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REVIEW

NAFLD as a Sexual Dimorphic Disease: Role of Gender and Reproductive Status in the Development and Progression of Nonalcoholic Fatty Liver Disease and Inherent Cardiovascular Risk

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ABSTRACT

Nonalcoholic fatty liver disease (NAFLD) spans steatosis through nonalcoholic steatohepatis, cirrhosis, and hepatocellular carcinoma (HCC) associated with striking systemic features and excess cardiovascular and liver-related mortality. The pathogenesis of NAFLD is complex and multifactorial. Endocrine derangements are closely linked with dysmetabolic traits. For example, in animal and human studies, female sex is protected from dysmetabolism thanks to young individuals' ability to partition fatty acids towards ketone body production rather than very low density lipoprotein (VLDL)-triacylglycerol, and to sex-specific browning of white adipose tissue. Ovarian senescence facilitates both the development of massive hepatic steatosis and the fibrotic progression of liver disease in an experimental overfed zebrafish model. Consistently, estrogen deficiency, by potentiating hepatic inflammatory changes, hastens the progression of disease in a dietary model of nonalcoholic steatohepatitis (NASH) developing in ovariectomized mice fed a high-fat diet. In humans, NAFLD more often affects men; and premenopausal women are equally protected from developing NAFLD as they are from cardiovascular disease. It would be expected that early menarche, definitely associated with estrogen activation, would produce protection against the risk of NAFLD. Nevertheless, it has been suggested that early menarche may confer an increased risk of NAFLD in adulthood, excess adiposity being the primary culprit of this association. Fertile age may be associated with more severe hepatocyte injury and inflammation, but also with a decreased risk of liver fibrosis compared to men and postmenopausal status. Later in life, ovarian senescence is strongly associated with severe steatosis and fibrosing NASH, which may occur in postmenopausal women. Estrogen deficiency is deemed to be responsible for these findings via the development of postmenopausal metabolic syndrome. Estrogen supplementation may at least theoretically protect from NAFLD development and progression, as suggested by

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some studies exploring the effect of hormonal replacement therapy on postmenopausal women, but the variable impact of different sex hormones in NAFLD (i.e., the pro-inflammatory effect of progesterone) should be carefully considered.

Keywords: Fibrosis; Hormones; Inflammation; Man; Menarche; Menopausal status; NASH; Physiopathology; Sex; Steatosis; Women

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) encompasses the whole spectrum of (predominantly) steatogenic liver disorders spanning steatosis through nonalcoholic steatohepatitis (NASH), cirrhosis, and hepatocellular carcinoma (HCC) [1–3], associated with striking systemic features [4, 5] and excess cardiovascular and liver-related mortality [6–9]. Histologically indistinguishable from alcoholic liver disease, and nevertheless observed in the non-alcoholic individual [10], NAFLD is closely linked with insulin resistance (IR) [11] and, bidirectionally, with the metabolic syndrome (MetS) of which it may be both a cause and a consequence [12, 13]. A leading cause of chronic liver disease worldwide and affecting one out of four individuals in the European and north-American general populations [14], NAFLD is highly prevalent in certain groups of individuals carrying either the full-blown or individual traits of the MetS [15]. Moreover, NAFLD carries an excess of health-related expenditures owing to its close connections with progressive liver disease and cardio-metabolic morbi-mortality [9, 16–19].

The pathogenesis of NAFLD has variably been conceptualized as two-hit [20], a one-hit [11], or a multistep process [21], and the last is presently the most widely accepted pathogenic model. In its original definition, a first hit leads to steatogenesis and a second one to fibrosis [22]. However, metabolic factors, and particularly IR, invariably account for most of the elementary NAFLD histologic lesions in humans, indirectly supporting the outdated and yet conceptually more parsimonious "one-hit" theory [11, 12].

Evidence both in animals and humans supports the notion that female sex is protected from dysmetabolic traits thanks to young individuals' ability to partition fatty acids towards ketone body production rather than very low density lipoprotein (VLDL)-triacylglycerol [23], and to sex-specific browning of white adipose tissue which contributes in protecting female mice from experimental NAFLD associated with methionine choline deficient diet [24].

In 1980, Ludwig reported that NASH was common among elderly women with metabolic comorbidity [25]. However, we now know that NAFLD more often affects men [15, 26] and that premenopausal women are equally protected from NAFLD development as they are from cardiovascular disease (CVD) [27, 28]. Recent studies in the overnourished zebrafish model have shown that ovarian senescence, via hypoestrogenemia, facilitates both the development of massive hepatic steatosis and the fibrotic progression of liver disease [29]. Consistently, estrogen deficiency, by potentiating hepatic inflammatory changes, hastens the progression of disease in a dietary model of NASH developing in ovariectomized mice fed a cholesterol-rich hyperlipidic diet [30]. Collectively, these findings suggest that hormonal changes, rather than those multiple physiologic derangements associated with aging per se [31], are a major determinant of progressive NAFLD in human menopause.

Obesity and obesity-related diseases, such as type 2 diabetes (T2D), MetS, and atherosclerosis, are complex conditions driven by genetic and environmental factors, in which a sexual dimorphism has been clearly established. Here, we have reviewed current evidence suggesting that NAFLD is a sexually dimorphic condition, too. We hypothesized that the higher incidence of disease in men and the worse outcome in postmenopausal women, i.e., the "sexual dimorphism" of NAFLD, might offer clues useful in expanding our understanding of the pathogenesis and providing hints for prevention and treatment of NAFLD. Given the potential research and clinical implications of sexual dimorphism in NAFLD, we carried out a

systematic review of the literature by using the following keywords: steatosis OR fatty liver AND gender OR sex OR menopause. On these grounds, this narrative review is aimed to highlight how sex modulates the development and progression of NAFLD and to pinpoint what the role of menarche and menopause is. Special emphasis is conferred to CVD risk. The relationship of polycystic ovary syndrome (PCOS) to NAFLD has recently been covered elsewhere [32] and is outside the scope of this review.

Compliance with Ethics Guidelines

This article is based on previously conducted studies and does not involve any new studies of human or animal subjects performed by any of the authors.

EPIDEMIOLOGY OF NAFLD

Gender and reproductive status modulate the risk of developing NAFLD and NASH with/without advanced fibrosis [15].

Risk of NAFLD

Incidence

Evidence from longitudinal studies suggests that the incidence of NAFLD is higher in the male as compared to the female gender (Table 1) [33–43]. One study investigating the incidence of NAFLD in women as a function of reproductive status found incidence to be higher in menopausal (7.5%)/postmenopausal (6.1%) women as compared to premenopausal (3.5%) women. However, postmenopausal status was associated with an increased risk of incident NAFLD at univariate but not at multivariate analysis adjusted for age, metabolic syndrome, and weight gain [37]. The incidence of NAFLD in women taking hormonal replacement therapy (HRT) (5.3%) was higher than in premenopausal (3.5%) women but lower than in menopausal women (7.5%). HRT was not associated with increased risk of incident NAFLD either at univariate or at multivariate analysis [37]. Indeed other longitudinal studies suggest that estrogens are a protective factor for the development of NAFLD. An Italian multicentric study on 5408 healthy women who had had hysterectomies, randomly assigned to receive tamoxifen (an estrogen inhibitor) or placebo for 5 years, showed that tamoxifen was associated with higher risk of development of NAFLD/NASH especially in overweight/obese women [44]. Finally a small double-blind, randomized placebo-controlled trial on 50 women with T2D showed that HRT containing low-dose estradiol and norethisterone significantly reduced serum levels of liver function enzymes, potentially owing to a reduced liver fat accumulation [45].

Overall, data from epidemiological longitudinal studies have shown a key role of weight gain, presence of MetS and its single traits as independent predictors of the development of NAFLD [33–43]. Notably, some studies have found that male sex was associated with incident NAFLD independently of age and metabolic factors [33, 35, 41]. Moreover, two Asian studies have reported a specific role of age according to gender and reproductive status in modulating the risk of incident NAFLD. One study showed that age was an independent predictor for developing NAFLD only in females [34] and the other one reported that age increased the risk for incident NAFLD in premenopausal but not in postmenopausal women [37].

