1 **Sexual dimorphism of metabolic dysfunction-associated steatotic liver**

2 **disease**

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Abstract

Metabolic dysfunction-associated steatotic liver disease (MASLD) is the most common chronic liver condition. MASLD is a sexually dimorphic condition, with its development and progression influenced by sex chromosomes and hormones. Estrogens typically protect against, while androgens promote MASLD. Therapeutic approaches for a gender-specific personalized medicine include estrogen replacement, androgen blockers, and novel drugs targeting hormonal pathways. However, the interactions between hormonal factors and inherited genetic variation impacts MASLD risk, necessitating more tailored therapies. Understanding sex disparities and the role of estrogens could improve MASLD interventions and management, while clinical trials addressing sex differences are crucial for advancing personalized treatment. This review explores the underappreciated impact of sexual dimorphism in MASLD and discusses the potential therapeutic application of sex-related hormones.

MASLD: the most prevalent chronic liver disease

Metabolic dysfunction-associated steatotic liver disease (MASLD; see Glossary) is the most common chronic liver condition (Box 1), and is [1–3][4][5,6] strongly associated with several extrahepatic metabolic manifestations such as obesity, type 2 diabetes mellitus (T2DM), insulin resistance, dyslipidemia, hypertension, and kidney disease [1]. Furthermore, patients with MASLD are likely to have higher risk incidence of cardiovascular disease (CVD) [7–9]. The pathophysiological mechanism linking MASLD to CVD is complex and multifaceted, involving metabolic, inflammatory, endothelial and vascular processes [10], and still awaits to be further clarified. With obesity shifting towards an early onset in life, increasing prevalence of T2DM, and an aging population, MASLD prevalence is projected to increase by up to 56% no later than 2030 worldwide [11].

In the general adult population, MASLD prevalence is higher in men than in women, and men are also more prone to develop metabolic dysfunction associated steatohepatitis (MASH), fibrosis and liver-related complications [3,12]. In particular, MASH-related hepatocellular carcinoma (HCC) is diagnosed 2-4 times more often in men than in women, although this ration is lower than in other liver diseases and there is also data showing that liver-related mortality for HCC in equally prevalent in women [13]. **Sex**-specific prevalence of MASLD is related to age, with an opposite trend between men and women. Indeed, men typically exhibit a growing incidence of MASLD during adulthood from young to middle-aged, with a decline noted after the age of 50-60 years. In contrast, in women the prevalence rises after 50 years, peaking at 60-69 years, and declines after 70 years [14]. As a result, postmenopausal women have a higher incidence of MASLD compared to age-matched men. This review discusses the impact of sexual dimorphism in MASLD, which is still undervalued in preclinical and clinical studies. Furthermore, we discuss the potential therapeutic application of sex-related hormones to prevent and improve the treatment of individuals with MASLD.

Key factors contributing to sex differences in MASLD

Similar to other non-reproductive diseases, sex has undoubtedly a profound impact on MASLD incidence in humans. The study of sexual differences is a rapidly growing area of medicine, but little is known in MASLD progression due to the presence of many key factors contributing to sex differences at different molecular levels.

Sex hormones

Extensive experimental data indicate that **sex** hormones influence mechanisms involved in the development and progression of MASLD, including metabolism, oxidative stress, cellular viability, immune response, and tissue regeneration. **Estrogens**, predominantly synthesized in the ovaries, exert a protective effect on the liver by regulating lipid metabolism, suppressing inflammation, and promoting hepatocellular regeneration [15] (Figure 1). In keeping, young women suffering from reproductive dysfunction characterized by altered estrogen levels or undergoing oophorectomy, show a higher prevalence of MASLD than young fertile women [16,17]. Similarly, progesterone, another female sex hormone, modulates insulin sensitivity and adipose tissue function thereby exerting a potential influence on liver health [18,19]. The estrogen pathway also plays a role in liver glucose metabolism and homeostasis by regulating insulin release, the expression of the glucose transporter 2 (GLUT2) gene and glycogen synthesis [20,21].

