al. [22] were first in documenting a histological evidence of NASH with varying degrees of fibrosis among young women with PCOS in a retrospective chart review study. Some case reports [15, 39] have also revealed that PCOS (especially if associated with severe hyperandrogenism) is associated with an increased risk of inflammatory hepatocellular adenoma, which is a potential precursor of non-cirrhotic hepatocellular carcinoma [40].

As shown in Table 1, most published studies have clearly demonstrated that PCOS women with NAFLD had a greater insulin resistance and a higher prevalence of the MetS (and its individual traits) compared with their counterparts without NAFLD; however, no significant differences were found in circulating levels of total testosterone and dehydroepiandrosterone sulfate (DHEAS) between the two groups of patients. For example, Qu et al. [37] reported that PCOS women with NAFLD had higher values of BMI, waist/hip ratio, insulin resistance, and triglycerides and lower HDL-cholesterol levels than their counterparts without NAFLD. In contrast, the two groups of women did not differ significantly with respect to plasma total testosterone, DHEAS, and estradiol levels. Similarly, Cussons et al. [25] did not find any significant differences in circulating levels of total testosterone, sex hormone-binding globulin (SHBG) or free androgen index (FAI) between PCOS women with and without NAFLD. Other investigators reported similar results [32, 38]. However, although in most published studies, there were no significant differences in circulating total testosterone levels between PCOS women with and without NAFLD, uncertainty still remains regarding the circulating levels of FAI. Indeed, FAI levels in PCOS women with NAFLD were observed to be similar to or higher than those of PCOS women without NAFLD (Table 1). Interestingly, in a case–control study involving 29 young obese women with PCOS and 22 age-, BMI-, and waist circumference-matched controls, Jones et al. [33] subdivided PCOS women according to their FAI levels and found that hyperandrogenic PCOS women had markedly higher intrahepatic fat content on magnetic resonance spectroscopy compared to both normo-androgenic PCOS women and controls (mean intrahepatic fat content: 12.9 vs. 0.6 vs. 1.9 %, respectively). It is important to note that these wide intergroup differences in intrahepatic fat content remained significant even after adjustment for BMI, visceral fat, and insulin resistance [30]. Vassilatou et al. reported higher FAI and lower SHBG levels in PCOS women with NAFLD than in those without NAFLD [26]. This study also revealed that PCOS was associated with NAFLD, independent of age, BMI, waist/hip ratio, insulin resistance, SHBG, and FAI [26]. However, other investigators did not observe any significant differences in FAI or SHBG levels between PCOS women with and without NAFLD (25, 38). Future studies in larger cohorts of carefully characterized PCOS patients will be needed to elucidate this controversial issue.

Importantly, PCOS women also have an elevation in circulating inflammatory biomarkers that is independent of overweight/obesity and that may be further amplified by the coexistence of NAFLD [12, 25, 31, 41, 42]. In PCOS patients, this subclinical inflammation persists during gestation and is exacerbated by pregnancy, and it is associated with adverse pregnancy outcomes [43]. These findings corroborate existing molecular evidence of the subclinical inflammation that may be, at least in part, implicated in PCOS pathogenesis [41, 42].

Table 1 Principal epidemiological studies that have assessed the relationship between NAFLD (as detected by imaging or histology) and PCOS, ordered by publication year

Authors, publication year (reference)	Study and patient characteristics	Diagnosis of NAFLD	Main findings of the study
Setji et al. [22]	Retrospective chart review of 200 United States women with PCOS	Liver enzymes and/or biopsy	15 % of PCOS women had elevated serum aminotransferase levels (>60 IU/l). Six women (mean age 29 years) with persistent aminotransferase elevations underwent liver biopsy. All six patients had NASH with varying degrees of fibrosis
Gambarin-Gelwan et al. [23]	Retrospective study of 88 United States overweight premenopausal women with PCOS	Ultrasonography	Of the 88 women, 48 (55 %) had NAFLD; 15 (39 %) of them were lean women. Significant differences were found in BMI, insulin resistance, and HDL-cholesterol between PCOS women with and without NAFLD. Most women had normal serum liver enzymes
Cerda et al. [24]	Case–control study of 41 Chilean obese women with PCOS and 31 age-, BMI-, and waist circumference-matched control women	Ultrasonography	PCOS women had a greater prevalence of NAFLD than matched controls (42 vs. 19 %). Significant differences were found in BMI and insulin resistance between PCOS women with and without NAFLD. A total of 64 % of PCOS women with NAFLD had moderately elevated serum aminotransferases
Cussons et al. [25]	Cross-sectional study of 25 Australian consecutive young obese women with PCOS without known hepatic diseases	Magnetic resonance spectroscopy	NAFLD was present in 12 of 25 women (48 %). Significant differences were found in BMI, systolic blood pressure, triglycerides, fasting insulin, free fatty acids, insulin resistance and C-reactive protein but not in total testosterone, SHBG, and FAI between PCOS women with and without NAFLD
Vassilatou et al. [26]	Case–control study of 57 Greek young overweight women with PCOS and 60 age- and BMI-matched healthy control women	Ultrasonography	Women with PCOS had a greater prevalence of NAFLD than matched controls (37 vs. 20 %). Significant differences were found in BMI, waist/hip ratio, insulin resistance, triglycerides, HDL-cholesterol, FAI (increased), and SHBG (reduced) but not in total testosterone, DHEAS, and delta-4-androstenedione levels between PCOS women with and without NAFLD. In multivariate regression analysis, PCOS was independently associated with NAFLD even after adjustment for age, BMI, waist/hip ratio, HDL-cholesterol, HOMA-IR, SHBG, and FAI
Brzozowska et al. [27]	Cross-sectional study of 14 consecutive obese Australian women with a diagnosis of NAFLD and elevated serum aminotransferase levels, who were screened for PCOS	Ultrasonography or biopsy (in 50 % of cases)	PCOS was present in 10 of 14 women (71 %). Five PCOS patients with NAFLD had documented liver fibrosis on biopsy, indicative of more advanced liver disease. No significant differences were found in BMI and other metabolic syndrome features between NAFLD women with and without PCOS
Gutierrez-Grobe et al. [28]	Case–control study of 50 overweight Mexican women with PCOS, 90 pre-menopausal women, and 57 post-menopausal control women	Ultrasonography	Prevalence of NAFLD in premenopausal women, postmenopausal women, and patients with PCOS was 32, 58, and 62 %, respectively. PCOS women had higher values of age, BMI, waist/hip ratio, and insulin resistance and lower HDL-cholesterol and estradiol levels compared with other two groups
Markou et al. [29]	Case–control study of 17 young lean Greek women with PCOS and 17 age-, BMI-, and waist circumference-matched healthy control women	Ultrasonography and computed tomography	PCOS women had no evidence of NAFLD according to either ultrasonography or computed tomography
Hossain et al. [30]	Cross-sectional study of 66 young obese United States women with biopsy-proven NAFLD (33 % with NASH) who were screened for PCOS (tertiary gastroenterology center)	Biopsy	PCOS was present in 34 of 66 women (51 %). PCOS women had a higher prevalence of NASH (44 vs. 21 %). No significant differences were found in BMI, waist circumference, and metabolic features between NAFLD women with and without PCOS