Prevalence

Despite preliminary studies reporting a higher risk of NAFLD in females, a large body of evidence now definitely supports the notion that the prevalence of NAFLD is higher in men than in women and that gender-specific differences exist in relation to age (Table 1) [33, 37, 41, 46–77]. Conflicting with the above notion, two large sample community Iranian studies reported a higher prevalence of NAFLD in women than in men [70, 74]. However, these findings might, at least in part, be accounted for by women having markedly higher rates of prevalence of obesity compared to men in these studies. Consistent with the view reported above, a higher prevalence of fatty liver has

Table 1 Gender impacts on incident and prevalent NAFLD. Evidence from longitudinal and cross-sectional studies

Study	Method		Findings		Conclusion
	Study population	Diagnosis of FL	Incidence (longitudinal studies)	Independent predictors	
Kojima et al. [33]	35,519 Japanese subjects (health check-up), 65.2% M, mean age 45.8 years, F-up 12 years	US	14.3%	M sex (OR 1.7), metabolic factors (BMI OR 6.3)	M sex and metabolic factors are independent risk factors for the incidence of NAFLD
Hamaguchi et al. [34]	3147 healthy Japanese (1694 M), aged 21–80 years, mean F-up 414 days	US	10%; in M (14%) > F (5%)	Age (only in F), weight gain, and MetS	NAFLD is more incident in men than in women
Suzuki et al. [35]	1537 Japanese subjects (1352 M), mean age 35 years, mean F-up 60 months	AST, ALT	31/1000 person-years	M sex overall and in subjects <40 years (HR 4.6); metabolic factors	M sex and metabolic factors are independent risk factors for the incidence of NAFLD
Tsuneto et al. [36]	1635 Nagasaki atomic bomb survivors (606 M), mean age 63 years, F-up 11.6 years	US	19.9/1000 person-years (22.3 in M > 18.6 in F); M (38%) > F (25%) before 50 years	Obesity, hypertension, and high-TG (gender NS)	Gender is not an independent risk factor for incident NAFLD
Hamaguchi et al. [37]	1603 Japanese women, aged 21–80 years, mean F-up 414 days	US	5% in F; in postmenopausal (6.1–7.5%) and under HRT (5.3%) > premenopausal (3.5%)	Age (only in premenopausal), weight gain, and MetS	There is a gradient in the incidence of NAFLD in women: postmenopausal > HRT > premenopausal
Zhou et al. [38]	507 Chinese NAFLD-free participants, median F-up 4 years	US	9.1%; in M 7.3% > F 9.7%	Age, metabolic factors (gender NS)	Gender is not an independent risk factor for incident NAFLD
Zelber-Sagi et al. [39]	147 Israeli subjects, mean age 51.2 years, F-up 7 years	US	19.0% (2.7%/year)	Weight gain and HOMA (gender NS)	Gender is not an independent risk factor for incident NAFLD
Sung et al. [40]	2589 Korean subjects, mean F-up 4.37 years	US	34.7/1000 person-years; in M (23.4%) > F (9.7%)	MetS traits (gender NS)	Gender was not an independent risk factor for incident NAFLD
Xu et al. [41]	5562 non-obese Chinese subjects, mean age 43 years, F-up 5 years	US	8.9%	M sex, younger age, and metabolic factors	M sex is a risk factor for the incidence of NAFLD at multivariate analysis

Table 1 continued

Study	Method		Findings		Conclusion
	Study population	Diagnosis of FL	Incidence (longitudinal studies)	Independent predictors	
Wong et al. [42]	565 Hong Kong subjects, mean age 48 years, median F-up 47 months	^{1}H NMR	3.4%/year	MetS (M sex predictor only at univariate analysis)	M sex is a risk factor for the incidence of NAFLD at univariate analysis
Yun et al. [43]	37,130 Korean subjects, mean age (M 39.4, F 38.6 years), 46% M, F-up 2 years	US	M (44.5/1000 person-years) > F (20.4/1000 person-years)	WC gain in both sexes	Visceral obesity strongly predicts NAFLD in either gender

	Study population	Diagnosis of FL	Prevalence (cross-sectional studies)	Independent predictors	
Kojima et al. [33]	39,151 Japanese subjects (health check-up), mean age 45.8 years, 61% M	US	M (26%) > F (12.7%)	NA	NAFLD is more prevalent in M gender
Shen et al. [46]	4009 Chinese administrative officers non-drinkers, aged 20–81 years, 64% M	US	M (13.3%) > F (2.7%) before 50 years; similar after 50 years	M sex, age >50 years, and metabolic traits	M sex and metabolic factors are independent risk factors for the prevalence of NAFLD
Fan et al. [47]	3175 Chinese subjects from Shanghai, mean age 52years, 38.4% M	US	M (19.2%) > F (11.3%) before 50 years; F > M after 50 years	M sex (OR 2.7) and metabolic factors	M sex and metabolic factors are independent risk factors for the prevalence of NAFLD

Table 1 continued

	Study population	Diagnosis of FL	Prevalence (cross-sectional studies)	Independent predictors	
Chen et al. [48]	3245 adults in a Taiwan rural village, aged ≥18 years, 45.3% M	US	M (19.7%) > F (10.7%)	M sex and metabolic factors; age ≥65 years negative predictor	M sex and metabolic factors are independent risk factors for the prevalence of NAFLD
Park et al. [49]	6648 Korean subjects, aged ≥20 years, 53% M	US	M (22.6%) > F (6.8%) before 50 years; similar after 50 years	Menopause status and increasing age in F	Menopause is a strong risk factor for prevalent NAFLD
Zelber-Sagi et al. [50]	352 Israeli subjects, mean age 51 years, 53.4% M	US	M (38%) > F (21%)	M sex (after adjustment for obesity)	M sex and metabolic factors are independent risk factors for prevalent NAFLD
Zhou et al. [51]	3543 inhabitants of 6 urban and rural areas in China, aged >7 years, 37% M	US	M (13.8%) > F (7.1%) before 50 years; the opposite after 50 years	M sex and metabolic factors	M sex and metabolic factors are independent risk factors for prevalent NAFLD
Li et al. [52]	9094 Chinese subjects (medical check-up), aged >18 years, 52% M	US	M (18.9%) > F (5.7%); increased with age in both sexes <50 years	M sex, increasing age, BMI, and other MetS features	M sex and metabolic factors are independent risk factors for prevalent NAFLD

Table 1 continued

	Study population	Diagnosis of FL	Prevalence (cross-sectional studies)	Independent predictors	
Caballeria et al. [53]	766 Spanish individuals, aged 17–83 years, 33.4% M	US	M (42.2%) > F (20.3%)	M sex, increasing age, and metabolic factors	M sex and metabolic factors are independent risk factors for prevalent NAFLD
Hu et al. [54]	7152 employees of Shanghai work-units, aged 18–65 years, 60.5% M	US	M > F in the same age group, peaking at 50–65 years; in F > 50 years doubled	High TG level was the strongest predictor in M while obesity the strongest one in F	Risk factors for prevalent NAFLD are gender-specific
Wong et al. [55]	922 individuals from Hong Kong population, aged 19–72 years, 58% F	^{1}H MRS	M (36.8%) > F (22.7%); in M peaking at 40 years; in F increasing after menopause	Metabolic factors (M sex and older age NS)	Gender is not an independent risk factor for prevalent NAFLD
Hamaguchi et al. [37]	4401 Japanese subjects (health check-up), aged 21-80 years, 51.6% men	US	M (24%) and postmenopausal F (15%) > remenopausal F (6%); increased with age in F only	Postmenopause and HRT NS (age and MetS adjusted); aging in premenopausal F only (weight gain and MetS adjusted)	Menopause and HRT are not independent risk factors for prevalent NAFLD
Eguchi et al. [56]	8352 Japanese subjects (health check-ups), aged 21–86 years, 51.8% M	US	M > F at all ages	Metabolic factors (age >50 years in F only)	Gender is not an independent risk factor for prevalent NAFLD

Table 1 continued

	Study population	Diagnosis of FL	Prevalence (cross-sectional studies)	Independent predictors	
Younossi et al. [57]	11,613 American participants (from NHANES III)	US	19%	F sex and younger age for lean NAFLD; the opposite for NAFLD with BMI ≥25	F sex is a risk factor for lean NAFLD
Lazo et al. [58]	12,454 American subjects (from NHANES III), aged 20–74 years	US	M (20.2%) > F (15.8%)	Prevalence in M (20.2%) > F (15.8%) (adjusted for age, race, BMI, T2D)	M gender is associated with an increased NAFLD prevalence
Wang et al. [59]	4226 Chinese subjects >60 years vs 3145 controls <60 years from the same cohort	US	M (32%) > F (9%) before 60 years; similar after 60 years	M sex (only in those <60 years) and metabolic factors	M sex and metabolic factors are independent risk factors for prevalent NAFLD
Xu et al. [41]	6905 non-obese (BMI < 25) Chinese subjects, 63% M	US	7.3%	M sex, younger age, and metabolic factors	M sex and metabolic factors are independent risk factors for prevalent NAFLD
Yan et al. [60]	3762 Chinese residents, aged 20–92 years, 61.9% M	US	M (45%) > F (30%); higher before 50 years but similar after	Metabolic factors (M sex predicted only at univariate analysis)	M sex is a risk factor for prevalent NAFLD at univariate analysis only