On the other hand, **androgens** also display a role in liver metabolism and perturbations in their signaling can lead to the development of liver disease. Preclinical studies in male rodents showed a protective role of androgens against hepatic fat accumulation and insulin resistance, whereas in female rodents androgens promote steatosis [22–24]. Similarly, in premenopausal women with biopsy-proven MASLD, increasing concentrations of free testosterone confer a two-fold higher risk of MASH [20,25].

Sex chromosomes

Although traditionally sexual dimorphism in metabolic disorders has been attributed to the effect of sexual hormones, sexual chromosomes may also play a direct role. Indeed, several studies have reported that X chromosome dosage is strictly correlated to higher food intake, body weight, and greater adipose tissue mass [26,27]. Furthermore, men affected by Klinefelter syndrome (XXY) show an increased prevalence of insulin resistance and metabolic disorders, as well as women with Turner syndrome are at increased risk of MASLD [28,29]. A deeper understanding of these differences can be gained by also considering the fat distribution storage, with greater subcutaneous fat in women and visceral fat in men, together with the lower ability to mobilize stored fat in women than in men, and the gynoid pattern of fat storage in women to provide energy resources during pregnancy (Figure 1).

Insulin resistance

Insulin resistance is a key driver of MASLD, and sexual hormones play a crucial role in the occurrence and development of insulin resistance [30]. Recently, it was showed that deletion of the nuclear **estrogen receptor alpha (ERα)** alters the central hepatic control of insulin sensitivity in female mice, as well as impairs the regulation of insulin secretion in males [31,32]. Conversely, several studies show that androgen excess in females and androgen deficiency in males, are associated with insulin resistance, leading to a perturbation in hepatic, adipose, and skeletal muscle lipid metabolism [33], that may further contribute to MASLD development and progression.

Oxidative stress

Disruption of mitophagy and macroautophagy, processes involved in the removal and recycling of damaged or low-functioning mitochondria, may contribute to hepatic fat accumulation, leading to oxidative stress, inflammation, and apoptosis. Female mice display differences in hepatic mitochondrial biogenesis and function, resulting in a more efficient use of increased dietary lipids for energy production. Indeed, recent studies show that loss of estrogen signaling contributes to hepatic oxidative damage induced by low expression of proliferative-activated-receptor-γ coactivator-1 (PGC-1) family, exacerbating steatohepatitis in mice fed a high fat-diet [34,35]. Furthermore, in a recent study performed in mice treated with a steatogenic diet, females had higher hepatic mitochondrial respiration, which was linked to enhanced protection against steatosis development compared to males [36]. Despite evidence for sexual dimorphism in oxidative stress and MASLD in rodent models, the available studies on hepatic oxidative stress in humans were still too limited to fully address this question.

Gut microbiota

The liver is constantly exposed to the highest concentration of intestinal microbiota-derived metabolites convoyed by the portal vein, which play a crucial role in the development of metabolic diseases [37]. Several studies have reported differences in the diversity and composition of gut microbiota between females and males [38] (Figure 1). A recent study, revealed an interaction between farnesoid X receptor (FXR) and sex hormones to regulate gut microbiota composition [39]. Furthermore, gut microbiota may affect sex hormone levels, also regulating the timing of puberty [40–42]. Recently, it has been reported that gut microbiota composition in postmenopausal women is more similar to that of men as compared to premenopausal women [43], with high proportion of

123 *Firmicutes/Bacteroidetes* and of the genera *Lachnospira* and *Roseburia*, and lower abundance of

124 genera *Prevotella*, *Parabacteroides* and *Bilophila* [43].

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Sex-specific hormonal drivers in MASLD

127 Sex differences in the liver can be influenced throughout life by hormonal status, particularly by the

128 levels of circulating estrogens and androgens, as well as to their ratio. Furthermore, the

129 concentrations of sexual hormones change with the age, as occur in women after menopause, when

130 the reduction of circulating estrogens can lead women liver phenotype similar to men.