Table 1 continued

Authors, publication year (reference)	Study and patient characteristics	Diagnosis of NAFLD	Main findings of the study
Ma et al. [31]	Cross-sectional study of 117 non-obese Chinese women with PCOS	Ultrasonography	NAFLD was present in 46 of 117 women (39 %). Significant differences were found in BMI, waist circumference, insulin resistance, metabolic syndrome features, white blood cell count, and SHBG (reduced) but not in total testosterone levels between PCOS women with and without NAFLD. Those with NAFLD also had increased carotid intima–media thicknesses
Gangale et al. [32]	Cross-sectional study of 140 non-diabetic overweight Italian women with PCOS	Ultrasonography	NAFLD was present in 81 of 140 women (58 %). Significant differences were found in insulin resistance, triglycerides and SHBG (reduced), and FAI (increased) but not in age, BMI, waist circumference, total testosterone, gonadotropins, DHEAS, androstenedione, 17-hydroxy-progesterone, estradiol, and prolactin between PCOS women with and without NAFLD. Most PCOS women had normal serum liver enzymes
Jones et al. [33]	Case–control study of 29 young obese UK women with PCOS and 22 age-, BMI-, and waist circumference-matched healthy control women. PCOS women were subsequently stratified into two groups according to FAI (hyper-androgenic vs. normo-androgenic women)	Magnetic resonance spectroscopy	PCOS women had a significantly higher intrahepatic fat content than controls (mean: 6.1 vs. 1.9 %). In a subgroup analysis, women with hyper-androgenic PCOS (i.e., FAI >7 %, $n = 19$) had a markedly higher hepatic fat content compared with both normo-androgenic PCOS women ($n = 10$) and healthy controls (mean hepatic fat content: 12.9 vs. 0.6 vs. 1.9 %, respectively), even after adjustment for BMI and insulin resistance
Zueff et al. [34]	Case–control study of 45 young obese Brazilian women with PCOS and 45 age-, BMI-, and waist circumference-matched healthy control women	Ultrasonography	PCOS women had a greater prevalence of NAFLD than matched control women (73 vs. 47 %)
Borruel et al. [35]	Case–control study of 55 overweight Spanish women with PCOS and 25 age- and BMI-matched healthy control women	Ultrasonography	Prevalence of NAFLD was not significantly higher in patients with PCOS than in matched control women (38 vs. 28 %)
Karoli et al. [36]	Case–control study of 54 overweight young Indian women with PCOS and 55 age- and BMI-matched healthy control women	Ultrasonography	PCOS women had a significantly higher prevalence of NAFLD than matched controls (67 vs. 25 %). Patients with PCOS and NAFLD had a higher waist/hip ratio, a higher insulin resistance, and lower HDL-cholesterol levels, but similar values of total testosterone and SHBG compared with their counterparts without NAFLD
Qu et al. [37]	Case–control study of 602 young lean Chinese women with PCOS and 588 control women who were comparable for age and BMI	Ultrasonography	PCOS women had a greater prevalence of NAFLD than matched controls (33 vs. 18 %). Significant differences were found in BMI, waist circumference, insulin resistance, triglycerides, and HDL-cholesterol but not in total testosterone, DHEAS, estradiol, and prolactin levels between PCOS women with and without NAFLD
Dawson et al. [38]	Cross-sectional study of 25 severely obese UK women with PCOS without known hepatic diseases	Ultrasonography	NAFLD was present in 13 of 25 women (52 %). Significant differences were found in BMI and waist circumference but not in insulin resistance, lipids, C-reactive protein, SHBG, total testosterone, and FAI between PCOS women with and without NAFLD

In most published studies, PCOS was diagnosed using the 2003 Rotterdam criteria, except for the studies by Setji et al. [22] and by Gambarin-Gelwan et al. [23], which used the 1990 National Institutes of Health criteria. In all studies, insulin resistance was estimated by homeostasis model assessment (HOMA-IR score), and FAI was calculated as the total testosterone concentration × 100/SHBG concentration

BMI body mass index, *DHEAS* dehydroepiandrosterone sulfate, *FAI* free androgen index, *SHBG* sex hormone-binding globulin

Collectively, the published studies indicate that the prevalence of NAFLD is remarkably high among young women with PCOS, independent of overweight/obesity and other MetS features. Accordingly, a recent meta-analysis, including seven case–control studies published through 2013, has confirmed that PCOS women exhibit an approximately fourfold increased rate of NAFLD compared to control women [44]. In addition, the young age of many PCOS women and the relatively advanced stage of NASH revealed by the biopsies from these patients clearly suggest the possibility of a significant risk for long-term liver-related complications in this group of patients. Therefore, the current body of evidence argues for more careful monitoring and evaluation of the presence of NAFLD in women with PCOS.

Putative mechanisms linking NAFLD to PCOS

The etiology of PCOS remains unknown. Similarly, to date, the underlying mechanisms linking NAFLD to PCOS are not fully understood. As schematically shown in Fig. 1, it is plausible to assume that the mechanisms underlying the association between NAFLD and PCOS are multifactorial, involve both genetic and acquired factors, and often overlap with metabolic disorders which commonly coexist in PCOS women, such as abdominal overweight/obesity and insulin resistance.

