MANAGEMENT OF ENDOCRINE DISEASE

Polycystic ovary syndrome and nonalcoholic fatty liver disease

Djuro Macut[1], Ivana Božić-Antić[1], Jelica Bjekić-Macut[2] and Konstantinos Tziomalos[3]

[1]Clinic of Endocrinology, Diabetes and Metabolic Diseases, [2]Department of Endocrinology, CHC Bezanijska Kosa, Faculty of Medicine, University of Belgrade, Belgrade, Serbia, and [3]First Propaedeutic Department of Internal Medicine, Medical School, Aristotle University of Thessaloniki, AHEPA Hospital, Thessaloniki, Greece

Correspondence should be addressed to D Macut
Email
djmacut@gmail.com

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.

European Journal of Endocrinology
(2017) **177**, R145–R158

European Journal of Endocrinology

Invited Author's profile

Djuro Macut, MD, PhD is an Associate Professor of Internal Medicine and Endocrinology at the Faculty of Medicine, University of Belgrade, and Deputy Director of the Clinic for Endocrinology, Diabetes and Metabolic Diseases, Belgrade, Serbia. His clinical interests are related to reproductive endocrinology, metabolic endocrinology, neuroendocrinology and neuroendocrine tumors, while the main research focus is currently oriented toward molecular basis and cardiometabolic outcomes of polycystic ovary syndrome, and the effects of different metabolites on neurophysiological functions.

www.eje-online.org
DOI: 10.1530/EJE-16-1063

Published by Bioscientifica Ltd.

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). ^{1}H-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,*

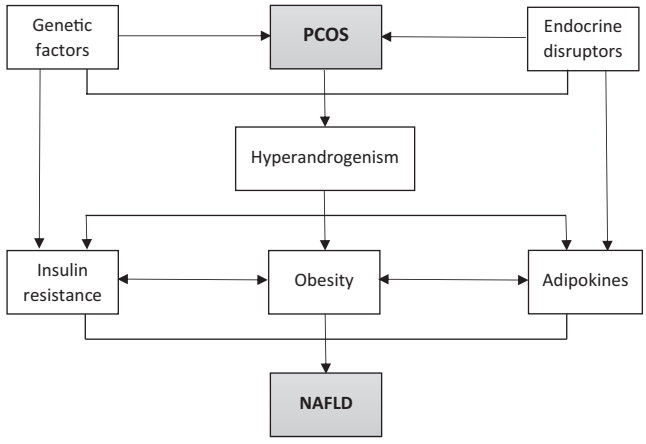

Figure 1
Factors involved in the development of nonalcoholic fatty liver disease (NAFLD) in patients with polycystic ovary syndrome (PCOS).

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-β1 (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 heterogenicity 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 (TNFα), 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 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.

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.

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.

Funding

This work was supported by Grants 41009 and 175032 from the Serbian Ministry of Science and Education.

References

1 Knochenhauer ES, Key TJ, Kahsar-Miller M, Waggoner W, Boots LR & Azziz R. Prevalence of the polycystic ovary syndrome in unselected black and white women of the southeastern United States: a prospective study. *Journal of Clinical Endocrinology and Metabolism* 1998 **83** 3078–3082. (doi:10.1210/jc.83.9.3078)

2 Diamanti-Kandarakis E, Kouli CR, Bergiele AT, Filandra FA, Tsianateli TC, Spina GG, Zapanti ED & Bartzis MI. A survey of the polycystic ovary syndrome in the Greek island of Lesbos: hormonal and metabolic profile. *Journal of Clinical Endocrinology and Metabolism* 1999 **84** 4006–4011. (doi:10.1210/jcem.84.11.6148)

3 Livadas S & Diamanti-Kandarakis E. Polycystic ovary syndrome: definitions, phenotypes and diagnostic approach. *Frontiers of Hormone Research* 2013 **40** 1–21. (doi:10.1159/000341673)

4 Macut D, Damjanovic S, Panidis D, Spanos N, Glisic B, Petakov M, Rousso D, Kourtis A, Bjekic J & Milic N. Oxidised low-density lipoprotein concentration – early marker of an altered lipid metabolism in young women with PCOS. *European Journal of Endocrinology* 2006 **155** 131–136. (doi:10.1530/eje.1.02187)

5 Schmidt J, Landin-Wilhelmsen K, Brannstrom M & Dahlgren E. Cardiovascular disease and risk factors in PCOS women of postmenopausal age: a 21-year controlled follow-up study. *Journal of Clinical Endocrinology and Metabolism* 2011 **96** 3794–3803. (doi:10.1210/jc.2011-1677)

6 Conway GS, Dewailly D, Diamanti-Kandarakis E, Escobar Morreale H, Franks S, Gambineri A, Kelestimur F, Macut D, Micic D, Pasquali R *et al*. The polycystic ovary syndrome: an endocrinological perspective from the European Society of Endocrinology. *European Journal of Endocrinology* 2014 **171** 489–498. (doi:10.1530/EJE-14-0252)

7 Welt CK & Carmina E. Clinical review: lifecycle of polycystic ovary syndrome (PCOS): from in utero to menopause. *Journal of Clinical Endocrinology and Metabolism* 2013 **98** 4629–4638. (doi:10.1210/jc.2013-2375)

8 Yildiz BO, Knochenhauer ES & Azziz R. Impact of obesity on the risk for polycystic ovary syndrome. *Journal of Clinical Endocrinology and Metabolism* 2008 **93** 162–168. (doi:10.1210/jc.2007-1834)

9 Panidis D, Macut D, Tziomalos K, Papadakis E, Mikhailidis K, Kandaraki EA, Tsourdi EA, Tantanasis T, Mavromatidis G & Katsikis I. Prevalence of metabolic syndrome in women with polycystic ovary syndrome. *Clinical Endocrinology* 2013 **78** 586–592. (doi:10.1111/cen.12008)

10 Mather KJ, Kwan F & Corenblum B. Hyperinsulinemia in polycystic ovary syndrome correlates with increased cardiovascular risk independent of obesity. *Fertility and Sterility* 2000 **73** 150–156. (doi:10.1016/S0015-0282(99)00468-9)

11 Toprak S, Yonem A, Cakir B, Guler S, Azal O, Ozata M & Corakci A. Insulin resistance in nonobese patients with polycystic ovary syndrome. *Hormone Research* 2001 **55** 65–70. (doi:10.1159/000049972)

12 Savic-Radojevic A, Bozic Antic I, Coric V, Bjekic-Macut J, Radic T, Zarkovic M, Djukic T, Pljesa-Ercegovac M, Panidis D, Katsikis I *et al*. Effect of hyperglycemia and hyperinsulinemia on glutathione peroxidase activity in non-obese women with polycystic ovary syndrome. *Hormones* 2015 **14** 101–108. (doi:10.14310/horm.2002.1525)