Table 1 continued

	Study population	Diagnosis of FL	Prevalence (cross-sectional studies)	Independent predictors	
Chiloiro et al. [61]	2974 South Italian subjects, aged 30–89 years, 56.5% M	US	M (37%) > F (26%)	MetS and its features, BMI, visceral, and subcutaneous fat in both M and F	Risk factors for prevalent NAFLD are not gender-specific
Foster et al. [62]	3056 American multiethnic subjects, mean age 63 years, 55% F	CT	NA	F sex protective in all and AAs; not in Caucasians and Hispanics. Younger age in all, Caucasians and Hispanics; not in AAs	F sex is spared from prevalent NAFLD
Yang et al. [63]	2256 Chinese subjects (health check-up), mean age 62.04 years, 87.9% M	US	M (28.4%) > F (20.3%)	Younger age, RDW and metabolic factors (gender NS)	Gender is not an independent risk factor for prevalent NAFLD
Saida et al. [64]	3110 Japanese subjects (health check-up), aged ≥30 years, 59.5% M	US	M (45%) > F (23%)	Weight gain ≥10 kg since the age of 20 years regardless of sex	Gender is not an independent risk factor for prevalent NAFLD
Wang et al. [65]	25,032 Chinese subjects (health check-up), aged 18–94 years, 62% M	US	M (32%) > F (13%); similar in both sexes >60 years	Metabolic parameters in both sexes; increasing age (≥60 years) only in F	Risk factors for prevalent NAFLD are not gender-specific

Table 1 continued

	Study population	Diagnosis of FL	Prevalence (cross-sectional studies)	Independent predictors	
Schneider et al. [66]	4037 non-Hispanic white, 2746 non-Hispanic black, and 2892 Mexican–American adults in the NHANES III	US	M (15%) > F (10%) among non-Hispanic whites only (age-adjusted)	Gender difference still significant after adjustment for BMI and WC	M gender is associated with an increased NAFLD prevalence independent of visceral obesity
Xiao et al. [67]	18,676 Chinese subjects, mean age 40.6 years, 55.4% M	US	M (33%) > F (8%)	M sex and metabolic factors	M sex is an independent risk factor for prevalent NAFLD
Martínez-Alvarado et al. [68]	846 Mexican subjects, mean age 53 years, 49.3% M	CT	M (36.5%) > F (28.4%)	Metabolic factors (MetS in F only)	Gender is not an independent risk factor for prevalent NAFLD
Liu et al. [69]	11,200 Chinese gallstone-free subjects, 46.7% M	US	M (31%) > F (13%)	NA	NAFLD is more prevalent in M gender
Amirkalali et al. [70]	5023 Iranian participants, mean age 45.35 years, 56.7% M	US	F (46%) > M (42%); peaking in M 40–60 years, in F >60 years	Age, MetS, and its factors (higher ORs in F)	Gender is not an independent risk factor for prevalent NAFLD
Nishioji et al. [71]	3271 Japanese subjects (health check-up), 44.2% M	US	M > F for all ages, except in obese ≥70 years	Metabolic factors in M and F	Risk factors for prevalent NAFLD are not gender-specific
Huang et al. [72]	8626 Chinese subjects ≥40 years	US	M (28.7%) ~ F (28.1%)	NA	No gender specific difference in prevalent NAFLD

Table 1 continued

	Study population	Diagnosis of FL	Prevalence (cross-sectional studies)	Independent predictors	
Fattahi et al. [73]	2980 Iranian subjects, aged ≥18 years, 31.3% M	US	M (32.9%) > F (27.4%)	NA	NAFLD is more prevalent in M gender
Motamed et al. [74]	5052 Iranian subjects, aged ≥18 years, 56.6% M	US	F (44.2%) > M (40.1%)	Age, metabolic factors; F sex protective	F sex is spared from prevalent NAFLD

AAs African-Americans, *BMI* body mass index, *F* female/s, *FL* fatty liver, *F-up* follow-up, *^{1}H MRS* proton magnetic resonance spectroscopy, *HOMA* homeostasis model assessment, *HRT* hormone replacement therapy, *M* male/s, *MetS* metabolic syndrome, *NA* not assessed, *NAFLD* nonalcoholic fatty liver disease, *NHANES III* National Health and Nutrition Examination Survey III, *NS* not significant, *OR* odds ratio, *RDW* red blood cell distribution width, *T2D* type 2 diabetes, *TG* triglycerides, *US* ultrasound, *WC* waist circumference

been also observed in male obese children and adolescents than in female ones [78].

Men commonly display an increasing prevalence of NAFLD during adulthood from young to middle-age, describing an "inverted U-shaped curve" which starts declining after the age of 50–60 years [47, 56]. In women of fertile age, the prevalence of NAFLD is lower than in men owing to the putative protective effect of estrogens which, however, wanes after the menopause. Accordingly, the prevalence of NAFLD in women rises after the age of 50 years, peaks at 60–69 years, and declines after 70 years [47, 54, 56]. As a result of this, after the fifth decade, postmenopausal women compared to men of the same age have a similar [46, 49, 60, 65] or even higher [47, 51] prevalence of NAFLD. In agreement with these findings, a multicenter study from northern Italy found that men with NAFLD were approximately 10 years younger than women with this condition [79], a finding compatible with the hypothesis that premenopausal women are "protected" from developing NAFLD. Conversely, NAFLD is more prevalent in postmenopausal [37, 80–82] and PCOS-affected women than premenopausal ones [80]. Consistently, a large cross-sectional population-based survey in northeast Germany on 808 women aged 40–59 years showed that menopause status was independently associated with hepatic steatosis after adjustment for metabolic factors [83]. Interestingly, women with NAFLD exhibit a significantly lower concentration of serum estradiol, which is the principal active estrogen, than NAFLD-free (premenopausal, postmenopausal, and PCOS) controls [80]. A lower prevalence of NAFLD, as well as of MetS, has been reported in postmenopausal women receiving HRT compared to those not receiving it, which suggests that HRT probably protects from NAFLD. Moreover, in this study postmenopausal women with NAFLD who received HRT had lower frequency of insulin resistance as well as MetS and showed reduced serum levels of liver enzymes and ferritin [84].

Most studies using multivariate analysis have shown that male sex is associated with an increased prevalence of NAFLD independently of age and metabolic conditions

[41, 46, 48, 50, 51, 53, 58, 62, 63, 66, 67, 74]. Some studies have shown an independent, positive association between NAFLD and increasing age in both sexes [46, 50–53, 70, 74] or in females only [65], while others reported an inverse association [41, 48, 62, 63]. At variance, other studies have shown that sex differences in the prevalence of NAFLD were mostly accounted for by metabolic factors [36, 37, 55, 56, 60, 63, 64]. Finally, some studies failed to investigate the role of gender given that a separate multivariate analysis according to sex was not performed [34, 49, 54, 56, 61, 65, 68, 70, 71].

In addition to and independent of the role of MetS and its components, menopausal status [49, 83, 85] and increasing age [49, 56, 82] have all been consistently identified as strong risk factors for NAFLD in women. Only in a few studies was the association between menopausal status and NAFLD no longer significant after adjustment for age and metabolic factors [37, 81], indicating that menopause predisposes to developing NAFLD via incident dysmetabolic traits which typically appear in the postmenopausal age.

A recent cross-sectional study conducted in a population of postmenopausal women concluded that MetS, abdominal obesity, and IR are risk factors for the development of NAFLD while higher adiponectin levels protect from developing it [86]. These findings indicate that NAFLD modifiers in this specific population of women closely mirror those found in the general population.

In lean subjects [body mass index (BMI) less than 25 kg/m^2)] NAFLD has been associated with younger age [41, 57] and either male [41] or female gender [57].

Risk of NASH and Fibrosis Progression

One large sample study based on an electronic health file database reported that the risk of "recorded" NAFLD/NASH diagnosis increased linearly with BMI, was higher in males than females and in those with T2D [87]. However, the proportion of true NASH cases among the "recorded" diagnosis of NAFLD/NASH was unclear.

A longitudinal study in subjects with biopsy-proven NAFLD found that sex was not an independent risk factor for the progression of fibrosis [88]. In agreement, a systematic review has shown that age and hepatic necroinflammation are the only independent predictors of the development of advanced fibrosis in NASH patients, while other parameters such as MetS features and sex are not [89].

However, conflicting with the above studies, data from cross-sectional studies (Table 2) [29, 57, 65, 90–97], which are based, in the majority of cases, on a histological diagnosis of NASH, tend to suggest that the risk of NASH and advanced fibrosis is indeed higher in females than males independent of metabolic factors [65, 90, 93, 94], and only a few studies conflict with the above findings [91, 92, 95].