Role of genetics

In 2011, Azizz et al. were first in proposing that PCOS is an ancient disorder arising from ancestral gene variants selected during the Paleolithic period [45]. According to this hypothesis, PCOS has persisted throughout human evolution despite reduced fecundity because of the benefits it confers to affected women, such as greater sturdiness, improved energy utilization (a rearing advantage for their children and kin), and a reduction in the risk of perinatal mortality [45]. This hypothesis suggests that genetics may play an important role in the pathogenesis of PCOS and that ancestral gene variants eventually found to be associated with PCOS are similar across ethnicities. Brower et al. recently confirmed the existence of common susceptibility genes in different ethnicities demonstrating that at least four of the PCOS susceptibility loci (i.e., all functionally involved in androgen synthesis, insulin action, and secretion) identified in the Chinese genome-wide association studies are also associated with PCOS in European individuals [46]. Although functional studies are needed to improve our understanding of the role these genes play in PCOS pathogenesis, however, it is known that insulin resistance is a major player in the pathogenesis of NAFLD [1, 3–5, 47–49]. Further supporting a role of genetic factors in PCOS, a recent study has shown that maternal factors, namely genetic and perhaps epigenetic determinants, significantly contribute to the metabolic phenotype (mainly impaired fasting glycaemia) in affected women [50]. Similarly, subclinical inflammation observed in PCOS women may be influenced by genetic polymorphisms of interleukin (IL)-18, IL-1a, IL-1b, IL-6, and plasminogen activator inhibitor-1 [51].

Abdominal obesity

It is likely that the mechanisms linking NAFLD with PCOS may result from the expanded and inflamed (dysfunctional) visceral adipose tissue, with the liver being both a target of the resulting systemic abnormalities and a major source of several pathogenic mediators, e.g., pro-inflammatory, pro-oxidant, and pro-coagulant factors [3–5, 47, 52]. These factors may further amplify both the metabolic/hormonal/inflammatory derangements and the vascular damage, which are observed in patients with PCOS. In addition, it is well known that NAFLD plays a key role in the development of atherogenic dyslipidemia and hepatic insulin resistance [3–7, 47–49].

Molecular basis of hepatic insulin resistance

With the exception of cases where NAFLD results from either familial hypobetalipoproteinemia or patatin-like phospholipase domain-containing 3 (*PNPLA-3*) gene polymorphisms, in which NAFLD is usually dissociated from insulin resistance [48–54], NAFLD directly causes hepatic insulin resistance in most cases [3, 7, 47–49]. Molecular and genetic studies have recently supported the view that the pathogenic mechanisms contributing to intrahepatocytic lipid compartmentation are determinants of whether hepatic steatosis is or is not associated with insulin resistance and the MetS [7]. The proposed mechanisms by which NAFLD causes hepatic insulin resistance implicate various lipid species, inflammatory signaling, and other cellular and subcellular changes. Experimental studies have elucidated a key role for hepatic diacylglycerol activation of protein kinase Cε (PKCε) in triggering hepatic insulin resistance [55]. Dysregulation of intrahepatic lipid droplet metabolism may influence the intracellular compartmentation of diacylglycerol, which dictates whether or not PKCε will translocate to the plasma membrane so promoting lipotoxicity and hepatic insulin resistance [55]. Irrespective of overweight/obesity, insulin resistance plays a central role in the development of characteristic features of PCOS in susceptible individuals. Indeed, in PCOS women, insulin resistance is closely associated with a prothrombotic state, subclinical inflammation, atherogenic dyslipidemia, hyperandrogenemia, and chronic anovulation [12, 41, 42, 56–59].

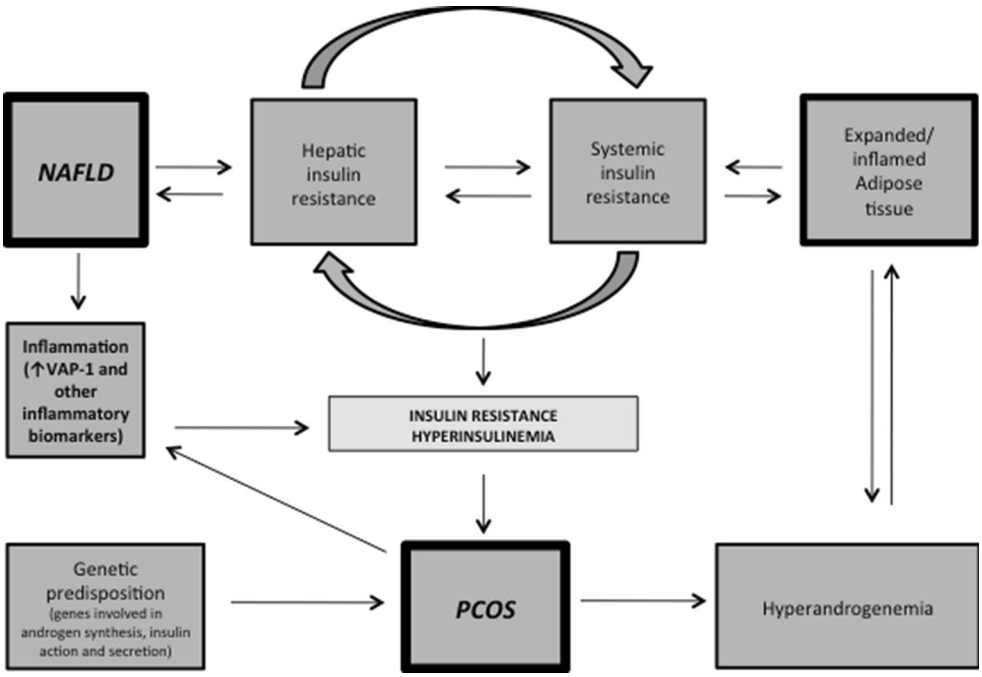

Fig. 1 Schematic representation of the putative pathogenic mechanisms shared by NAFLD and PCOS

Hyperandrogenemia and insulin resistance

Linking metabolism with subclinical inflammation and endocrine imbalances associated with PCOS, insulin resistance may also exacerbate the hyperandrogenic state seen in PCOS by increasing ovarian androgen production and decreasing hepatic SHBG synthesis [59]. In its turn, hyperandrogenemia may induce a low-grade inflammatory state [60] and contribute to the development of the MetS phenotype in PCOS women [61] by stimulating the maturation of adipocytes, thus promoting the development of abdominal obesity [51]. In a multi-ethnic cohort study of 5734 adult individuals, Lazo et al. recently reported that post-menopausal women who were in the highest tertile of bioavailable testosterone were more likely to have NAFLD on ultrasound than women in the lowest tertile after adjusting for age, race/ethnicity, smoking, waist-to-hip ratio, hypertension, plasma lipids, insulin resistance, and hormone replacement therapy use [62]. Further research is needed to elucidate the molecular mechanisms leading from hyperandrogenemia to the development and progression of NAFLD.