13 Moran LJ, Misso ML, Wild RA & Norman RJ. Impaired glucose tolerance, type 2 diabetes and metabolic syndrome in polycystic ovary syndrome: a systematic review and meta-analysis. *Human Reproduction Update* 2010 **16** 347–363. (doi:10.1093/humupd/dmq001)

14 Burra P. Liver abnormalities and endocrine diseases. *Best Practice and Research: Clinical Gastroenterology* 2013 **27** 553–563. (doi:10.1016/j.bpg.2013.06.014)

15 Macut D, Tziomalos K, Bozic-Antic I, Bjekic-Macut J, Katsikis I, Papadakis E, Andric Z & Panidis D. Non-alcoholic fatty liver disease is associated with insulin resistance and lipid accumulation product in women with polycystic ovary syndrome. *Human Reproduction* 2016 **31** 1347–1353. (doi:10.1093/humrep/dew076)

16 Younossi ZM, Stepanova M, Afendy M, Fang Y, Younossi Y, Mir H & Srishord M. Changes in the prevalence of the most common causes of chronic liver diseases in the United States from 1988 to 2008. *Clinical Gastroenterology and Hepatology* 2011 **9** 524. e521–530.e521; quiz e560. (doi:10.1016/j.cgh.2011.03.020)

17 Chalasani N, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, Charlton M & Sanyal AJ. 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. *Hepatology* 2012 **55** 2005–2023. (doi:10.1002/hep.25762)

18 Williams CD, Stengel J, Asike MI, Torres DM, Shaw J, Contreras M, Landt CL & Harrison SA. Prevalence of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis among a largely

middle-aged population utilizing ultrasound and liver biopsy: a prospective study. *Gastroenterology* 2011 **140** 124–131. (doi:10.1053/j.gastro.2010.09.038)

19 Vernon G, Baranova A & Younossi ZM. Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. *Alimentary Pharmacology and Therapeutics* 2011 **34** 274–285. (doi:10.1111/j.1365-2036.2011.04724.x)

20 Tziomalos K, Athyros VG & Karagiannis A. Non-alcoholic fatty liver disease in type 2 diabetes: pathogenesis and treatment options. *Current Vascular Pharmacology* 2012 **10** 162–172. (doi:10.2174/157016112799305012)

21 Leite NC, Salles GF, Araujo AL, Villela-Nogueira CA & Cardoso CR. Prevalence and associated factors of non-alcoholic fatty liver disease in patients with type-2 diabetes mellitus. *Liver International* 2009 **29** 113–119. (doi:10.1111/j.1478-3231.2008.01718.x)

22 Bae JC, Rhee EJ, Lee WY, Park SE, Park CY, Oh KW, Park SW & Kim SW. Combined effect of nonalcoholic fatty liver disease and impaired fasting glucose on the development of type 2 diabetes: a 4-year retrospective longitudinal study. *Diabetes Care* 2011 **34** 727–729. (doi:10.2337/dc10-1991)

23 Day CP & James OF. Steatohepatitis: a tale of two 'hits'? *Gastroenterology* 1998 **114** 842–845. (doi:10.1016/S0016-5085(98)70599-2)

24 Gastaldelli A, Cusi K, Pettiti M, Hardies J, Miyazaki Y, Berria R, Buzzigoli E, Sironi AM, Cersosimo E, Ferrannini E *et al*. Relationship between hepatic/visceral fat and hepatic insulin resistance in nondiabetic and type 2 diabetic subjects. *Gastroenterology* 2007 **133** 496–506. (doi:10.1053/j.gastro.2007.04.068)

25 Kotronen A, Peltonen M, Hakkarainen A, Sevastianova K, Bergholm R, Johansson LM, Lundbom N, Rissanen A, Ridderstrale M, Groop L *et al*. Prediction of non-alcoholic fatty liver disease and liver fat using metabolic and genetic factors. *Gastroenterology* 2009 **137** 865–872. (doi:10.1053/j.gastro.2009.06.005)

26 Donnelly KL, Smith CI, Schwarzenberg SJ, Jessurun J, Boldt MD & Parks EJ. Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. *Journal of Clinical Investigation* 2005 **115** 1343–1351. (doi:10.1172/JCI23621)

27 Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, Ferrell LD, Liu YC, Torbenson MS, Unalp-Arida A *et al*. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology* 2005 **41** 1313–1321. (doi:10.1002/hep.20701)

28 Neuschwander-Tetri BA, Clark JM, Bass NM, Van Natta ML, Unalp-Arida A, Tonascia J, Zein CO, Brunt EM, Kleiner DE, McCullough AJ *et al*. Clinical, laboratory and histological associations in adults with nonalcoholic fatty liver disease. *Hepatology* 2010 **52** 913–924. (doi:10.1002/hep.23784)

29 Paradis V, Perlemuter G, Bonvoust F, Dargere D, Parfait B, Vidaud M, Conti M, Huet S, Ba N, Buffet C *et al*. High glucose and hyperinsulinemia stimulate connective tissue growth factor expression: a potential mechanism involved in progression to fibrosis in nonalcoholic steatohepatitis. *Hepatology* 2001 **34** 738–744. (doi:10.1053/jhep.2001.28055)

30 Chalasani N, Deeg MA & Crabb DW. Systemic levels of lipid peroxidation and its metabolic and dietary correlates in patients with nonalcoholic steatohepatitis. *American Journal of Gastroenterology* 2004 **99** 1497–1502. (doi:10.1111/j.1572-0241.2004.30159.x)

31 Targher G. Relationship between high-sensitivity C-reactive protein levels and liver histology in subjects with non-alcoholic fatty liver disease. *Journal of Hepatology* 2006 **45** 879–881; author reply 881–872. (doi:10.1016/j.jhep.2006.09.005)

32 Wieckowska A, Zein NN, Yerian LM, Lopez AR, McCullough AJ & Feldstein AE. In vivo assessment of liver cell apoptosis as a novel biomarker of disease severity in nonalcoholic fatty liver disease. *Hepatology* 2006 **44** 27–33. (doi:10.1002/hep.21223)

33 Manousou P, Kalambokis G, Grillo F, Watkins J, Xirouchakis E, Pleguezuelo M, Leandro G, Arvaniti V, Germani G, Patch D *et al*. Serum ferritin is a discriminant marker for both fibrosis and inflammation in histologically proven non-alcoholic fatty liver disease patients. *Liver International* 2011 **31** 730–739. (doi:10.1111/j.1478-3231.2011.02488.x)