As is easily foreseeable, obese and postmenopausal women, compared to premenopausal and non-obese women, suffer from a remarkably higher prevalence of NAFLD and NASH as a result of a worse metabolic profile [82]. At variance, a recent study reported that, compared to men and postmenopausal women, the risk of lobular inflammation and hepatocyte injury was significantly increased both in premenopausal women and in those taking synthetic hormones such as oral contraceptives and HRT [97]. Given the supposed effects of estrogens on metabolic health and liver injury, the findings of this study appear counterintuitive. It is noteworthy that the study by Yang et al. has several limitations, including some important sources of potential bias, such as the restricted enrollment at tertiary academic centers, menopausal category and synthetic hormone use were self-reported, and information on cumulative estrogen and/or progesterone exposure and serum hormonal levels were lacking. Of note, the authors highlight that, despite increased liver injury and inflammation, premenopausal women were at decreased risk of liver fibrosis compared with men and postmenopausal women. Moreover, sensitivity analyses separately assessing the impact of progesterone use and estrogen use clearly suggested that only the former was associated with liver damage. Collectively these findings provide

Table 2 Impact of gender on NASH/fibrosis prevalence

Study	Method Cross-sectional studies		Findings		Conclusion
	Study population	Diagnosis of NASH	Prevalence	Independent predictors	
Singh et al. [90]	71 consecutive Asian-Indian NASH patients, aged 9–57 years, 76.1% M	Biopsy	NA	F sex for fibrosis stage	F sex is an independent risk factor for fibrosis
Hossain et al. [91]	432 American NAFLD patients, mean age 43.6 years, 22.9% M	Biopsy	26.8% NASH and 17.4% moderate-to severe fibrosis	M sex for NASH and moderate-to-severe fibrosis in addition to ethnicity, ALT, AST, metabolic factors	M gender is a strong independent risk factor for both NASH and fibrosis
Al-hamoudi et al. [92]	1312 Saudi Arabian inpatients, mean age 44.7 years, 51.0% M	US	16.6% NASH (by ALT >60 U/L)	M sex, young age and low total CH predicted high ALT in NAFLD	M gender is a strong independent risk factor for hypertransaminasemic NAFLD
Bambha et al. [93]	1026 adults (NASH CRN Database), mean age 48.8 years, 37% M	Biopsy	61% NASH and 29% advanced fibrosis	F sex for NASH and advanced fibrosis; increasing age for advanced fibrosis; plus metabolic factors	F sex is an independent risk factor for NASH and fibrosis
Younossi et al. [57]	11,613 American participants (from NHANES III)	US	12% NASH (by grade 2–3 US-FL + high ALT, AST, and/or T2D/IR)	Younger age and metabolic factors (gender NS)	Gender is not an independent risk factor for NASH
Tapper et al. [94]	358 NAFLD patients, 62.9% M	Biopsy	NASH and advanced fibrosis > in F vs M (45% vs 30%; 23% vs 14%)	F sex, BMI, and ALT for NASH; age, AST, and APRI for advanced fibrosis	F sex is an independent risk factor for NASH and fibrosis

Table 2 continued

Study	MethodCross-sectional studies		Findings		Conclusion
	Study population	Diagnosis of NASH	Prevalence	Independent predictors	
Wang et al. [65]	25,032 Chinese subjects (health check-up), aged 18–94 years, 62% M	US	NASH with advanced fibrosis (by BAAT score, AST/ALT) > F vs M	NA	Prevalence of NASH with advanced fibrosis is higher in F sex
Yang et al. [95]	541 American patients with NASH, mean age 48 years, 35.1% M	Biopsy	100% NASH; 22% advanced fibrosis	M sex, postmenopausal F status (premenopausal F reference; borderline P), pre-T2D/T2D for fibrosis	M sex and postmenopausal status are independent risk factors for fibrosing NASH
Turola et al. [29]	244 females and 244 age-matched males with NAFLD	Biopsy	F2–F4 fibrosis	Menopause, metabolic factors, and NASH for F2–F4 fibrosis in F with NAFLD	Menopause is strongly associated with fibrosing NASH
Klair et al. [96]	488 postmenopausal NAFLD subjects	Biopsy	Advanced fibrosis 38.4% in premature menopause F vs 32.7% in other F	Premature menopause and time from menopause for advanced fibrosis (adjusted for age, race, metabolic factors)	The longer the duration of postmenopausal status the higher the risk of NASH
Yang et al. [97]	1112 American NAFLD patients (160 premenopausal and 489 postmenopausal F; 463 M)	Biopsy	NASH > in pre/postmenopausal F (62%) vs M (50%)	Lobular inflammation risk increased in (1) premenopausal F > M and postmenopausal F, (2) oral contraceptives and HRT users (adjusted for covariates of liver metabolic stress)	Fertile age and estrogen use may predispose to more necroinflammatory NASH variants

ALT alanine aminotransferase, *AST* aspartate aminotransferase, *APRI* AST-to-platelet ratio index, *BMI* body mass index, *F* female/s, *FL* fatty liver, *F-up* follow-up, *HRT* hormone replacement therapy, *M* male/s, *MetS* metabolic syndrome, *NA* not assessed, *NAFLD* nonalcoholic fatty liver disease, *NASH* nonalcoholic steatohepatitis, *NHANES III* National Health and Nutrition Examination Survey III, *NS* not significant, *T2D* type 2 diabetes, *US* ultrasound, *WC* waist circumference, *years* years

novel hints regarding a potential multifaceted impact of sex hormones in NAFLD.

Consistent with the hypothesis that estrogens do exert a beneficial effect on NAFLD, menopause has been independently associated with significant fibrosis both in women with NAFLD and in an experimental zebrafish steatosis model [29]. A recent study has shown that men display a higher risk of advanced fibrosis compared to premenopausal women, while after menopause both sexes show a similar severity of liver fibrosis, suggesting that estrogens protect from the development of fibrosis [95]. A subsequent study limited to non-obese women with biopsy-proven NAFLD confirmed that, even in non-obese NAFLD patients, postmenopausal women still had more severe fibrosis, when compared to premenopausal subjects [98]. Accordingly, a study conducted in 488 postmenopausal women with biopsy-proven NAFLD has shown that, independently of age and metabolic factors, the longer the estrogen deficiency in postmenopausal status is (i.e., premature menopause and time from menopause), the higher the risk of fibrosis is [96].

The role of gender in influencing liver-related mortality in NAFLD is still uncertain, although some studies have reported that men are at an increased risk [99–101].

Risk of Hepatocellular Carcinoma

A universal feature of HCC is the striking male prevalence, with a male/female ratio averaging 2:1 to 7:1; the latter proportion is more often found in HBV-related HCC [102]. Nevertheless, compared to viral etiologies of HCC, NAFLD-related HCC was found to be associated with the lowest male/female ratios in one study [103]. NAFLD-related HCC may also occur in non-cirrhotic livers, but this seems to be more likely in men [104]. The sexual dimorphism in HCC is also maintained regarding prognosis, with women showing better survival rates [102, 105]. However, menopause seems to attenuate these advantages [102, 105].

In summary, the incidence of NAFLD is higher in men than in women, and some longitudinal studies indicate that male gender is an independent predictor of NAFLD development. The prevalence of NAFLD is globally higher in men than women, but after menopause women display a similar or even a higher prevalence compared to men, a finding supporting a protective effect of estrogens. In cross-sectional studies, male gender and menopausal status have often been associated with the risk of NAFLD, independent of age and metabolic factors. The prevalence of NASH and advanced fibrosis has been found to be higher in postmenopausal women than in men; however, longitudinal studies have failed to support a role for gender in influencing the progression of liver fibrosis. Finally, HCC is definitely more common in men than in women in cases due to viral etiology; NAFLD may probably lower the male/female ratio in the risk of developing HCC and it is possible that gender affects the prognosis of HCC.

NAFLD AND CVD

NAFLD is increasingly recognized as a multisystem disease [5]. A growing body of evidence suggests that NAFLD (assessed by liver enzymes, imaging, or biopsy) is associated with increased incidence and prevalence of subclinical and clinical CVD, mainly coronary heart disease (CHD), independently of age, gender, and metabolic factors, as recently reviewed in detail elsewhere [106, 107]. In the general population, male sex is more prone to incident CHD under the age of 50 years compared to women but, after menopause, the incidence in women dramatically increases to approach that of men [108].

Gender-Specific Risk of CVD in Studies on NAFLD and CVD

Several longitudinal studies investigating the association between CVD and NAFLD have reported multivariate analysis models, which have been adjusted for multiple confounders, including sex; as a result of this, the influence of gender in the risk of CVD cannot be evaluated in such studies (reviewed in [106, 107]). A large

population-based American study (NHANES III) found that NAFLD assessed with ultrasound, together with male sex, age, race, and metabolic factors independently predicted incident CVD [109]. Another study addressing CHD as a pre-specified outcome found that patients with NAFLD had a higher 10-year risk for CHD, as calculated by the Framingham risk score, than the matched control population. This estimated risk was higher in men than in women and more CVD events were reported among men than women at the end of 10 year follow-up, although this finding was not statistically significant [110].