Subclinical inflammation

As reported previously, it is well established that both PCOS and NAFLD are closely associated with a low-grade inflammatory state [3–7, 41, 42]. It is also known that the circulating levels of adiponectin, a cytokine with anti-inflammatory and anti-atherogenic properties, are typically reduced in NAFLD patients [63–65]. Francque et al. have recently shown that serum adiponectin levels are positively associated with liver peroxisome proliferator-activated receptor (PPAR)-α gene expression, which, in its turn, is negatively associated with the presence and severity of NASH [66]. Interestingly, lower levels of high-molecular weight adiponectin have been recently reported to be associated with hyperandrogenemia and insulin resistance in women with PCOS [67]. However, a small-sized trial found that treatments with metformin or pioglitazione did not significantly affect pre-treatment adiponectin levels [68].

Research agenda

Collectively, the findings discussed above suggest that PCOS and NAFLD share common pathogenic mechanisms and are part of a complex and intriguing network of genetic, clinical, and pathophysiological features (i.e., endocrine, metabolic, and inflammatory changes) (as summarized in schematic Fig. 1).

Although the liver is a master regulator of glucose and lipid metabolism and is the primary source of several coagulation and inflammatory factors, the close interconnections of PCOS and NAFLD with abdominal adiposity and insulin resistance make it extremely difficult to dissect the specific contribution of NAFLD *per se* to the hormonal, metabolic, hemostatic, and inflammatory manifestations of PCOS [3–5, 11]. It is, therefore, reasonable to speculate that the research advances achieved for either disease may

be fruitfully applied to the other one. For instance, the molecular basis underlying the subclinical inflammation in NAFLD is increasingly elucidated. The adhesion molecule vascular adhesion protein-1 (VAP-1) is a membrane-bound amine oxidase that promotes leukocyte recruitment to the liver, and its soluble form exerts insulin-like effects and may initiate oxidative stress. A recent study has elegantly shown that hepatic VAP-1 expression is increased in patients with chronic liver disease and that serum VAP-1 levels are elevated in patients with NAFLD compared to those in control individuals. In murine hepatic injury models, the absence or blockade of functional VAP-1 reduced inflammatory cell recruitment to the liver and attenuated hepatic fibrosis [69]. Moreover, liver disease was reduced in animals expressing a catalytically inactive form of VAP-1, implicating enzyme activity in disease pathogenesis [69]. Although VAP-1 was not directly measured, a recent case–control study reported that the levels of messenger RNAs for intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 were significantly higher in lean PCOS women than in age-matched controls and were associated with insulin resistance, independent of body weight [70].

Another promising area of NAFLD and PCOS research is mechanistic studies exploring the increased cardiovascular risk observed in patients with either disease. Recent data have shown that cholesterol efflux capacity, a new biomarker that characterizes a key step in reverse cholesterol transport, is inversely associated with the risk of future cardiovascular events in a large population-based cohort of adults [71]. Interestingly, a recent study has reported that PCOS women have a decreased cholesterol efflux capacity and reduced atherogenic lipid particle number and size, independently of body weight [72]. Similar evaluations among NAFLD patients will be needed in the near future, because these patients, like PCOS women, are at high risk for cardiovascular disease [3–6, 47, 52, 73].

Finally, the prevalence and significance of two important drivers/correlates of NAFLD, namely gallstones [74, 75] and altered gut microbiota [76], appear to have been insufficiently investigated in PCOS women and are a promising research area. Ruhl et al. investigated the relationship of gallstones and cholecystectomy with NAFLD among participants of the Third National Health and Nutrition Examination Survey, a national, population-based cohort study [77]. These investigators found that NAFLD was independently associated with cholecystectomy, but not with gallstones, after adjusting for several potential confounders, thus suggesting that cholecystectomy itself may be a novel risk factor for NAFLD. To date, a PubMed search (assessed on March, 7th, 2015) using the key words "cholecystectomy and PCOS" did not find any results.

In carefully reviewing available evidence, Machado et al. have concluded that NAFLD, obesity, and high fat diet are all associated with a specific gut microbiota, which is particularly enriched in firmicutes and is deemed to be a noxious agent to the liver via small intestinal bacterial overgrowth, increased intestinal permeability and increased endotoxemia and activation of the toll-like receptor-4 signaling cascade [78]. These pathophysiological mechanisms are of potential interest in that manipulation of gut microbiota with probiotics might offer the promise as a potential treatment for NAFLD. However, a PubMed search using the key words "PCOS and intestinal dysbiosis" found only a review article [79], but otherwise retrieved no original studies.

Finally, the anti-mullerian hormone, which has been advocated as a potentially useful adjunct both in the diagnosis of PCOS and in the evaluation of disease severity [80, 81], has never been investigated among patients with NAFLD.

Conclusions

PCOS represents a unique model of a disease exposing young women to an increased risk of developing serious cardio-metabolic and hepatic diseases. Although the current clinical evidence is restricted to cross-sectional and case-control studies, the published data fully support a significant association between NAFLD and PCOS. Notably, in most published studies, this association appears to be independent of coexisting MetS features. In addition, given the young age at which NAFLD may occur in PCOS, these women are at significant risk for progressive hepatic injury over the course of their lives. However, further studies are needed to better clarify the association between NAFLD and PCOS and the putative biological mechanisms underlying this association. Specific soluble mediators of this novel 'hepato-ovarian axis' need to be further elucidated in order to discover innovative drugs and treatments.

In the interim, clinicians should be aware of the link between NAFLD and PCOS and consider screening for NAFLD in PCOS patients who have other metabolic risk factors. The optimal method of screening is presently unknown. However, given the limitations of serum liver enzymes for the screening of NAFLD [1, 2, 20], we believe that liver ultrasonography and transient elastography combined with the use of non-invasive fibrosis scoring systems (e.g., NAFLD fibrosis score) may be useful as first-line options in identifying both patients with NAFLD and those with suspected NASH to submit to liver biopsy among those with PCOS [1, 47, 82–84]. Conversely, a high index of suspicion may help in identifying PCOS cases among female patients with documented NAFLD, who

exhibit compatible clinical features. Finally, all these patients should undergo regular follow-up not only for liver-related complications but also for cardio-metabolic diseases [1–4, 6, 52, 73]. There are no proven treatments for NAFLD to date, but a personalized pathogenesis-based approach to treatment should be considered for this multifactorial disease. More research is needed to determine the best approach to management of NAFLD in women with PCOS.