34 Loria P, Adinolfi LE, Bellentani S, Bugianesi E, Grieco A, Fargion S, Gasbarrini A, Loguercio C, Lonardo A, Marchesini G *et al*. 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. *Digestive and Liver Disease* 2010 **42** 272–282. (doi:10.1016/j.dld.2010.01.021)

35 Myers RP, Fong A & Shaheen AA. Utilization rates, complications and costs of percutaneous liver biopsy: a population-based study including 4275 biopsies. *Liver International* 2008 **28** 705–712. (doi:10.1111/j.1478-3231.2008.01691.x)

36 Ratziu V, Charlotte F, Heurtier A, Gombert S, Giral P, Bruckert E, Grimaldi A, Capron F & Poynard T. Sampling variability of liver biopsy in nonalcoholic fatty liver disease. *Gastroenterology* 2005 **128** 1898–1906. (doi:10.1053/j.gastro.2005.03.084)

37 Hernaez R, Lazo M, Bonekamp S, Kamel I, Brancati FL, Guallar E & Clark JM. Diagnostic accuracy and reliability of ultrasonography for the detection of fatty liver: a meta-analysis. *Hepatology* 2011 **54** 1082–1090. (doi:10.1002/hep.24452)

38 Papagianni M, Sofogianni A & Tziomalos K. Non-invasive methods for the diagnosis of nonalcoholic fatty liver disease. *World Journal of Hepatology* 2015 **7** 638–648. (doi:10.4254/wjh.v7.i4.638)

39 McPherson S, Jonsson JR, Cowin GJ, O'Rourke P, Clouston AD, Volp A, Horsfall L, Jothimani D, Fawcett J, Galloway GJ *et al*. Magnetic resonance imaging and spectroscopy accurately estimate the severity of steatosis provided the stage of fibrosis is considered. *Journal of Hepatology* 2009 **51** 389–397. (doi:10.1016/j.jhep.2009.04.012)

40 Permutt Z, Le TA, Peterson MR, Seki E, Brenner DA, Sirlin C & Loomba R. Correlation between liver histology and novel magnetic resonance imaging in adult patients with non-alcoholic fatty liver disease – MRI accurately quantifies hepatic steatosis in NAFLD. *Alimentary Pharmacology and Therapeutics* 2012 **36** 22–29. (doi:10.1111/j.1365-2036.2012.05121.x)

41 Lupsor M, Badea R, Stefanescu H, Grigorescu M, Serban A, Radu C, Crisan D, Sparchez Z, Iancu S & Maniu A. Performance of unidimensional transient elastography in staging non-alcoholic steatohepatitis. *Journal of Gastrointestinal and Liver Diseases* 2010 **19** 53–60.

42 Hashemi SA, Alavian SM & Gholami-Fesharaki M. Assessment of transient elastography (FibroScan) for diagnosis of fibrosis in non-alcoholic fatty liver disease: a systematic review and meta-analysis. *Caspian Journal of Internal Medicine* 2016 **7** 242–252.

43 Kwok R, Tse YK, Wong GL, Ha Y, Lee AU, Ngu MC, Chan HL & Wong VW. Systematic review with meta-analysis: non-invasive assessment of non-alcoholic fatty liver disease – the role of transient elastography and plasma cytokeratin-18 fragments. *Alimentary Pharmacology and Therapeutics* 2014 **39** 254–269. (doi:10.1111/apt.12569)

44 Castera L, Foucher J, Bernard PH, Carvalho F, Allaix D, Merrouche W, Couzigou P & de Ledinghen V. Pitfalls of liver stiffness measurement: a 5-year prospective study of 13 369 examinations. *Hepatology* 2010 **51** 828–835.

45 Boursier J, Isselin G, Fouchard-Hubert I, Oberti F, Dib N, Lebigot J, Bertrais S, Gallois Y, Cales P & Aube C. Acoustic radiation force impulse: a new ultrasonographic technology for the widespread

noninvasive diagnosis of liver fibrosis. *European Journal of Gastroenterology and Hepatology* 2010 **22** 1074–1084. (doi:10.1097/MEG.0b013e328339e0a1)

46 Poynard T, Munteanu M, Luckina E, Perazzo H, Ngo Y, Royer L, Fedchuk L, Sattonnet F, Pais R, Lebray P *et al*. Liver fibrosis evaluation using real-time shear wave elastography: applicability and diagnostic performance using methods without a gold standard. *Journal of Hepatology* 2013 **58** 928–935. (doi:10.1016/j.jhep.2012.12.021)

47 Kim D, Kim WR, Talwalkar JA, Kim HJ & Ehman RL. Advanced fibrosis in nonalcoholic fatty liver disease: noninvasive assessment with MR elastography. *Radiology* 2013 **268** 411–419. (doi:10.1148/radiol.13121193)

48 Lerchbaum E, Gruber HJ, Schwetz V, Giuliani A, Moller R, Pieber TR & Obermayer-Pietsch B. Fatty liver index in polycystic ovary syndrome. *European Journal of Endocrinology* 2011 **165** 935–943. (doi:10.1530/EJE-11-0614)

49 McPherson S, Stewart SF, Henderson E, Burt AD & Day CP. Simple non-invasive fibrosis scoring systems can reliably exclude advanced fibrosis in patients with non-alcoholic fatty liver disease. *Gut* 2010 **59** 1265–1269. (doi:10.1136/gut.2010.216077)

50 Demir M, Lang S, Nierhoff D, Drebber U, Hardt A, Wedemeyer I, Schulte S, Quasdorff M, Goeser T, Tox U *et al*. Stepwise combination of simple noninvasive fibrosis scoring systems increases diagnostic accuracy in nonalcoholic fatty liver disease. *Journal of Clinical Gastroenterology* 2013 **47** 719–726. (doi:10.1097/MCG.0b013e3182819a89)

51 Angulo P, Hui JM, Marchesini G, Bugianesi E, George J, Farrell GC, Enders F, Saksena S, Burt AD, Bida JP *et al*. The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. *Hepatology* 2007 **45** 846–854. (doi:10.1002/hep.21496)

52 Harrison SA, Oliver D, Arnold HL, Gogia S & Neuschwander-Tetri BA. Development and validation of a simple NAFLD clinical scoring system for identifying patients without advanced disease. *Gut* 2008 **57** 1441–1447. (doi:10.1136/gut.2007.146019)

53 Francque SM, Verrijken A, Mertens I, Hubens G, Van Marck E, Pelckmans P, Michielsen P & Van Gaal L. Noninvasive assessment of nonalcoholic fatty liver disease in obese or overweight patients. *Clinical Gastroenterology and Hepatology* 2012 **10** 1162–1168; quiz e1187. (doi:10.1016/j.cgh.2012.06.019)