Many cross-sectional studies in which the diagnosis of NAFLD was based on liver imaging studies (either ultrasound or CT scanning) showed an independent association of male sex with coronary artery calcifications [111] as well as significant CHD [112–115].

Gender Differences in the Association Between NAFLD and CVD

Studies in which the diagnosis of NAFLD was based on surrogate indices, such as otherwise unexplained raised liver enzymes, found that sex modulates the association of NAFLD with incident CVD/mortality. For example, most population-based cohort and meta-analytic studies reported an independent association between raised GGT and incident CVD in both sexes [116]; conversely, a population-based cohort study from Germany found that increased GGT was associated with higher risk of all-cause and CVD mortality only in men but not in women, and this association was stronger in men who also had ultrasound scanning findings compatible with steatosis [101]. Increased ALT has been variably associated with incident CVD either in both sexes [117, 118] or in men only [119]. A recent study found that ALT levels independently predicted insulin sensitivity only in women, suggesting that this gender-specific association might explain the sex difference in the predictive role of increased ALT for CVD [120]. Finally, a recent large Korean cross-sectional study reported that men had more prevalent ultrasonographic fatty liver disease, carotid plaques, and increased carotid intima-media thickness (IMT) values than women, but ultrasonographic fatty liver disease independently predicted subclinical carotid atherosclerosis (IMT and plaques) in women only [121].

No gender difference has been reported in the association between NAFLD assessed with liver ultrasound and incident CVD/mortality. A study based on a national Danish registry showed that patients with a hospital discharge diagnosis of NAFLD had a higher all-cause mortality, including liver and CVD related, which was similar among sexes [122]. A study conducted in a community-based Japanese cohort of 1637 apparently healthy subjects found that the diagnosis of NAFLD based on ultrasound findings was a predictor of CVD in both men and women [123].

The only study carried out in post-menopausal women found a significant correlation between NAFLD (based on CT scanning findings) and prevalence of coronary artery calcification (CAC); however, NAFLD was not independently associated with CAC in these postmenopausal women [124].

In summary, male patients carry an increased risk of CVD; moreover, NAFLD seems to be associated with CVD independently of metabolic factors in both sexes. Few data are available in postmenopausal women and studies should specifically be conducted to ascertain whether NAFLD is a specific/independent cardiovascular risk factor in this population of patients.

ROLE OF GENDER, REPRODUCTIVE STATUS, AND AGE IN THE HETEROGENEITY OF NAFLD PATHOBIOLOGY

Although NAFLD may be found in either gender from infancy to old adulthood, gender and reproductive status modulate the susceptibility to development and progression of disease [125]. Indeed age, sex, and fertility exert a variable impact on those general pathogenic mechanisms which are involved in NAFLD.

Here we will specifically examine how body fat distribution, obesity and local hypercorticolism, steatogenesis and lipidomics, oxidative stress and antioxidant mechanisms, endotoxins, immune response, and fibrogenesis are affected by gender and sex hormones as a result of reproductive status and age.

Factors Associated with NAFLD are Different in Men and in Women

A pioneering study supporting the notion that gender-dimorphic risk factors are associated with NAFLD was published in 2000. This study, by evaluating 199 individuals, found that elevated BMI was an independent predictor of fatty liver in either sex. Glucose area under the curve and a central-type body fat distribution predicted fatty liver only in women [126]. Similarly, insulin sensitivity has also been reported to be strongly associated with gender-dimorphic risk factors, i.e., fasting insulin and leptin levels (but none of the liver enzymes) in men versus BMI and ALT in women [120]. Of note, Suzuki et al. demonstrated that the associations between anthropometric measures (regional adiposity) and degree of fibrosis clearly differ between premenopausal women and postmenopausal women/men [127].

NAFLD Epidemiology and Physiopathology are Modulated by Age at Menarche and Postmenopausal Status

In women, a complex interaction including genetic polymophisms, dietary habits, endogenous sex hormones, age at menarche, menopausal status, dysmetabolic traits, and HRT modulates the risk of developing NAFLD, NASH, and fibrosis [84, 128–130].

Early menarche has been associated with higher alanine aminotransferase, C-reactive protein, triglyceride levels, BMI, waist circumference, adult diabetes, cardiovascular morbidity and mortality, advanced liver disease, and HCC [131]. A recent Chinese study conducted in postmenopausal women has identified early menarche as a potential risk factor for NAFLD later in life; consistently, late menarche protects from NAFLD [130]. These associations were significantly attenuated after adjustment for current BMI or HOMA-IR, suggesting that obesity and insulin resistance may partly mediate the association between age at menarche and NAFLD [130]. The biological mechanism linking early menarche with increased risk of NAFLD is far from being clearly elucidated. It has been suggested that early maturation may determine a longer duration of positive energy balance and a greater accumulation of body fat [132]. Consistently, a large cross-sectional study among middle-aged Korean women confirmed that the inverse association between age at menarche and NAFLD was partially mediated by adiposity [133]. Again, a recent study from the American CARDIA cohort showed that early menarche was associated with NAFLD and visceral and subcutaneous abdominal ectopic fat depots in middle adulthood; these associations were attenuated after adjustment for weight gain between young and middle adulthood [131]. Finally, a Chinese study suggested that the presence of central obesity and MetS, but not NAFLD, after menopause was predicted by longer duration of menstruation and early menarche [134].

Compared to the fertile age, menopause increases the risk of NAFLD and liver fibrosis [135] via long-standing estrogen deficiency associated with ovarian senescence and dysmetabolic features such as T2D, hypertriglyceridemia, and central obesity [29, 81]. Consistently, HRT protects from NAFLD development [84], and oophorectomy in young women with endometrial cancer independently increases the risk of NAFLD together with the development of T2D and hypercholesterolemia [136]. These findings are in agreement with a study suggesting that, in HCV infection, increasing severity of fibrosis is associated with a higher BMI, advanced steatosis, and the menopause and that, conversely, menopausal women receiving HRT exhibit a lower stage of fibrosis [137]. Collectively, these data support the notion that estrogens have antifibrogenic properties in humans. This antifibrotic activity may occur by triggering anti-inflammatory, antioxidant, and antiapoptotic molecular

pathways [138], which are mimicked by exercise training [139].

Of concern, however, young women in their reproductive age and those exposed to female synthetic hormones (oral contraceptive or HRT) are not completely spared the risk of developing NAFLD and, indeed, they tend to have more severe hepatocyte injury and inflammation. Notably, despite the possibility of enhanced hepatocellular damage, premenopausal women have been consistently reported at decreased risk of liver fibrosis compared to men and postmenopausal women. Moreover, sex hormones exert complex and variable effects on human NAFLD; indeed, the detrimental pro-inflammatory impact may be conveyed by progesterone, but not estrogen [97].

Table 3 summarizes the physiological role of estrogen, progesterone, and androgens according to gender, age, reproductive status, and obesity [140–157].

Obesity and Local Hypercortisolism

Obesity, which is closely linked with NAFLD, mimics hypercortisolism. It is of interest that despite the prevalence of obesity being higher among women than men, the former are somewhat protected from the associated cardiometabolic consequences; however, this wanes after menopause, suggesting a role for estrogens. Mouse models suggest that sexually dimorphic expression and activity of glucocorticoid metabolizing enzymes may have a role in the differential metabolic responses to obesity in males and females [158].

Biochemical, genetic, and therapeutic studies have provided robust evidence for 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) being key in the pathogenesis of NAFLD [159–161]. It is of interest, therefore, that 11β-HSD1 gene expression is regulated in a tissue-specific and sexually dimorphic manner. In particular, intact rats exhibit hepatic 11β-HSD1 mRNA levels 18-fold lower in the female than the male [162].

Body Fat Distribution

Regional adiposity displays a typical sexual dimorphism in humans. Women have a larger capacity to store fat in the subcutaneous compartment [163]. Men generally have twice as much visceral fat compared to women for any given fat mass value [164]. This is relevant given that, compared to subcutaneous adipose tissue depots, visceral adipose tissue depots in general display greater secretory capacity and a more pro-inflammatory profile [165]. Moreover, visceral adipose depots release free fatty acids (FFAs) directly into the portal blood and thus potentially overload the liver [166]. Not surprisingly, those phenotypes which occur whenever, due to hormonal effect, peripheral adiposity is relatively restricted and visceral adiposity is expanded (i.e., male obesity and postmenopausal women) have all been associated with NASH, and fibrosing NASH [127, 167–169].