Conflict of interest Nothing to declare.

References

1. F. Nascimbeni, R. Pais, S. Bellentani, C.P. Day, V. Ratziu, P. Loria, A. Lonardo, From NAFLD in clinical practice to answers from guidelines. J. Hepatol. **59**, 859–871 (2013)
2. N. Chalasani, Z. Younossi, J.E. Lavine, A.M. Diehl, E.M. Brunt, K. Cusi, M. Charlton, A.J. Sanyal, American Association for the Study of Liver Diseases; American College of Gastroenterology; American Gastroenterological Association. The diagnosis and management of non-alcoholic fatty liver disease: practice guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. Am. J. Gastroenterol. **107**, 811–826 (2012)
3. Q.M. Anstee, G. Targher, C.P. Day, Progression of NAFLD to diabetes mellitus, cardiovascular disease or cirrhosis. Nat. Rev. Gastroenterol. Hepatol. **10**, 330–344 (2013)
4. G. Targher, C.P. Day, E. Bonora, Risk of cardiovascular disease in patients with nonalcoholic fatty liver disease. N. Engl. J. Med. **363**, 1341–1350 (2010)
5. G. Targher, C.D. Byrne, Diagnosis and management of nonalcoholic fatty liver disease and its hemostatic/thrombotic and vascular complications. Semin. Thromb. Hemost. **39**, 214–228 (2013)
6. A. Lonardo, S. Ballestri, G. Targher, P. Loria, Diagnosis and management of cardiovascular risk in nonalcoholic fatty liver disease. Expert Rev. Gastroenterol. Hepatol. **9**, 629–650 (2015)
7. A. Lonardo, S. Ballestri, G. Marchesini, P. Angulo, P. Loria, NAFLD a precursor of the metabolic syndrome. Dig. Liver Dis. **47**, 181–190 (2015)
8. A. Lonardo, C. Carani, N. Carulli, P. Loria, 'Endocrine NAFLD'a hormonocentric perspective of nonalcoholic fatty liver disease pathogenesis. J. Hepatol. **44**, 1196–1207 (2006)
9. Y. Yilmaz, Z.M. Younossi, Obesity-associated nonalcoholic fatty liver disease. Clin. Liver Dis. **18**, 19–31 (2014)
10. M. Machado, P. Marques-Vidal, H. Cortez-Pinto, Hepatic histology in obese patients undergoing bariatric surgery. J. Hepatol. **45**, 600–606 (2006)
11. P. Loria, L. Carulli, M. Bertolotti, A. Lonardo, Endocrine and liver interaction: the role of endocrine pathways in NASH. Nat. Rev. Gastroenterol. Hepatol. **6**, 236–247 (2009)
12. G. Targher, G. Zoppini, E. Bonora, P. Moghetti, Hemostatic and fibrinolytic abnormalities in polycystic ovary syndrome. Semin. Thromb. Hemost. **40**, 600–618 (2014)
13. C.E. Kelley, A.J. Brown, A.M. Diehl, T.L. Setji, Review of nonalcoholic fatty liver disease in women with polycystic ovary syndrome. World J. Gastroenterol. **20**, 14172–14184 (2014)
14. G. Daskalopoulos, A. Karkanaki, A. Piouka, N. Prapas, D. Panidis, P. Gkeleris, V.G. Athyros, Excess metabolic and cardiovascular risk is not manifested in all phenotypes of polycystic ovary syndrome: implications for diagnosis and treatment. Curr. Vasc. Pharmacol **14**(1), 109–119 (2015)
15. F. Nascimbeni, S. Ballestri, L. Di Tommaso, M. Piccoli, A. Lonardo, Inflammatory hepatocellular adenomatosis, metabolic syndrome, polycystic ovary syndrome and non-alcoholic steatohepatitis: chance tetrad or association by necessity? Dig. Liver Dis. **46**, 288–289 (2014)
16. J.D. Yang, M.F. Abdelmalek, H. Pang, C.D. Guy, A.D. Smith, A.M. Diehl, A. Suzuki, Gender and menopause impact severity of fibrosis among patients with nonalcoholic steatohepatitis. Hepatology **59**, 1406–1414 (2014)
17. H. Tomiyama, A. Yamashina, T. Arai, K. Hirose, Y. Koji, T. Chikamori, S. Hori, Y. Yamamoto, N. Doba, S. Hinohara, Influences of age and gender on results of noninvasive brachial-ankle pulse wave velocity measurement: a survey of 12517 subjects. Atherosclerosis **166**, 303–309 (2003)
18. D. Roulot, J.L. Costes, J.F. Buyck, U. Warzocha, N. Gambier, S. Czernichow, H. Le Clesiau, M. Beaugrand, Transient elastography as a screening tool for liver fibrosis and cirrhosis in a community-based population aged over 45 years. Gut **60**, 977–984 (2011)
19. C. Bunchorntavakul, R. Bahirwani, D. Drazek, M.C. Soulen, E.S. Siegelman, E.E. Furth, K. Olthoff, A. Shaked, K.R. Reddy, Clinical features and natural history of hepatocellular adenomas: the impact of obesity. Aliment Pharmacol. Ther. **34**, 664–674 (2011)
20. P. Loria, L.E. Adinolfi, S. Bellentani, E. Bugianesi, A. Grieco, S. Fargion, A. Gasbarrini, C. Loguercio, A. Lonardo, G. Marchesini, F. Marra, M. Persico, D. Prati, Baroni GS; NAFLD Expert Committee of the Associazione Italiana per lo Studio del Fegato. Practice guidelines for the diagnosis and management of nonalcoholic fatty liver disease. A decalogue from the Italian Association for the Study of the Liver (AISF) Expert Committee. Dig. Liver Dis. **42**, 272–282 (2010)
21. R.S. Legro, S.A. Arslanian, D.A. Ehrmann, K.M. Hoeger, M.H. Murad, R. Pasquali, C.K. Welt, Endocrine Society, Diagnosis and treatment of polycystic ovary syndrome: an Endocrine Society clinical practice guideline. J. Clin. Endocrinol. Metab. **98**, 4565–4592 (2013)
22. T.L. Setji, N.D. Holland, L.L. Sanders, K.C. Pereira, A.M. Diehl, A.J. Brown, Nonalcoholic steatohepatitis and nonalcoholic fatty liver disease in young women with polycystic ovary syndrome. J. Clin. Endocrinol. Metab. **91**, 1741–1747 (2006)
23. M. Gambarin-Gelwan, S.V. Kinkhabwala, T.D. Schiano, C. Bodian, H.C. Yeh, W. Futterweit, Prevalence of nonalcoholic fatty liver disease in women with polycystic ovary syndrome. Clin. Gastroenterol. Hepatol. **5**, 496–501 (2007)
24. C. Cerda, R.M. Pérez-Ayuso, A. Riquelme, A. Soza, P. Villaseca, T. Sir-Petermann, M. Espinoza, M. Pizarro, N. Solis, J.F. Miquel, M. Arrese, Nonalcoholic fatty liver disease in women with polycystic ovary syndrome. J. Hepatol. **47**, 412–417 (2007)
25. A.J. Cussons, G.F. Watts, T.A. Mori, B.G. Stuckey, Omega-3 fatty acid supplementation decreases liver fat content in polycystic ovary syndrome: a randomized controlled trial employing proton magnetic resonance spectroscopy. J. Clin. Endocrinol. Metab. **94**, 3842–3848 (2009)
26. E. Vassilatou, S. Lafoyianni, A. Vryonidou, D. Ioannidis, L. Kosma, K. Katsoulis, E. Papavassiliou, I. Tzavara, Increased androgen bioavailability is associated with non-alcoholic fatty liver disease in women with polycystic ovary syndrome. Hum. Reprod. **25**, 212–220 (2010)
27. M.M. Brzozowska, G. Ostapowicz, M.D. Weltman, An association between non-alcoholic fatty liver disease and polycystic ovarian syndrome. J. Gastroenterol. Hepatol. **24**, 243–247 (2009)
28. Y. Gutierrez-Grobe, G. Ponciano-Rodríguez, M.H. Ramos, M. Uribe, N. Méndez-Sánchez, Prevalence of nonalcoholic fatty