54 Sumida Y, Yoneda M, Hyogo H, Yamaguchi K, Ono M, Fujii H, Eguchi Y, Suzuki Y, Imai S, Kanemasa K *et al*. A simple clinical scoring system using ferritin, fasting insulin, and type IV collagen 7S for predicting steatohepatitis in nonalcoholic fatty liver disease. *Journal of Gastroenterology* 2011 **46** 257–268. (doi:10.1007/s00535-010-0305-6)

55 Guha IN, Parkes J, Roderick P, Chattopadhyay D, Cross R, Harris S, Kaye P, Burt AD, Ryder SD, Aithal GP *et al*. Noninvasive markers of fibrosis in nonalcoholic fatty liver disease: validating the European Liver Fibrosis Panel and exploring simple markers. *Hepatology* 2008 **47** 455–460. (doi:10.1002/hep.21984)

56 Tanwar S, Trembling PM, Guha IN, Parkes J, Kaye P, Burt AD, Ryder SD, Aithal GP, Day CP & Rosenberg WM. Validation of terminal peptide of procollagen III for the detection and assessment of nonalcoholic steatohepatitis in patients with nonalcoholic fatty liver disease. *Hepatology* 2013 **57** 103–111. (doi:10.1002/hep.26030)

57 Vassilatou E, Lafoyianni S, Vryonidou A, Ioannidis D, Kosma L, Katsoulis K, Papavassiliou E & Tzavara I. Increased androgen bioavailability is associated with non-alcoholic fatty liver disease in women with polycystic ovary syndrome. *Human Reproduction* 2010 **25** 212–220. (doi:10.1093/humrep/dep380)

58 Jones H, Sprung VS, Pugh CJ, Daousi C, Irwin A, Aziz N, Adams VL, Thomas EL, Bell JD, Kemp GJ *et al*. 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. *Journal of Clinical Endocrinology and Metabolism* 2012 **97** 3709–3716. (doi:10.1210/jc.2012-1382)

59 Baranova A, Tran TP, Birerdinc A & Younossi ZM. Systematic review: association of polycystic ovary syndrome with metabolic syndrome and non-alcoholic fatty liver disease. *Alimentary Pharmacology and Therapeutics* 2011 **33** 801–814. (doi:10.1111/j.1365-2036.2011.04579.x)

60 Manneras L, Cajander S, Holmang A, Seleskovic Z, Lystig T, Lonn M & Stener-Victorin E. A new rat model exhibiting both ovarian and metabolic characteristics of polycystic ovary syndrome. *Endocrinology* 2007 **148** 3781–3791. (doi:10.1210/en.2007-0168)

61 Nikolic M, Macut D, Djordjevic A, Velickovic N, Nestorovic N, Bursac B, Antic IB, Macut JB, Matic G & Vojnovic Milutinovic D. Possible involvement of glucocorticoids in 5alpha-dihydrotestosterone-induced PCOS-like metabolic disturbances in the rat visceral adipose tissue. *Molecular and Cellular Endocrinology* 2015 **399** 22–31. (doi:10.1016/j.mce.2014.08.013)

62 Hogg K, Wood C, McNeilly AS & Duncan WC. The in utero programming effect of increased maternal androgens and a direct fetal intervention on liver and metabolic function in adult sheep. *PLoS ONE* 2011 **6** e24877. (doi:10.1371/journal.pone.0024877)

63 Anstee QM, Targher G & Day CP. Progression of NAFLD to diabetes mellitus, cardiovascular disease or cirrhosis. *Nature Reviews Gastroenterology and Hepatology* 2013 **10** 330–344. (doi:10.1038/nrgastro.2013.41)

64 Birkenfeld AL & Shulman GI. Nonalcoholic fatty liver disease, hepatic insulin resistance, and type 2 diabetes. *Hepatology* 2014 **59** 713–723. (doi:10.1002/hep.26672)

65 Qu Z, Zhu Y, Jiang J, Shi Y & Chen Z. The clinical characteristics and etiological study of nonalcoholic fatty liver disease in Chinese women with PCOS. *Iranian Journal of Reproductive Medicine* 2013 **11** 725–732.

66 Gangale MF, Miele L, Lanzone A, Sagnella F, Martinez D, Tropea A, Moro F, Morciano A, Ciardulli A, Palla C *et al*. Long-term metformin treatment is able to reduce the prevalence of metabolic syndrome and its hepatic involvement in young hyperinsulinaemic overweight patients with polycystic ovarian syndrome. *Clinical Endocrinology* 2011 **75** 520–527. (doi:10.1111/j.1365-2265.2011.04093.x)

67 Dawson AJ, Sathyapalan T, Smithson JA, Vince RV, Coady AM, Ajjan R, Kilpatrick ES & Atkin SL. A comparison of cardiovascular risk indices in patients with polycystic ovary syndrome with and without coexisting nonalcoholic fatty liver disease. *Clinical Endocrinology* 2014 **80** 843–849. (doi:10.1111/cen.12258)

68 Hossain N, Stepanova M, Afendy A, Nader F, Younossi Y, Rafiq N, Goodman Z & Younossi ZM. Non-alcoholic steatohepatitis (NASH) in patients with polycystic ovarian syndrome (PCOS). *Scandinavian Journal of Gastroenterology* 2011 **46** 479–484. (doi:10.3109/00365521.2010.539251)

69 Pascal N, Amouzou EK, Sanni A, Namour F, Abdelmouttaleb I, Vidailhet M & Gueant JL. Serum concentrations of sex hormone binding globulin are elevated in kwashiorkor and anorexia nervosa but not in marasmus. *American Journal of Clinical Nutrition* 2002 **76** 239–244.

70 Lazo M, Zeb I, Nasir K, Tracy RP, Budoff MJ, Ouyang P & Vaidya D. Association between endogenous sex hormones and liver fat in a multiethnic study of atherosclerosis. *Clinical Gastroenterology and Hepatology* 2015 **13** 1686.e1682–1693.e1682. (doi:10.1016/j.cgh.2014.12.033)

71 Dai R, Yan D, Li J, Chen S, Liu Y, Chen R, Duan C, Wei M, Li H & He T. Activation of PKR/eIF2alpha signaling cascade is associated with dihydrotestosterone-induced cell cycle arrest and apoptosis in human liver cells. *Journal of Cellular Biochemistry* 2012 **113** 1800–1808. (doi:10.1002/jcb.24051)

72 Tan S, Bechmann LP, Benson S, Dietz T, Eichner S, Hahn S, Janssen OE, Lahner H, Gerken G, Mann K *et al*. Apoptotic markers indicate nonalcoholic steatohepatitis in polycystic ovary syndrome. *Journal of Clinical Endocrinology and Metabolism* 2010 **95** 343–348. (doi:10.1210/jc.2009-1834)