Steatogenesis and Lipidomics are Affected by Sex Hormones

Steatosis will invariably occur as a result of an imbalance among enhanced steatogenesis and decreased capacity of oxidation of fatty acids [170]. Moreover, qualitative changes in the hepatic lipidomics may concur with lipotoxicity. Data suggest that all these mechanisms are under the control of sex hormones. For example ovariectomy in rats is associated with increased intrahepatic steatogenesis which occurs through a decreased synthesis of peroxisome proliferator-activated receptor (PPAR), and an increased transcription of genes encoding sterol regulatory element-binding protein 1 [(SREBP-1), a nuclear transcription factor and master regulator of the endogenous synthesis of cholesterol, fatty acids, triglycerides, and phospholipids] and stearoyl coenzyme A desaturase 1 [(SCD1), the rate-limiting enzyme for generating monounsaturated fatty acids (MUFA) from saturated fatty acids protects from hepatocyte lipotoxicity]; all these effects of

Table 3 Physiological role of hormones

	Men	Women	Both sexes	Obesity/type 2 diabetes
Estradiol	Estradiol in men is essential for modulating libido, erectile function, and spermatogenesis [140] A study conducted in healthy men suggests that estradiol protects from NAFLD [141]	Endogenous estrogens are master regulators of lipid metabolism and inhibit inflammation, vascular cell growth, and plaque progression in premenopausal women [142] The loss of estrogens which occurs postmenopausally is associated with a modest increase in LDL cholesterol with either no change or a small decrease in HDL cholesterol. Estrogen administration decreases LDL cholesterol and Lp(a) levels while increasing triglycerides and HDL cholesterol levels, but these effects are dependent on the dose and route of administration [143]	Estrogens improve inflammation related to metabolic dysfunction ("metaflammation"). Further to a direct downregulation of inflammatory pathways, this effect is also mediated by metabolic amelioration [144]	Obesity is associated with hyperestrogenism which, in turn, increases the risk of breast cancer in men [145]
Progesterone	Progesterone has important effects in regulating male fertility by affecting the energetic homeostasis of sperm [146]	Progesterone has a major role in the ovarian and menstrual cycle; moreover it exerts an immuno regulatory function; regulates the contraction of human intestinal smooth muscle cells and the motility of various human cell types [147] Progesterone is an independent predictor of insulin resistance in girls [148]	Progesterone has been potentially implicated as a therapeutic adjunct in many clinical conditions such as traumatic brain injury, Alzheimer's disease, and diabetic neuropathy [147]	Increasing levels of progesterone have been associated with the development of systemic insulin resistance [149] Little is known regarding the role, if any, of serum progesterone in NAFLD

Table 3 continued

	Men	Women	Both sexes	Obesity/type 2 diabetes
Androgens	The synthesis of testosterone is key to male fertility. A negative feedback finely regulates the secretion of hormones at the levels of hypothalamic-pituitary–gonadal axis. Congenital or acquired disturbances of this axis will lead to hypogonadism and thus impair male fertility [150]. Testosterone has no significant correlation with NAFLD in a study from China [141]. However, it is an independent predictor of insulin resistance in boys [148]	Androgens have important biological roles in young women, influencing bone and muscle mass, vascular health, cognition, mood, well-being, and libido [151] However, testosterone deficiency in young women may pass underdiagnosed because of generally nonspecific symptoms and inaccuracy of testosterone measurement [152]. The primary indication for the prescription of testosterone for women is loss of libido [153]	Sarcopenia, namely the decline in muscle mass and strength which occurs with ageing, has been associated with a deficiency in both 17β-estradiol and testosterone, two sex hormones which act on satellite cells. These remain quiescent throughout life and are activated in response to stressful events, enabling them to guide repair and regeneration of the skeletal muscle [154]	Obese men tend to be hypogonadic as a result of the functional suppression of the hypothalamic–pituitary–testicular axis [155]. However, weight loss obtained with either a low-calorie diet or bariatric surgery is associated with the normalization of sex hormone levels exhibiting a significant increase in bound and unbound testosterone and gonadotropin levels and a decrease in estradiol [156]. A study from Japan reported that testosterone levels were inversely associated with diabetes among men but not among women [157]

ovariectomy are prevented by 177β-estradiol replacement, indicating a role for estrogens in the prevention of hepatic fat accumulation [171]. Consistently, hypoestrogenemia is associated with hepatic steatosis through changes in gene expression of molecules related to fat oxidation and lipogenesis; resistance and endurance training prevent this both in rats and human models [172–175]. Moreover, a normal activity of stearoyl-CoA desaturase (SCD), the rate-limiting enzyme for generating monounsaturated fatty acids from saturated fatty acids, protects from hepatocyte lipotoxicity. Indeed, in mouse models of NAFLD, either genetic manipulation or dietary changes that inhibit the activity of SCD promote fibrosing NASH via hepatocyte lipotoxicity, despite inhibiting obesity and improving insulin resistance [176, 177]. Interestingly, ovarian hormones are also involved in MUFA biosynthesis, via SCD1 [178].

Excess 16:0 fatty acids associated with de novo lipogenesis from high carbohydrate diet inhibits the synthesis of highly unsaturated fatty acids; this may potentially account for the improved metabolic profile which results from supplementing a hypercaloric diet with preformed eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) [179]. For example, findings from a genetically engineered NASH mouse model fed a Western diet have shown that dietary DHA was superior to EPA in attenuating Western diet-induced hyperlipidemia and hepatic injury and at reversing those deleterious metabolic and histological effects induced in the liver by a Western diet [180]. Interestingly, human (women using the contraceptive pill or HRT and transsexual subjects) and experimental data in rats consistently indicate that sex hormones act to modify plasma and tissue n-3 PUFA content, possibly by altering the expression of desaturase and elongase enzymes in the liver [181]. Collectively, these findings suggest that enzyme activities which are key in the development of a potentially hepatotoxic lipidomic signature are critically controlled by sex hormones. Finally, it is of clinical importance that the therapeutic activity of PPAR-α agonists in obesity and fatty acid oxidation is modulated by sex and estrogens [182].

Oxidative Stress and Antioxidant Mechanisms

Reactive oxygen species (ROS) result from the oxidation of fatty acids within mitochondria and peroxisomes. The antioxidant defenses which physiologically constrain oxidative stress are hindered by nutritional deficiencies or changes in the intestinal microbiome that limit availability of choline [183]; by aging and the content of cysteine in diet, which, in turn, will affect the intrahepatic synthesis of glutathione [184]; and by sex and menopause which both affect the normal metabolism of choline [185].

Endotoxin

Quali-quantitative changes in intestinal microbiota (dysbiosis), which are often associated with dietary indiscretions, have consistently been associated with increased gut permeability which could lead to increased translocation of bacterial products from the intestinal lumen into the portal circulation, thereby triggering chronic inflammation [170]. It this context, it is relevant that in an experimental model of liver failure due to endotoxemia after hepatectomy, sexually mature female rats are more exposed than males to endotoxin-induced liver injury and that ovariectomy abrogates this sexual dimorphism [186]. Whether this also applies to human NAFLD, however, remains to be proven.

Interindividual Variation in Immune Responses is Another Key Liver Disease Modifier in NAFLD

Whether metabolic inflammation is a gender-dimorphic phenomenon has not been explored in a systematic and organized manner. Obesity, however, will typically exhibit changes in the innate and adaptive immune mechanisms [187]. However, how mechanistically such obesity-related dysregulation of immune defenses will impact on the pathogenesis of NASH is not fully elucidated. Experimental data obtained in the *ob*/*ob* mouse model suggest a role for natural killer T cells (NKT cells) which may at least partly account for the

interindividual variation in immune responses being a key modifier in the development and progression of NAFLD [125]. In this respect, it should be highlighted that sex is a major determinant in the immune response. Supporting this notion, one study in young children reported that immune responses to vaccines were consistently higher or equivalent in girls compared with boys [188].

Liver Fibrosis

Liver fibrosis is the end-stage result of various liver injuries. In recent times, importance has been given to the Hedgehog signaling pathway on the grounds that activation of this pathway will stimulate the proliferation and differentiation of hepatic stellate cells (HSCs) as myofibroblasts (MF-HSCs), and, conversely, inhibiting Hedgehog activity in myofibroblasts derived from HSCs causes them to revert to a more quiescent (namely less fibrogenic) phenotype [125]. Accordingly, Hedgehog ligands and other factors that control fate decisions in HSCs are critical determinants of the development of cirrhosis due to various causes as well of the course of NASH [125]. For example, hepatic expression of Hedgehog ligands and Hedgehog pathway activity progressively increase from simple steatosis, to NASH, and reach highest levels in NASH-cirrhosis [189]. Of major significance to the pathogenesis of NAFLD, the Hedgehog pathway is strongly regulated by lipids [190], and conversely, Hedgehog signaling is a master regulator of body fat distribution and glucose metabolism [191, 192]. These findings suggest that interindividual variation in Hedgehog signaling might contribute to variability in both hepatic and extrahepatic outcomes of the metabolic syndrome [125].

Experimental overexpression of Hedgehog ligand in hepatocytes is able to induce a fibrogenic liver response and to promote hepatocarcinogenesis in a transgenic mouse model [193]. At least in part by modulating intermediary metabolism [194, 195], Hedgehog also interacts with pathways which regulate growth and differentiation [194, 196–198], such as Wnt/β-catenin signaling, which are of potential significance for the development of hepatocellular carcinoma [199].