liver disease in premenopausal, postmenopausal and polycystic ovary syndrome women: the role of estrogens. Ann. Hepatol. **9**, 402–409 (2010)
29. A. Markou, I.I. Androulakis, C. Mourmouris, A. Tsikkini, C. Samara, S. Sougioultzis, G. Piaditis, G. Kaltsas, Hepatic steatosis in young lean insulin resistant women with polycystic ovary syndrome. Fertil. Steril. **93**, 1220–1226 (2010)
30. N. Hossain, M. Stepanova, A. Afendy, F. Nader, Y. Younossi, N. Rafiq, Z. Goodman, Z.M. Younossi, Non-alcoholic steatohepatitis (NASH) in patients with polycystic ovarian syndrome (PCOS). Scand. J. Gastroenterol. **46**, 479–484 (2011)
31. R.C. Ma, K.H. Liu, P.M. Lam, L.P. Cheung, W.H. Tam, G.T. Ko, M.H. Chan, C.S. Ho, C.W. Lam, W.C. Chu, P.C. Tong, W.Y. So, J.C. Chan, C.C. Chow, Sonographic measurement of mesenteric fat predicts presence of fatty liver among subjects with polycystic ovary syndrome. J. Clin. Endocrinol. Metab. **96**, 799–807 (2011)
32. M.F. Gangale, L. Miele, A. Lanzone, F. Sagnella, D. Martinez, A. Tropea, F. Moro, A. Morciano, A. Ciardulli, C. Palla, M. Pompili, C. Cefalo, A. Grieco, R. Apa, Long-term metformin treatment is able to reduce the prevalence of metabolic syndrome and its hepatic involvement in young hyper insulinaemic overweight patients with polycystic ovarian syndrome. Clin. Endocrinol. (Oxf). **75**, 520–527 (2011)
33. H. Jones, V.S. Sprung, C.J. Pugh, C. Daousi, A. Irwin, N. Aziz, V.L. Adams, E.L. Thomas, J.D. Bell, G.J. Kemp, D.J. Cuthbertson, Polycystic ovary syndrome with hyperandrogenism is characterized by an increased risk of hepatic steatosis compared to nonhyperandrogenic PCOS phenotypes and healthy controls, independent of obesity and insulin resistance. J. Clin. Endocrinol. Metab. **97**, 3709–3716 (2012)
34. L.F. Zueff, W.P. Martins, C.S. Vieira, R.A. Ferriani, Ultrasonographic and laboratory markers of metabolic and cardiovascular disease risk in obese women with polycystic ovary syndrome. Ultrasound Obstet. Gynecol. **39**, 341–347 (2012)
35. S. Borruel, E. Fernández-Durán, M. Alpañés, D. Martí, F. Alvarez-Blasco, M. Luque-Ramírez, H.F. Escobar-Morreale, Global adiposity and thickness of intraperitoneal and mesenteric adipose tissue depots are increased in women with polycystic ovary syndrome (PCOS). J. Clin. Endocrinol. Metab. **98**, 1254–1263 (2013)
36. R. Karoli, J. Fatima, A. Chandra, U. Gupta, F.U. Islam, G. Singh, Prevalence of hepatic steatosis in women with polycystic ovary syndrome. J. Hum. Reprod. Sci. **6**, 9–14 (2013)
37. Z. Qu, Y. Zhu, J. Jiang, Y. Shi, Z. Chen, The clinical characteristics and etiological study of nonalcoholic fatty liver disease in Chinese women with PCOS. Iran J. Reprod. Med. **11**, 725–732 (2013)
38. A.J. Dawson, T. Sathyapalan, J.A. Smithson, R.V. Vince, A.M. Coady, R. Ajjan, E.S. Kilpatrick, S.L. Atkin, A comparison of cardiovascular risk indices in patients with polycystic ovary syndrome with and without coexisting nonalcoholic fatty liver disease. Clin. Endocrinol. (Oxf). **80**, 843–849 (2014)
39. C. Toso, L. Rubbia-Brandt, F. Negro, P. Morel, G. Mentha, Hepatocellular adenoma and polycystic ovary syndrome. Liver Int. **23**, 35–37 (2003)
40. T.C. Liu, N. Vachharajani, W.C. Chapman, E.M. Brunt, Noncirrhotic hepatocellular carcinoma: derivation from hepatocellular adenoma? Clinico-pathologic analysis. Mod. Pathol. **27**, 420–432 (2014)
41. H.F. Escobar-Morreale, M. Luque-Ramírez, F. González, Circulating inflammatory markers in polycystic ovary syndrome: a systematic review and meta-analysis. Fertil. Steril. **95**, 1048–1058 (2011)
42. F. González, Inflammation in polycystic ovary syndrome: underpinning of insulin resistance and ovarian dysfunction. Steroids **77**, 300–305 (2012)