73 Baranova A, Tran TP, Afendy A, Wang L, Shamsaddini A, Mehta R, Chandhoke V, Birerdinc A & Younossi ZM. Molecular signature of adipose tissue in patients with both non-alcoholic fatty liver disease (NAFLD) and polycystic ovarian syndrome (PCOS). *Journal of Translational Medicine* 2013 **11** 133. (doi:10.1186/1479-5876-11-133)

74 Croston GE, Milan LB, Marschke KB, Reichman M & Briggs MR. Androgen receptor-mediated antagonism of estrogen-dependent low density lipoprotein receptor transcription in cultured hepatocytes. *Endocrinology* 1997 **138** 3779–3786. (doi:10.1210/endo.138.9.5404)

75 Blouin K, Boivin A & Tchernof A. Androgens and body fat distribution. *Journal of Steroid Biochemistry and Molecular Biology* 2008 **108** 272–280. (doi:10.1016/j.jsbmb.2007.09.001)

76 Xu A, Chan KW, Hoo RL, Wang Y, Tan KC, Zhang J, Chen B, Lam MC, Tse C, Cooper GJ *et al*. Testosterone selectively reduces the high molecular weight form of adiponectin by inhibiting its secretion from adipocytes. *Journal of Biological Chemistry* 2005 **280** 18073–18080. (doi:10.1074/jbc.M414231200)

77 O'Connor A, Phelan N, Tun TK, Boran G, Gibney J & Roche HM. High-molecular-weight adiponectin is selectively reduced in women with polycystic ovary syndrome independent of body mass index and severity of insulin resistance. *Journal of Clinical Endocrinology and Metabolism* 2010 **95** 1378–1385. (doi:10.1210/jc.2009-1557)

78 Cankaya S, Demir B, Aksakal SE, Dilbaz B, Demirtas C & Goktolga U. Insulin resistance and its relationship with high molecular weight adiponectin in adolescents with polycystic ovary syndrome and a maternal history of polycystic ovary syndrome. *Fertility and Sterility* 2014 **102** 826–830. (doi:10.1016/j.fertnstert.2014.05.032)

79 Heiker JT, Kosel D & Beck-Sickinger AG. Molecular mechanisms of signal transduction via adiponectin and adiponectin receptors. *Biological Chemistry* 2010 **391** 1005–1018. (doi:10.1515/BC.2010.104)

80 Jung TW, Lee YJ, Lee MW & Kim SM. Full-length adiponectin protects hepatocytes from palmitate-induced apoptosis via inhibition of c-Jun NH2 terminal kinase. *FEBS Journal* 2009 **276** 2278–2284. (doi:10.1111/j.1742-4658.2009.06955.x)

81 Xu A, Wang Y, Keshaw H, Xu LY, Lam KS & Cooper GJ. The fat-derived hormone adiponectin alleviates alcoholic and nonalcoholic fatty liver diseases in mice. *Journal of Clinical Investigation* 2003 **112** 91–100. (doi:10.1172/JCI200317797)

82 Targher G, Bertolini L, Rodella S, Zoppini G, Scala L, Zenari L & Falezza G. Associations between plasma adiponectin concentrations and liver histology in patients with nonalcoholic fatty liver disease. *Clinical Endocrinology* 2006 **64** 679–683. (doi:10.1111/j.1365-2265.2006.02527.x)

83 Hui JM, Hodge A, Farrell GC, Kench JG, Kriketos A & George J. Beyond insulin resistance in NASH: TNF-alpha or adiponectin? *Hepatology* 2004 **40** 46–54. (doi:10.1002/hep.20280)

84 Polyzos SA, Toulis KA, Goulis DG, Zavos C & Kountouras J. Serum total adiponectin in nonalcoholic fatty liver disease: a systematic review and meta-analysis. *Metabolism* 2011 **60** 313–326. (doi:10.1016/j.metabol.2010.09.003)

85 Ma H, Gomez V, Lu L, Yang X, Wu X & Xiao SY. Expression of adiponectin and its receptors in livers of morbidly obese patients with non-alcoholic fatty liver disease. *Journal of Gastroenterology and Hepatology* 2009 **24** 233–237. (doi:10.1111/j.1440-1746.2008.05548.x)

86 Kaser S, Moschen A, Cayon A, Kaser A, Crespo J, Pons-Romero F, Ebenbichler CF, Patsch JR & Tilg H. Adiponectin and its receptors in non-alcoholic steatohepatitis. *Gut* 2005 **54** 117–121. (doi:10.1136/gut.2003.037010)

87 Francque S, Verrijken A, Caron S, Prawitt J, Paumelle R, Derudas B, Lefebvre P, Taskinen MR, Van Hul W, Mertens I *et al*. PPARalpha gene expression correlates with severity and histological treatment response in patients with non-alcoholic steatohepatitis. *Journal of Hepatology* 2015 **63** 164–173. (doi:10.1016/j.jhep.2015.02.019)

88 Polyzos SA, Aronis KN, Kountouras J, Raptis DD, Vasiloglou MF & Mantzoros CS. Circulating leptin in non-alcoholic fatty liver disease: a systematic review and meta-analysis. *Diabetologia* 2016 **59** 30–43. (doi:10.1007/s00125-015-3769-3)

89 Polyzos SA, Kountouras J & Mantzoros CS. Leptin in nonalcoholic fatty liver disease: a narrative review. *Metabolism* 2014 **64** 60–78. (doi:10.1016/j.metabol.2014.10.012)

90 Ikejima K, Honda H, Yoshikawa M, Hirose M, Kitamura T, Takei Y & Sato N. Leptin augments inflammatory and profibrogenic responses in the murine liver induced by hepatotoxic chemicals. *Hepatology* 2001 **34** 288–297. (doi:10.1053/jhep.2001.26518)

91 Saxena NK, Ikeda K, Rockey DC, Friedman SL & Anania FA. Leptin in hepatic fibrosis: evidence for increased collagen production in stellate cells and lean littermates of ob/ob mice. *Hepatology* 2002 **35** 762–771. (doi:10.1053/jhep.2002.32029)

92 Chitturi S, Farrell G, Frost L, Kriketos A, Lin R, Fung C, Liddle C, Samarasinghe D & George J. Serum leptin in NASH correlates with hepatic steatosis but not fibrosis: a manifestation of lipotoxicity? *Hepatology* 2002 **36** 403–409. (doi:10.1053/jhep.2002.34738)

93 Chalasani N, Crabb DW, Cummings OW, Kwo PY, Asghar A, Pandya PK & Considine RV. Does leptin play a role in the pathogenesis of human nonalcoholic steatohepatitis? *American Journal of Gastroenterology* 2003 **98** 2771–2776. (doi:10.1111/j.1572-0241.2003.08767.x)