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132 *Role of Estrogens and Estrogen Receptors*

133   The pivotal role of estrogen signaling in counteracting MASLD-promoting pathways was

134 highlighted by different approaches, including in estrogen-deficient scenarios such as in post-

135 menopausal women and males with aromatase gene mutations.

136   In female mice undergoing **ovariectomy (OVX)** as a **menopause** model, estrogen deficiency

137 leads to hepatic insulin resistance and increased fat accumulation due to enhanced lipid synthesis

138 and reduced catabolism **[44]**. Prolonged estrogen deficiency in both OVX female mice on a high-fat

139 diet and post-menopausal women with MASLD seems to amplify pro-inflammatory responses and

140 oxidative stress, worsening liver inflammation and MASLD progression **[45]**. Conversely, estrogen

141 replacement in OVX females reduces liver fat deposition by enhancing insulin sensitivity, suppressing

142 fat synthesis, promoting lipid export, and boosting fat breakdown, while fostering anti-inflammatory

143 responses **[46]**.

144   Estrogen deficiency in males, either due to aromatase gene mutations or knockout models,

145 disrupts liver glucose and lipid metabolism, inducing hepatic dysfunction and steatosis. Aromatase

146 knockout (ArKO) male mice develop hepatic steatosis that can be reversed by estrogen treatment

147 that reduces fat synthesis and uptake while enhances breakdown **[47]**.

148 Moreover, perinatal exposure of rodents to **endocrine-disrupting chemicals (EDCs)** predisposes

149 both males and females to an elevated risk of MASLD later in life **[48,49]**, potentially by interfering

150 with the physiological estrogen-dependent programming of hepatic metabolism and impairing the

151 achievement of hepatic sexual differentiation **[50]**.

152   In the liver, estrogens primarily exert their effects through ERα. Studies using ERα knockout

153 mice and liver specific ERα knockout (LERKO) mouse models highlighted the significance of hepatic

154 ERα in regulating metabolic processes. Male and female whole body ERαKO mice exhibited

increased body weight, visceral adiposity, glucose production, insulin resistance, and hepatic steatosis with sustained inflammatory signaling **[51]**. The specific relevance of hepatic ERα in regulating female hepatic metabolism is highlighted by studies showing enhanced liver lipid deposition in LERKO females, that cannot be reversed by estrogen supplementation or dietary interventions **[52–54]**. Although expressed at lower levels in males, hepatic ERα is also crucial for estrogen-mediated programming of hepatic metabolism, contributing to sexual dimorphism. Lack of hepatic ERα signaling has opposite consequences in males and females exposed to excess dietary lipids, with LERKO males displaying alterations in plasma lipid profile that may predispose to atherosclerosis and cardiovascular diseases **[52]**.

Role of Androgens and Androgen Receptors

The role of androgen signaling in MASLD is multifaceted and subject to debate, as low androgen levels in mostly older men (possibly by preventing sarcopenia) and high levels in women have been linked to MASLD **[45,55]**. In males, androgen deficiency leads to hepatic steatosis, that can be reversed by testosterone replacement **[56]**, although it should be noted that exogenous androgen supplementation can lead to liver damage in healthy males. In male rodents, removal of androgens through **orchidectomy (ORX)** leads to hepatic steatosis. In diet-induced models of MASLD, androgen deficiency from ORX increases lipid droplet formation, liver inflammation, and hepatocyte apoptosis. As for humans, testosterone replacement mitigates MASLD in castrated male rodents and mice with impaired androgen signaling **[57]**.

Conversely, prenatal and post-natal exposure of females to androgens increases the risk of developing MASLD later in life **[58]**. High androgen levels exacerbate MASLD progression in obese pre-menopausal women as well as in women with polycystic ovary syndrome (PCOS) **[59,60]**.

In addition to androgens, low levels of glycoprotein sex hormone-binding globulin (SHBG), which transports testosterone and other steroids in the circulation, may also play a role in MASLD **[61]**. Indeed, in women with PCOS, a high-free androgen index is associated with an elevated risk of MASLD, independently of obesity.