Treatment with compounds, e.g., vitamin E, that suppress Hedgehog ligand production and reduce the hepatic accumulation of Hedgehog-responsive myofibroblasts has proven beneficial in human NASH [200].

Further studies are necessary to establish whether and to what extent sex hormones affect Hedgehog signaling and how manipulation of cellular energy homeostasis might be exploited to prevent and manage fibrosing NASH, cirrhosis, and HCC in individuals with NAFLD.

CAN GENDER DIMORPHISM OF NAFLD BE EXPLOITED FOR THERAPEUTIC PURPOSES?

In principle, sex differences found in NAFLD may be accounted for by the effects of sex chromosomes; sex hormones; and by differences in dietary and lifestyle habits [201, 202]. A better understanding of the physiopathological peculiarities of NAFLD in women may contribute to developing tailored therapeutic interventions [203]. Accordingly, the roles of estrogen and HRT, the metabolism of choline, and the effect of weight reduction and exercising in women are discussed in detail hereafter.

Estrogens

As detailed in Table 3, estrogens exert several beneficial metabolic effects. Experimental studies suggest that estrogens promote the accumulation of peripheral gluteofemoral subcutaneous adipose tissue and, within the liver, promote FFA beta-oxidation and prevent the accumulation of triglycerides; moreover, they regulate energy homeostasis, enhance insulin sensitivity, and exert a protective role on the function of pancreatic beta-cells [201]. Finally, estrogens seem to have antisteatotic, antioxidant, and antifibrogenic properties in the liver [204, 205]. Estrogens may protect from liver steatosis and fibrosis in female mice via upregulation of miRNA-125b and miRNA-29,

respectively [206, 207]. A recent experimental study showed that the estrogen/estrogen receptor alpha signaling plays essential roles in ROS detoxification and in the reduction of oxidative damage in the liver via partnering with hepatic PPARG coactivator 1 alpha, thus halting the transition from simple steatosis to steatohepatitis [205]. Estrogens are also known to inhibit stellate cell activation and fibrogenesis in experimental models [204]. Of note, a study showed that the natural estrogen 17β-estradiol prevents deoxycholic acid-induced hepatocellular damage in several cell lines. This hepatoprotective effect of estrogen was sustained by mechanisms which were unlikely to be mediated by nuclear estrogen receptor alpha [208]. Interestingly, it has recently been demonstrated that a non-nuclear estrogen receptor plays a key role in reducing hepatic steatosis in female mice [209]. Similarly, estrogen deficiency leads to fat redistribution toward visceral fat accumulation and its inherently unfavorable metabolic derangements, and is thus able to induce the development and progression of NAFLD/NASH both in mice and humans. Of note, both aromatase knockout mice, which are unable to synthesize endogenous estrogen, and estrogen receptor alpha knockout mice develop hepatic steatosis [210–212]. Patients with breast cancer treated with tamoxifen, a potent estrogen antagonist, develop progressive liver steatosis and NASH [213]. Surgical and physiological menopausal status have been invariably associated with excess risk of NAFLD progression and liver fibrosis, and duration of estrogen deficiency has been directly related to increased fibrosis risk in postmenopausal women with NAFLD [95, 96, 98, 136].

Hormonal Replacement Therapy

Despite the above premises, the role of HRT in reverting the metabolic alterations associated with low levels of estrogens is a matter of dispute. In animal models, both estrogen and raloxifene, a second-generation selective estrogen receptor modulator, have improved inflammation and ballooning so halting the progression of liver fibrosis in diet-induced NASH in female ovariectomized mice [30, 214]. However, the theoretical benefits of HRT in liver disease remain to be proven in humans. A seminal randomized placebo-controlled study suggested that HRT containing low-dose estradiol and norethisterone was able to reduce liver enzyme concentrations in women with T2D, potentially through the reduction of accumulation of fat in the liver [45]. Accordingly, a north American study using data of the NHANES III survey showed that postmenopausal women who received HRT had a significantly lower risk of NAFLD than postmenopausal women who did not (OR 0.69, 95% CI 0.48–0.99) [215]. However, whether HRT reduces the risk of NASH and/or fibrosis among postmenopausal women remains uncertain. Two studies showed no, or borderline, beneficial effects of HRT on fibrosis among postmenopausal women with NAFLD [95, 96]. Worryingly, a recent study reported that synthetic hormone use was associated with more severe hepatocellular injury and lobular inflammation. Further analysis showed that progesterone, but not estrogen use, was associated with worse liver histological lesions, suggesting a multifaceted impact of sex hormones on NASH features [97].

Choline

Several lines of evidence support a key role of choline as an essential nutrient and cellular component. Choline is the major source of methyl groups in the diet and is a precursor of phosphatidylcholine, which is an important component of cell membranes and VLDL, required for the export of lipids from the liver into the bloodstream. Thus, depletion of choline inhibits hepatic triglyceride export and induces fatty liver in both experimental models and humans [185, 203, 216, 217]. Of note, postmenopausal women are more prone than premenopausal women and men to develop NAFLD as a consequence of choline deficiency [128, 185, 217], and decreased dietary choline intake has been significantly associated with increased fibrosis in postmenopausal women

with NAFLD [218]. Indeed, menopausal status determines a differential susceptibility to choline deficiency arising from the estrogen-inducible expression and activity of phosphatidylethanolamine-*N*-methyltransferase (PEMT), a key enzyme in de novo choline biosynthetic pathway. Following menopause, the endogenous supply of choline decreases as a result of estrogen deficiency; consequently, the dietary requirement of choline is increased [185, 203, 216, 217]. Consistently, choline supplementation has been reported to improve or revert liver steatosis in patients receiving long-term total parenteral nutrition [219]. However, no interventional studies have addressed whether choline supplementation is able to prevent NAFLD or reduce NAFLD progression; moreover, future studies investigating the role of preventive or therapeutic choline supplementation in postmenopausal women are eagerly awaited.

Weight Reduction and Exercise

Lifestyle changes, i.e., physical activity and balanced diet, are the milestone of intervention for NAFLD treatment. In general, weight loss induced by lifestyle changes improves IR and features of the MetS, including NAFLD. Of note, a modest weight loss of as little as 2–3 kg is associated with NAFLD reversal [39], and weight loss of greater than 7–10% significantly improves histological features of NASH, including fibrosis [220]. However, significant weight reduction achieved through dietary restrictions results in negative effects on lean muscle and bone mass in postmenopausal women. Therefore, while weight reduction in postmenopausal women with metabolic abnormalities and risk factors for NAFLD should be strongly recommended, special attention should be paid to how weight loss is obtained in older women to prevent the worsening of loss of lean mass [139, 203]. An integrated approach consisting of dietary changes along with regular exercise is mandatory to prevent the untoward effect of losing lean mass. Both resistance training and aerobic exercise are effective in preventing muscle and bone loss during weight loss [203], and equally reduce liver fat content [221, 222]. Postmenopausal women with higher levels of physical activity seem to have lower total body and visceral fat and to be less likely to gain fat mass during menopause [139, 223]. However, only few studies have specifically assessed the role of physical activity in NAFLD prevention/reversal in postmenopausal women. Data have shown that exercise training effectively reduces liver enzymes in overweight postmenopausal women, probably as a reflection of liver fat content [224], and aerobic physical activity reduces cardiovascular risk factors in postmenopausal women with NAFLD [225]. Further studies are needed to define the ideal structure and duration of exercise-based interventions in postmenopausal women with NAFLD or at risk of developing it.

In summary, there are no codified therapeutic interventions approved for tackling NAFLD in women. Given the theoretical beneficial metabolic and hepatic effects of estrogens, it is intriguing to speculate that HRT might possibly play a role in the prevention and treatment of metabolic alterations associated with menopause, including NAFLD. However, studies in humans are lacking and the potential metabolic benefits should be weighed, in clinical practice, against the detrimental cardiovascular and neoplastic risk associated with long-term HRT [226, 227]. Moreover, the multifaceted and variable impact of different sex hormones in NAFLD should be carefully considered. Dietary changes associated with either aerobic or resistance physical exercise should be considered as the milestone of treatment of NAFLD. Future studies will have to evaluate the effects on NAFLD prevention/treatment of supplementing choline to a balanced diet in postmenopausal women. Finally, the physiopathological peculiarities of NAFLD in women and the gender-based differences in the kinetics and dynamics of drugs and xenobiotics should be taken into account when developing new treatment strategies and proposing interventional trials for NAFLD.