94 Musso G, Gambino R, Durazzo M, Biroli G, Carello M, Faga E, Pacini G, De Michieli F, Rabbione L, Premoli A *et al*. Adipokines in NASH: postprandial lipid metabolism as a link between adiponectin and liver disease. *Hepatology* 2005 **42** 1175–1183. (doi:10.1002/hep.20896)

95 Pagano C, Soardo G, Pilon C, Milocco C, Basan L, Milan G, Donnini D, Faggian D, Mussap M, Plebani M *et al*. Increased serum resistin in nonalcoholic fatty liver disease is related to liver disease severity and not to insulin resistance. *Journal of Clinical Endocrinology and Metabolism* 2006 **91** 1081–1086. (doi:10.1210/jc.2005-1056)

96 Senates E, Colak Y, Yesil A, Coskunpinar E, Sahin O, Kahraman OT, Erkalma Senates B & Tuncer I. Circulating resistin is elevated in patients with non-alcoholic fatty liver disease and is associated with steatosis, portal inflammation, insulin resistance and nonalcoholic steatohepatitis scores. *Minerva Medica* 2012 **103** 369–376.

97 Auguet T, Terra X, Porras JA, Orellana-Gavalda JM, Martinez S, Aguilar C, Lucas A, Pellitero S, Hernandez M, Del Castillo D *et al*. Plasma visfatin levels and gene expression in morbidly obese women with associated fatty liver disease. *Clinical Biochemistry* 2013 **46** 202–208. (doi:10.1016/j.clinbiochem.2012.11.006)

98 Wong VW, Hui AY, Tsang SW, Chan JL, Tse AM, Chan KF, So WY, Cheng AY, Ng WF, Wong GL *et al*. Metabolic and adipokine profile of Chinese patients with nonalcoholic fatty liver disease. *Clinical Gastroenterology and Hepatology* 2006 **4** 1154–1161. (doi:10.1016/j.cgh.2006.06.011)

99 Wang Y, Xie X & Zhu W. Serum adiponectin and resistin levels in patients with polycystic ovarian syndrome and their clinical implications. *Journal of Huazhong University of Science and Technology: Medical Sciences* 2010 **30** 638–642. (doi:10.1007/s11596-010-0556-8)

100 Pangaribuan B, Yusuf I, Mansyur M & Wijaya A. Serum adiponectin and resistin in relation to insulin resistance and markers of hyperandrogenism in lean and obese

women with polycystic ovary syndrome. *Therapeutic Advances in Endocrinology and Metabolism* 2011 **2** 235–245. (doi:10.1177/2042018811423770)

101 Aller R, de Luis DA, Fernandez L, Calle F, Velayos B, Olcoz JL, Izaola O, Sagrado MG, Conde R & Gonzalez JM. Influence of insulin resistance and adipokines in the grade of steatosis of nonalcoholic fatty liver disease. *Digestive Diseases and Sciences* 2008 **53** 1088–1092. (doi:10.1007/s10620-007-9981-3)

102 Jamali R, Razavizade M, Arj A & Aarabi MH. Serum adipokines might predict liver histology findings in non-alcoholic fatty liver disease. *World Journal of Gastroenterology* 2016 **22** 5096–5103. (doi:10.3748/wjg.v22.i21.5096)

103 Polyzos SA, Kountouras J & Mantzoros CS. Adipokines in nonalcoholic fatty liver disease. *Metabolism* 2015 **65** 1062–1079. (doi:10.1016/j.metabol.2015.11.006)

104 Polak K, Czyzyk A, Simoncini T & Meczekalski B. New markers of insulin resistance in polycystic ovary syndrome. *Journal of Endocrinological Investigation* 2017 **40** 1–8. (doi:10.1007/s40618-016-0523-8)

105 Sahin Ersoy G, Altun Ensari T, Vatansever D, Emirdar V & Cevik O. Novel adipokines WISP1 and betatrophin in PCOS: relationship to AMH levels, atherogenic and metabolic profile. *Gynecological Endocrinology* 2017 **33** 119–123. (doi:10.1080/09513590.2016.1223286)

106 Cengiz M, Ozenirler S & Kocabiyik M. Serum beta-trophin level as a new marker for noninvasive assessment of nonalcoholic fatty liver disease and liver fibrosis. *European Journal of Gastroenterology and Hepatology* 2016 **28** 57–63. (doi:10.1097/MEG.0000000000000502)

107 Grant RW & Dixit VD. Adipose tissue as an immunological organ. *Obesity* 2015 **23** 512–518. (doi:10.1002/oby.21003)

108 Ben-Jonathan N, Hugo ER & Brandebourg TD. Effects of bisphenol A on adipokine release from human adipose tissue: Implications for the metabolic syndrome. *Molecular and Cellular Endocrinology* 2009 **304** 49–54. (doi:10.1016/j.mce.2009.02.022)

109 Newbold RR. Impact of environmental endocrine disrupting chemicals on the development of obesity. *Hormones* 2010 **9** 206–217. (doi:10.14310/horm.2002.1271)

110 Lim JS, Lee DH & Jacobs DR Jr. Association of brominated flame retardants with diabetes and metabolic syndrome in the U.S. population, 2003–2004. *Diabetes Care* 2008 **31** 1802–1807. (doi:10.2337/dc08-0850)

111 Polyzos SA, Kountouras J, Deretzi G, Zavos C & Mantzoros CS. The emerging role of endocrine disruptors in pathogenesis of insulin resistance: a concept implicating nonalcoholic fatty liver disease. *Current Molecular Medicine* 2012 **12** 68–82. (doi:10.2174/156652412798376161)

112 Kandaraki E, Chatzigeorgiou A, Livadas S, Palioura E, Economou F, Koutsilieris M, Palimeri S, Panidis D & Diamanti-Kandarakis E. Endocrine disruptors and polycystic ovary syndrome (PCOS): elevated serum levels of bisphenol A in women with PCOS. *Journal of Clinical Endocrinology and Metabolism* 2011 **96** E480–E484. (doi:10.1210/jc.2010-1658)

113 Cussons AJ, Watts GF, Mori TA & Stuckey BG. Omega-3 fatty acid supplementation decreases liver fat content in polycystic ovary syndrome: a randomized controlled trial employing proton magnetic resonance spectroscopy. *Journal of Clinical Endocrinology and Metabolism* 2009 **94** 3842–3848. (doi:10.1210/jc.2009-0870)

114 Brzozowska MM, Ostapowicz G & Weltman MD. An association between non-alcoholic fatty liver disease and polycystic ovarian syndrome. *Journal of Gastroenterology and Hepatology* 2009 **24** 243–247. (doi:10.1111/j.1440-1746.2008.05740.x)