43. S. Palomba, A. Falbo, G. Chiossi, F. Orio, A. Tolino, A. Colao, G.B. La Sala, F. Zullo, Low-grade chronic inflammation in pregnant women with polycystic ovary syndrome: a prospective controlled clinical study. J. Clin. Endocrinol. Metab. **99**, 2942–2951 (2014)
44. M. Ramezani-Binabaj, M. Motalebi, H. Karimi-sari, M.S. Rezaee-Zavareh, S.M. Alavian, Are women with polycystic ovarian syndrome at a high risk of nonalcoholic fatty liver disease? A meta-analysis. Hepat. Mon. **14**, e23235 (2014)
45. R. Azziz, D.A. Dumesic, M.O. Goodarzi, Polycystic ovary syndrome: an ancient disorder? Fertil. Steril. **95**, 1544–1548 (2011)
46. M.A. Brower, M.R. Jones, J.I. Rotter, R.M. Krauss, R.S. Legro, R. Azziz, M.O. Goodarzi, Further investigation in Europeans of susceptibility variants for polycystic ovary syndrome discovered in genome-wide association studies of Chinese individuals. J. Clin. Endocrinol. Metab. **100**, E182–E186 (2015)
47. G. Targher, C.D. Byrne, Clinical review: nonalcoholic fatty liver disease: a novel cardiometabolic risk factor for type 2 diabetes and its complications. J. Clin. Endocrinol. Metab. **98**, 483–495 (2013)
48. A. Lonardo, S. Lombardini, F. Scaglioni, L. Carulli, M. Ricchi, D. Ganazzi, L.E. Adinolfi, G. Ruggiero, N. Carulli, P. Loria, Hepatic steatosis and insulin resistance: does etiology make a difference? J. Hepatol. **44**, 190–196 (2006)
49. A.L. Birkenfeld, G.I. Shulman, Nonalcoholic fatty liver disease, hepatic insulin resistance, and type 2 diabetes. Hepatology **59**, 713–723 (2014)
50. K. Kobaly, P. Vellanki, R.K. Sisk, L. Armstrong, J.Y. Lee, J. Lee, M.G. Hayes, M. Urbanek, R.S. Legro, A. Dunaif, Parent-of-origin effects on glucose homeostasis in polycystic ovary syndrome. J. Clin. Endocrinol. Metab. **99**, 2961–2966 (2014)
51. E. Deligeoroglou, N. Vrachnis, N. Athanasopoulos, Z. Iliodromiti, S. Sifakis, S. Iliodromiti, C. Siristatidis, G. Creatsas, Mediators of chronic inflammation in polycystic ovarian syndrome. Gynecol. Endocrinol. **28**, 974–978 (2012)
52. M.J. Armstrong, L.A. Adams, A. Canbay, W.K. Syn, Extrahepatic complications of nonalcoholic fatty liver disease. Hepatology **59**, 1174–1197 (2014)
53. K. Kantartzis, A. Peter, F. Machicao, J. Machann, S. Wagner, I. Königsrainer, A. Königsrainer, F. Schick, A. Fritsche, H.U. Häring, N. Stefan, Dissociation between fatty liver and insulin resistance in humans carrying a variant of the patatin-like phospholipase 3 gene. Diabetes **58**, 2616–2623 (2009)
54. A. Amaro, E. Fabbrini, M. Kars, P. Yue, K. Schechtman, G. Schonfeld, S. Klein, Dissociation between intrahepatic triglyceride content and insulin resistance in familial hypobetalipoproteinemia. Gastroenterology **139**, 149–153 (2010)
55. R.J. Perry, V.T. Samuel, K.F. Petersen, G.I. Shulman, The role of hepatic lipids in hepatic insulin resistance and type 2 diabetes. Nature **510**, 84–91 (2014)
56. F. Tosi, R. Dorizzi, R. Castello, C. Maffeis, G. Spiazzi, G. Zoppini, M. Muggeo, P. Moghetti, Body fat and insulin resistance independently predict increased serum C-reactive protein in hyperandrogenic women with polycystic ovary syndrome. Eur. J. Endocrinol. **161**, 737–745 (2009)
57. F. Tosi, D. Di Sarra, J.M. Kaufman, C. Bonin, R. Moretta, E. Bonora, E. Zanolin, P. Moghetti, Total body fat and central fat mass independently predict insulin resistance but not hyperandrogenemia in women with polycystic ovary syndrome. J. Clin. Endocrinol. Metab. **100**, 661–669 (2015)
58. J.J. Kim, Y.M. Choi, Dyslipidemia in women with polycystic ovary syndrome. Obstet. Gynecol. Sci. **56**, 137–142 (2013)
59. R.L. Rosenfield, Clinical review: adolescent anovulation: maturational mechanisms and implications. J. Clin. Endocrinol. Metab. **98**, 3572–3583 (2013)
60. F. González, C.L. Sia, D.M. Bearson, H.E. Blair, Hyperandrogenism induces a proinflammatory TNFα response to glucose