Both male and female **androgen receptor (AR)** knockout (ARKO) mice develop hepatic steatosis **[62]**. Conversely, the specific contribution of hepatic AR is different between the sexes,

with male liver-specific androgen receptor knockout (LARKO) mice being more susceptible to hepatic steatosis and insulin resistance than their female counterparts **[63]**.

Interaction of genetic and hormonal drivers in MASLD: relevance for sex/gender medicine

Mounting epidemiological evidence suggests that there is a complex interaction between inherited genetic variation and sex hormones in the pathogenesis of MASLD, MASH and progression to cirrhosis.

In a cross-sectional study conducted in a cohort of 1,153 non-Hispanic Europeans, researchers showed that the protective effect of loss-of-function *HSD17B13* rs72613567 variant, the main protective MASH variant, on the risk of MASH was stronger in post-menopausal women **[64]**. More recently, another study analyzing a Polish cohort of adult women affected by PCOS **[65]**, observed that carriers *HSD17B13* rs72613567 gene variant in homozygosity have lower concentrations of total testosterone, 17-OH progesterone, and androstenedione. As the wild-type HSD17B13 protein is also involved in the metabolism of sexual hormones in hepatocytes, these data suggest that reduced levels of intra-hepatic sexual hormones may be involved in mediating the protective effect of loss-of-function variant on steatohepatitis and fibrogenesis. However, whether HSD17B13 regulates sexual hormones or vice versa remains unclear.

Notably, the main MASLD inherited genetic risk factor, namely the *PNPLA3* p.I148M variant, displays a marked sexual dimorphism on MASLD risk. Indeed, an initial meta-analysis of the literature reported a larger effect of p.I148M variant MASLD in women **[66]**. Another study analyzing 756 European subjects with histological MASLD observed a larger effect of the PNPLA3 variant with an increased incidence of liver-related events in the subgroup of non-obese women older than 50 years, than in men **[67]**. Recently, it was formally demonstrated that there is a multiplicative interaction between carriage of the *PNPLA3* p.I148M variant and the development of MASLD **[68]**. The most potent estrogen, **17β-estradiol** was shown to induce the *PNPLA3* p.I148M protein variant expression through ERα in hepatocytes causing a direct interference in lipid metabolism, and contributing to the sex differences in the susceptibility of MASLD **[68]**. Indeed, the *PNPLA3* promoter present a specific ERα binding site enhancing the expression of PNPLA3 in presence of estradiol, with the

consequent lipid droplet accumulation and fibrogenesis, as well as an increased risk of the entire spectrum of MASLD in women carrying p.I148M variant.

These findings highlight HSD17B13 and PNPLA3 as promising targets to design new precision medicine approaches to treat MASLD/MASH in individuals with altered hormones levels. Indeed, hepatic PNPLA3 silencing in carriers of the p.I148M variant is currently under evaluation in clinical trials in patients with fibrosing MASH **[69]**.

From basic research to the clinic

Despite the strong sexual dimorphism in MASLD, sex is still rarely considered as a key biological factor in the design of preclinical and clinical studies, resulting in delayed initiation and inappropriate choice of therapeutic treatments. Furthermore, a major challenge faced by translational research is the development and selection of the optimal models that can help to dissect the molecular and mechanistic insights into liver disease, taking into consideration the sex relevance. To date, animal models of MASLD, namely rodents, have been recognized as useful tools to identify the relevant sex-dependent metabolic pathways involved in the MASLD pathophysiology [70–72] (Box2).

A recent study showed that a dietary formula modified in essential amino acid content rescued the hepatic transcriptomic profile and prevented hepatic steatosis in OVX control but not LERKO females [54], highlighting the importance of hepatic ERα in regulating liver metabolism in response to hormonal and nutritional inputs, especially amino acids.