115 Karoli R, Fatima J, Chandra A, Gupta U, Islam FU & Singh G. Prevalence of hepatic steatosis in women with polycystic ovary syndrome. *Journal of Human Reproductive Sciences* 2013 **6** 9–14. (doi:10.4103/0974-1208.112370)

116 Ramezani-Binabaj M, Motalebi M, Karimi-Sari H, Rezaee-Zavareh MS & Alavian SM. Are women with polycystic ovarian syndrome at a high risk of non-alcoholic Fatty liver disease; a meta-analysis. *Hepatitis Monthly* 2014 **14** e23235. (doi:10.5812/hepatmon.23235)

117 Hamaguchi M, Kojima T, Takeda N, Nakagawa T, Taniguchi H, Fujii K, Omatsu T, Nakajima T, Sarui H, Shimazaki M *et al*. The metabolic syndrome as a predictor of nonalcoholic fatty liver disease. *Annals of Internal Medicine* 2005 **143** 722–728. (doi:10.7326/0003-4819-143-10-200511150-00009)

118 Targher G, Rossini M & Lonardo A. Evidence that non-alcoholic fatty liver disease and polycystic ovary syndrome are associated by necessity rather than chance: a novel hepato-ovarian axis? *Endocrine* 2016 **51** 211–221. (doi:10.1007/s12020-015-0640-8)

119 Setji TL, Holland ND, Sanders LL, Pereira KC, Diehl AM & Brown AJ. Nonalcoholic steatohepatitis and nonalcoholic Fatty liver disease in young women with polycystic ovary syndrome. *Journal of Clinical Endocrinology and Metabolism* 2006 **91** 1741–1747. (doi:10.1210/jc.2005-2774)

120 Kahal H, Abouda G, Rigby AS, Coady AM, Kilpatrick ES & Atkin SL. Glucagon-like peptide-1 analogue, liraglutide, improves liver fibrosis markers in obese women with polycystic ovary syndrome and nonalcoholic fatty liver disease. *Clinical Endocrinology* 2014 **81** 523–528. (doi:10.1111/cen.12369)

121 Legro RS, Arslanian SA, Ehrmann DA, Hoeger KM, Murad MH, Pasquali R & Welt CK. Diagnosis and treatment of polycystic ovary syndrome: an Endocrine Society clinical practice guideline. *Journal of Clinical Endocrinology and Metabolism* 2013 **98** 4565–4592. (doi:10.1210/jc.2013-2350)

122 Gambarin-Gelwan M, Kinkhabwala SV, Schiano TD, Bodian C, Yeh HC & Futterweit W. Prevalence of nonalcoholic fatty liver disease in women with polycystic ovary syndrome. *Clinical Gastroenterology and Hepatology* 2007 **5** 496–501. (doi:10.1016/j.cgh.2006.10.010)

123 Ayonrinde OT, Adams LA, Doherty DA, Mori TA, Beilin LJ, Oddy WH, Hickey M, Sloboda DM, Olynyk JK & Hart R. Adverse metabolic phenotype of adolescent girls with non-alcoholic fatty liver disease plus polycystic ovary syndrome compared with other girls and boys. *Journal of Gastroenterology and Hepatology* 2016 **31** 980–987. (doi:10.1111/jgh.13241)

124 Cree-Green M, Bergman BC, Coe GV, Newnes L, Baumgartner AD, Bacon S, Sherzinger A, Pyle L & Nadeau KJ. Hepatic steatosis is common in adolescents with obesity and PCOS and relates to de novo lipogenesis but not insulin resistance. *Obesity* 2016 **24** 2399–2406. (doi:10.1002/oby.21651)

125 Gutierrez-Grobe Y, Ponciano-Rodriguez G, Ramos MH, Uribe M & Mendez-Sanchez N. Prevalence of non alcoholic fatty liver disease in premenopausal, posmenopausal and polycystic ovary syndrome women. The role of estrogens. *Annals of Hepatology* 2010 **9** 402–409.

126 Meigs JB, Rutter MK, Sullivan LM, Fox CS, D'Agostino RB Sr & Wilson PW. Impact of insulin resistance on risk of type 2 diabetes and cardiovascular disease in people with metabolic syndrome. *Diabetes Care* 2007 **30** 1219–1225. (doi:10.2337/dc06-2484)

127 Dunaif A, Segal KR, Futterweit W & Dobrjansky A. Profound peripheral insulin resistance, independent of obesity, in polycystic ovary syndrome. *Diabetes* 1989 **38** 1165–1174. (doi:10.2337/diab.38.9.1165)

128 Abdul-Ghani MA, Tripathy D & DeFronzo RA. Contributions of beta-cell dysfunction and insulin resistance to the pathogenesis of impaired glucose tolerance and impaired fasting glucose. *Diabetes Care* 2006 **29** 1130–1139. (doi:10.2337/dc05-2179)

129 DeFronzo RA & Tripathy D. Skeletal muscle insulin resistance is the primary defect in type 2 diabetes. *Diabetes Care* 2009 **32** (Supplement 2) S157–S163. (doi:10.2337/dc09-S302)

130 Abdul-Ghani MA, Matsuda M & DeFronzo RA. Strong association between insulin resistance in liver and skeletal muscle in non-diabetic subjects. *Diabetic Medicine* 2008 **25** 1289–1294. (doi:10.1111/j.1464-5491.2008.02597.x)

131 Targher G & Byrne CD. Clinical Review: nonalcoholic fatty liver disease: a novel cardiometabolic risk factor for type 2 diabetes and its complications. *Journal of Clinical Endocrinology and Metabolism* 2013 **98** 483–495. (doi:10.1210/jc.2012-3093)

132 Escobar-Morreale HF, Luque-Ramirez M & Gonzalez F. Circulating inflammatory markers in polycystic ovary syndrome: a systematic review and metaanalysis. *Fertility and Sterility* 2011 **95** 1048–1058.e1041–1042. (doi:10.1016/j.fertnstert.2010.11.036)

133 Kim JJ & Choi YM. Dyslipidemia in women with polycystic ovary syndrome. *Obstetrics and Gynecology Science* 2013 **56** 137–142. (doi:10.5468/ogs.2013.56.3.137)

134 Tan S, Vollmar N, Benson S, Sowa JP, Bechmann LP, Gerken G, Fuhrer D & Canbay A. Liver injury indicating fatty liver but not serologic NASH marker improves under metformin treatment in polycystic ovary syndrome. *International Journal of Endocrinology* 2015 **2015** 254169. (doi:10.1155/2015/254169)

135 Staehr P, Hother-Nielsen O, Landau BR, Chandramouli V, Holst JJ & Beck-Nielsen H. Effects of free fatty acids per se on glucose production, gluconeogenesis, and glycogenolysis. *Diabetes* 2003 **52** 260–267. (doi:10.2337/diabetes.52.2.260)