ingestion in a receptor-dependent fashion. J. Clin. Endocrinol. Metab. **99**, E848–E854 (2014)
61. M.W. O'Reilly, A.E. Taylor, N.J. Crabtree, B.A. Hughes, F. Capper, R.K. Crowley, P.M. Stewart, J.W. Tomlinson, W. Arlt, Hyperandrogenemia predicts metabolic phenotype in polycystic ovary syndrome: the utility of serum androstenedione. J. Clin. Endocrinol. Metab. **99**, 1027–1036 (2014)
62. M. Lazo, I. Zeb, K. Nasir, R.P. Tracy, M.J. Budoff, P. Ouyang, D. Vaidya, Association between endogenous sex hormones and liver fat in a multi-ethnic study of atherosclerosis. Clin. Gastroenterol. Hepatol. (2015). doi:10.1016/j.cgh.2014.12.033
63. G. Targher, L. Bertolini, L. Zenari, Hypoadiponectinemia is closely associated with nonalcoholic hepatic steatosis in obese subjects. Diabetes Care **27**, 2085–2086 (2004)
64. G. Targher, L. Bertolini, L. Scala, F. Poli, L. Zenari, G. Falezza, Decreased plasma adiponectin concentrations are closely associated with nonalcoholic hepatic steatosis in obese individuals. Clin. Endocrinol. (Oxf). **61**, 700–703 (2004)
65. G. Targher, L. Bertolini, S. Rodella, G. Zoppini, L. Scala, L. Zenari, G. Falezza, Associations between plasma adiponectin concentrations and liver histology in patients with nonalcoholic fatty liver disease. Clin. Endocrinol. (Oxf). **64**, 679–683 (2006)
66. S. Francque, A. Verrijken, S. Caron, J. Prawitt, R. Paumelle, B. Derudas, P. Lefebvre, M.R. Taskinen, W. Van Hul, I. Mertens, G. Hubens, E. Van Marck, P. Michielsen, L. Van Gaal, B. Staels, PPARα gene expression correlates with severity and histological treatment response in patients with non-alcoholic steatohepatitis. J. Hepatol. (2015). doi:10.1016/j.jhep.2015.02.019
67. S. Cankaya, B. Demir, S.E. Aksakal, B. Dilbaz, C. Demirtas, U. Goktolga, Insulin resistance and its relationship with high molecular weight adiponectin in adolescents with polycystic ovary syndrome and a maternal history of polycystic ovary syndrome. Fertil. Steril. **102**, 826–830 (2014)
68. T.P. Ciaraldi, V. Aroda, S.R. Mudaliar, R.R. Henry, Inflammatory cytokines and chemokines, skeletal muscle and polycystic ovary syndrome: effects of pioglitazone and metformin treatment. Metabolism **62**, 1587–1596 (2013)
69. C.J. Weston, E.L. Shepherd, L.C. Claridge, P. Rantakari, S.M. Curbishley, J.W. Tomlinson, S.G. Hubscher, G.M. Reynolds, K. Aalto, Q.M. Anstee, S. Jalkanen, M. Salmi, D.J. Smith, C.P. Day, D.H. Adams, Vascular adhesion protein-1 promotes liver inflammation and drives hepatic fibrosis. J. Clin. Invest. **125**, 501–520 (2015)
70. K.M. Seow, C.C. Juan, P.H. Wang, L.T. Ho, J.L. Hwang, Expression levels of vascular cell adhesion molecule-1 in young and nonobese women with polycystic ovary syndrome. Gynecol. Obstet. Invest. **73**, 236–241 (2012)
71. A. Rohatgi, A. Khera, J.D. Berry, E.G. Givens, C.R. Ayers, K.E. Wedin, I.J. Neeland, I.S. Yuhanna, D.R. Rader, J.A. de Lemos, P.W. Shaul, HDL cholesterol efflux capacity and incident cardiovascular events. N. Engl. J. Med. **371**, 2383–2393 (2014)
72. A. Roe, J. Hillman, S. Butts, M. Smith, D. Rader, M. Playford, N.N. Mehta, A. Dokras, Decreased cholesterol efflux capacity and atherogenic lipid profile in young women with PCOS. J. Clin. Endocrinol. Metab. **99**, E841–E847 (2014)
73. S. Ballestri, A. Lonardo, S. Bonapace, C.D. Byrne, P. Loria, G. Targher, Risk of cardiovascular, cardiac and arrhythmic complications in patients with non-alcoholic fatty liver disease. World J. Gastroenterol. **20**, 1724–1745 (2014)
74. A. Lonardo, S. Lombardini, F. Scaglioni, S. Ballestri, A.M. Verrone, M. Bertolotti, L. Carulli, D. Ganazzi, N. Carulli, P. Loria, Fatty liver, carotid disease and gallstones: a study of age-related associations. World J. Gastroenterol. **12**, 5826–5833 (2006)
75. A.L. Fracanzani, L. Valenti, M. Russello, L. Miele, C. Bertelli, A. Bellia, C. Masetti, C. Cefalo, A. Grieco, G. Marchesini, S. Fargion, Gallstone disease is associated with more severe liver damage in patients with non-alcoholic fatty liver disease. PLoS One **7**, e41183 (2012)
76. A.R. Moschen, S. Kaser, H. Tilg, Non-alcoholic steatohepatitis: a microbiota-driven disease. Trends Endocrinol. Metab. **24**, 537–545 (2013)
77. C.E. Ruhl, J.E. Everhart, Relationship of non-alcoholic fatty liver disease with cholecystectomy in the US population. Am. J. Gastroenterol. **108**, 952–958 (2013)
78. M.V. Machado, H. Cortez-Pinto, Gut microbiota and nonalcoholic fatty liver disease. Ann. Hepatol. **11**, 440–449 (2012)
79. K. Tremellen, K. Pearce, Dysbiosis of Gut Microbiota (DOGMA): a novel theory for the development of polycystic ovarian syndrome. Med. Hypotheses **79**, 104–112 (2012)
80. A.B. Sopher, G. Grigoriev, D. Laura, T. Cameo, J.P. Lerner, R.J. Chang, D.J. McMahon, S.E. Oberfield, Anti-Mullerian hormone may be a useful adjunct in the diagnosis of polycystic ovary syndrome in nonobese adolescents. J. Pediatr. Endocrinol. Metab. **27**, 1175–1179 (2014)
81. A. Köninger, L. Koch, P. Edimiris, A. Enekwe, J. Nagarajah, S. Kasimir-Bauer, R. Kimmig, T. Strowitzki, B. Schmidt, Anti-Mullerian hormone: an indicator for the severity of polycystic ovarian syndrome. Arch. Gynecol. Obstet. **290**, 1023–1030 (2014)
82. S. Ballestri, A. Lonardo, D. Romagnoli, L. Carulli, L. Losi, C.P. Day, P. Loria, Ultrasonographic fatty liver indicator, a novel score which rules out NASH and is correlated with metabolic parameters in NAFLD. Liver Int. **32**, 1242–1252 (2012)
83. L. Castera, V. Vilgrain, P. Angulo, Noninvasive evaluation of NAFLD. Nat. Rev. Gastroenterol. Hepatol. **10**, 666–675 (2013)
84. S. Ballestri, D. Romagnoli, F. Nascimbeni, G. Francica, A. Lonardo, Role of ultrasound in the diagnosis and treatment of NAFLD and its complications. Expert Rev. Gastroenterol. Hepatol. **19**, 1–25 (2015)