CONCLUSION

Consistent with the notion that NAFLD and NASH are strongly linked with hormonal

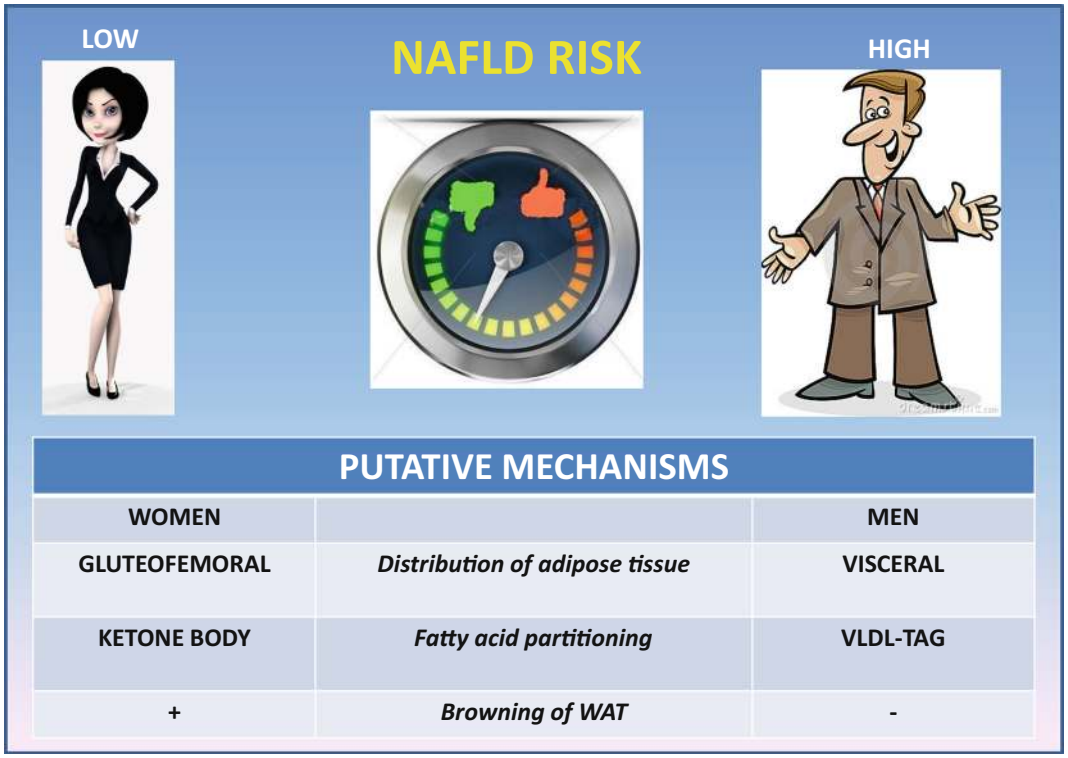

Fig. 1 Physiopathological grounds accounting for male sex as a strong predictor of NAFLD. Male gender and menopausal status have been associated with the risk of NAFLD independently of age and metabolic factors in cross-sectional studies. On the basis of human studies and extrapolation of notions from animal studies, it can be speculated that female sex is protected from dysmetabolism thanks to young individuals' ability to partition fatty acids towards ketone body production rather than VLDL-TAG [23], and to sex-specific browning of white adipose tissue which contributes in protecting female mice from experimental NAFLD associated with methionine choline deficient diet [24]. However, after menopause women display a similar or even higher prevalence of NAFLD compared to men, supporting a protective effect of estrogens. Finally, risk factors associated with NAFLD development are different in men compared to women. *TAG* triacylglycerols, *WAT* white adipose tissue

influences [228], this narrative review article was driven by the hypothesis that what we called the "sexual dimorphism" of NAFLD might be useful in identifying clues of pathogenic significance and providing hints for prevention and treatment of NAFLD. On these grounds, a systematic research of the literature was conducted. What we found was that not only are men at an increased risk of developing NAFLD (Fig. 1) but also significant age-related changes in NAFLD epidemiology in women may potentially bear physiopathological, clinical, and therapeutic significance. NAFLD epidemiology and physiopathology are modulated by age at menarche and postmenopausal status (Fig. 2). It would be expected that early menarche, definitely associated with estrogen activation, would produce protection against the risk of NAFLD. Nevertheless, it has been suggested that early menarche may confer an increased risk of NAFLD in adulthood, excess adiposity being the primary culprit of this association. Fertile age may be associated with more severe hepatocyte injury and inflammation, but also with a decreased risk of liver fibrosis compared to men and postmenopausal status. Ovarian senescence is strongly associated with severe steatosis and fibrosing NASH which may occur in postmenopausal women. Estrogen deficiency is deemed to be responsible for these findings via the development of postmenopausal metabolic syndrome. Estrogen supplementation may at least theoretically protect from NAFLD development

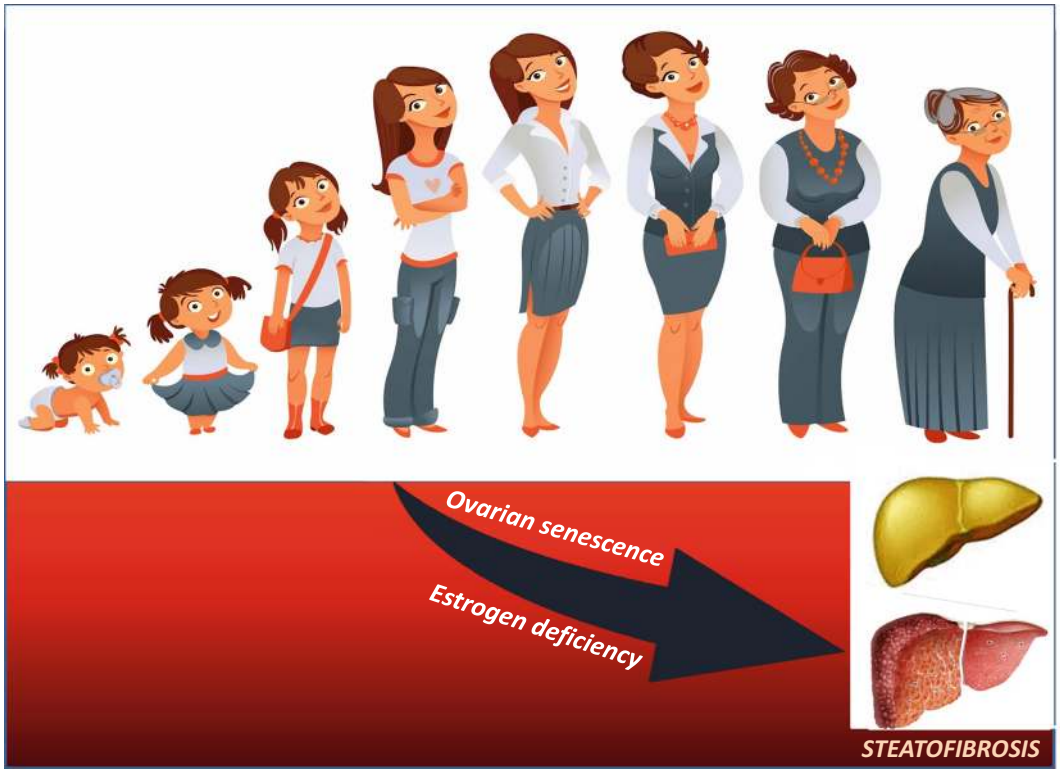

Fig. 2 Hormonal changes are a major determinant of progressive NAFLD in human menopause. NAFLD epidemiology and physiopathology are modulated by age at menarche and postmenopausal status. For example, early menarche may confer an increased risk of NAFLD in adulthood partly mediated by excess adiposity [130, 131, 133]. Moreover, ovarian senescence, via estrogen deficiency, may eventually be conducive to both massive liver steatosis and its fibrotic evolution via dysmetabolic traits including T2D, hypertriglyceridemia, and visceral obesity which are often found postmenopausally [29, 81, 135]

and progression, as suggested by some studies exploring the effect of HRT on postmenopausal women, but the variable impact of different sex hormones in NAFLD (i.e., the pro-inflammatory effect of progesterone) should be carefully taken into account when proposing treatment with synthetic hormones.

Taken collectively, these data may generate innovative hypotheses to be tested in appropriate clinical and experimental studies on NAFLD physiopathology and treatment.

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study evaluating the Safety and Efficacy of Obethicolic Acid in Subjects with NASH" (EudraCT 2015-002560-16). Alessandra Marrazzo is involved as a researcher in the "Phase 3, Double Blind, Randomized, Long-Term, Placebo-controlled, Multicenter study evaluating the Safety and Efficacy of Obethicolic Acid in Subjects with NASH" (EudraCT 2015-002560-16). Amedeo Lonardo is involved as a researcher in the "Phase 3, Double Blind, Randomized, Long-Term, Placebo-controlled, Multicenter study evaluating the Safety and Efficacy of Obethicolic Acid in Subjects with NASH" (EudraCT 2015-002560-16). Dante Romagnoli serves as a consultant for AbbVie.

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Data Availability. All data generated or analyzed during this study are included in this published article.

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