136 Lonardo A, Ballestri S, Marchesini G, Angulo P & Loria P. Nonalcoholic fatty liver disease: a precursor of the metabolic syndrome. *Digestive and Liver Disease* 2015 **47** 181–190. (doi:10.1016/j.dld.2014.09.020)

137 Vilar-Gomez E, Martinez-Perez Y, Calzadilla-Bertot L, Torres-Gonzalez A, Gra-Oramas B, Gonzalez-Fabian L, Friedman SL, Diago M & Romero-Gomez M. Weight loss through lifestyle modification significantly reduces features of nonalcoholic steatohepatitis. *Gastroenterology* 2015 **149** 367.e365–378.e365; quiz e314–e365. (doi:10.1053/j.gastro.2015.04.005)

138 Ryan MC, Itsiopoulos C, Thodis T, Ward G, Trost N, Hofferberth S, O'Dea K, Desmond PV, Johnson NA & Wilson AM. The Mediterranean diet improves hepatic steatosis and insulin sensitivity in individuals with non-alcoholic fatty liver disease. *Journal of Hepatology* 2013 **59** 138–143. (doi:10.1016/j.jhep.2013.02.012)

139 Hallsworth K, Thoma C, Hollingsworth KG, Cassidy S, Anstee QM, Day CP & Trenell MI. Modified high-intensity interval training reduces liver fat and improves cardiac function in non-alcoholic fatty liver disease: a randomized controlled trial. *Clinical Science* 2015 **129** 1097–1105. (doi:10.1042/CS20150308)

140 Katsagoni CN, Georgoulis M, Papatheodoridis GV, Panagiotakos DB & Kontogianni MD. Effects of lifestyle interventions on clinical characteristics of patients with non-alcoholic fatty liver disease: a meta-analysis. *Metabolism* 2017 **68** 119–132. (doi:10.1016/j.metabol.2016.12.006)

141 Sanyal AJ, Chalasani N, Kowdley KV, McCullough A, Diehl AM, Bass NM, Neuschwander-Tetri BA, Lavine JE, Tonascia J, Unalp A *et al*. Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. *New England Journal of Medicine* 2010 **362** 1675–1685. (doi:10.1056/NEJMoa0907929)

142 Bjelakovic G, Nikolova D, Gluud LL, Simonetti RG & Gluud C. Antioxidant supplements for prevention of mortality in healthy participants and patients with various diseases. *Cochrane Database of Systematic Reviews* 2012 **14** CD007176. (doi:10.1002/14651858.CD007176.pub2)

143 Zhu ZN, Jiang YF & Ding T. Risk of fracture with thiazolidinediones: an updated meta-analysis of randomized clinical trials. *Bone* 2014 **68** 115–123. (doi:10.1016/j.bone.2014.08.010)

144 Turner RM, Kwok CS, Chen-Turner C, Maduakor CA, Singh S & Loke YK. Thiazolidinediones and associated risk of bladder cancer: a systematic review and meta-analysis. *British Journal of Clinical Pharmacology* 2014 **78** 258–273. (doi:10.1111/bcp.12306)

145 Neuschwander-Tetri BA, Loomba R, Sanyal AJ, Lavine JE, Van Natta ML, Abdelmalek MF, Chalasani N, Dasarathy S, Diehl AM, Hameed B *et al*. Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT): a multicentre, randomized, placebo-controlled trial. *Lancet* 2015 **385** 956–965. (doi:10.1016/S0140-6736(14)61933-4)

146 Armstrong MJ, Gaunt P, Aithal GP, Barton D, Hull D, Parker R, Hazlehurst JM, Guo K, Abouda G, Aldersley MA *et al*. Liraglutide safety and efficacy in patients with non-alcoholic steatohepatitis (LEAN): a multicentre, double-blind, randomized, placebo-controlled phase 2 study. *Lancet* 2016 **387** 679–690. (doi:10.1016/S0140-6736(15)00803-X)

147 Rakoski MO, Singal AG, Rogers MA & Conjeevaram H. Meta-analysis: insulin sensitizers for the treatment of non-alcoholic steatohepatitis. *Alimentary Pharmacology and Therapeutics* 2010 **32** 1211–1221. (doi:10.1111/j.1365-2036.2010.04467.x)

148 Sumida Y, Seko Y & Yoneda M. Novel antidiabetic medications for non-alcoholic fatty liver disease with type 2 diabetes mellitus. *Hepatology Research* 2016 **47** 266–280. (doi:10.1111/hepr.12856)

149 Brown AJ, Tendler DA, McMurray RG & Setji TL. Polycystic ovary syndrome and severe nonalcoholic steatohepatitis: beneficial effect of modest weight loss and exercise on liver biopsy findings. *Endocrine Practices* 2005 **11** 319–324. (doi:10.4158/EP.11.5.319)

150 Preiss D, Sattar N, Harborne L, Norman J & Fleming R. The effects of 8 months of metformin on circulating GGT and ALT levels in obese women with polycystic ovarian syndrome. *International Journal of Clinical Practice* 2008 **62** 1337–1343. (doi:10.1111/j.1742-1241.2008.01825.x)

151 Ismail TA, Soliman MM & Nassan MA. Molecular and immunohistochemical effects of metformin in a rat model of type 2 diabetes mellitus. *Experimental and Therapeutic Medicine* 2015 **9** 1921–1930. (doi:10.3892/etm.2015.2354)

152 Tokubuchi I, Tajiri Y, Iwata S, Hara K, Wada N, Hashinaga T, Nakayama H, Mifune H & Yamada K. Beneficial effects of metformin on energy metabolism and visceral fat volume through a possible mechanism of fatty acid oxidation in human subjects and rats. *PLoS ONE* 2017 **12** e0171293. (doi:10.1371/journal.pone.0171293)

153 Ohki T, Isogawa A, Toda N & Tagawa K. Effectiveness of ipragliflozin, a sodium-glucose co-transporter 2 inhibitor, as a second-line treatment for non-alcoholic fatty liver disease patients with type 2 diabetes mellitus who do not respond to incretin-based therapies including glucagon-like peptide-1 analogs and dipeptidyl peptidase-4 inhibitors. *Clinical Drug Investigation* 2016 **36** 313–319. (doi:10.1007/s40261-016-0383-1)

154 Selen Alpergin ES, Bolandnazar Z, Sabatini M, Rogowski M, Chiellini G, Zucchi R & Assadi-Porter FM. Metabolic profiling reveals reprogramming of lipid metabolic pathways in treatment of polycystic ovary syndrome with 3-iodothyronamine. *Physiological Reports* 2017 **5** e13097. (doi:10.14814/phy14812.13097)

Received 23 December 2016
Revised version received 26 April 2017
Accepted 4 May 2017