Estrogen supplementation has demonstrated a protective effect in patients with MASLD and T2DM and seemed to reduce transaminases in post-menopausal women with T2DM [73]. The incidence of MASLD in women taking hormonal replacement therapy was higher than in premenopausal women, but lower than in menopausal women [74]. Furthermore, in a randomized 6 months placebo-controlled trial involving forty-five women taking low-dose continuous combined hormone replacement therapy (HRT) versus placebo, HRT reduced serum enzymes, potentially due to a lowering of liver fat accumulation [20]

Considering that the rise in testosterone levels observed in women after menopause may increase the risk of MASLD, androgen-blocking drugs have been also evaluated: the mineralocorticoid receptor antagonist spironolactone together with vitamin E has shown to improve markers of hepatic fat and insulin resistance in patients with histologically proven MASLD [75]. In keeping, men with T2DM and reduced serum testosterone levels receiving testosterone

replacement therapy displayed lower liver fat than the group not receiving androgen supplementation, as well as reduced markers of liver damage [76].

However, the hazards of HRT also need to be taken into account: some evidence suggests that estrogen-only HRT may increase the risk of endometrial cancer in menopausal women, breast cancer, dementia, blood clots and stroke with long-term use [77]. On the other hand, testosterone supplementation can accelerate the development of prostatic hyperplasia and cancer and increases the risk of breast cancer and cardiovascular disease [78]. Therefore, improved formulations and better-balanced regimes need to be developed. In this respect, phytoestrogens are commonly present in diet and exert many biological functions such as influence lipid and glucose metabolism, the intestinal flora, inflammation and oxidative stress, so that they have been considered as an alternative, but additional studies are needed [79]. The most studied among phytoestrogens, genistein, ameliorated MASLD in several animal models [80]. Based on these beneficial effects, this compound has been further tested as a promising clinical drug for MASLD treatment. A double-blind randomized controlled trial showed that oral supplementation with 250 mg of genistein for 8 weeks reduced insulin resistance, oxidative stress, and inflammation together with an improvement of fat metabolism in patients with MASLD, although the impact on liver histology and disease progression remains to be determined [81].

Another potential therapeutic strategy may be represented by the targeting of ERs by selective estrogen receptor modulators (SERMs), with distinct agonism/antagonism degree at different target tissues. However, the use of tamoxifen, indicated for the treatment of ER-positive breast cancer is associated with increased risk of developing MASH in women with metabolic risk factors [82]. It is still unclear whether the underlying mechanism encompasses the upregulation of the *PNPLA3* p.I148M protein in women carrying the variant. Interestingly, a third-generation SERM, bazedoxifene (BZA), has shown estrogen antagonistic activity in breast and uterus, and estrogen agonistic activity in bone [83].

A new therapeutic approach, named tissue-selective estrogen complex (TSEC), that consists of the combination of conjugated estrogens (CEs) with BZA, has been shown to have beneficial effects in lipid profile and there is an ongoing randomized open label phase 2b clinical trial addressing the effects of combined CE/BZA to prevent metabolic disorders in obese postmenopausal women (NCT04821141[i]) [20].

Although sex may have a large impact on drug responses, many Phase I and Phase II clinical trials are still conducted predominantly among men [84,85], with the implication that women have

277 a 1.5-to-2-fold greater risk to develop adverse drug reaction to the dose determined in men [86].

278 Therefore, the underestimated sex differences in drug dosing screening can have serious

279 implications, potentially slowing the development of new drugs and hindering the effective use of

280 existing ones already in clinical practice.

281   All in all, these findings and therapeutic approaches testify the important sex dimension of

282 MASLD and encourage to proceed with the search for personalised and sex-tailored treatments for

283 the disease.

284

Resmetirom: the first MASH drug therapy approved

286   Recently FDA approved the first molecule, called resmetirom (formerly known as MGL-3196,

287 which will be marketed under the name 'Rezdiffra'), able to reduce hepatic fat in MASH individuals

288 [87]. Resmetirom was developed to specifically target the thyroid hormone receptor (THR)-β, which

289 is responsible for regulating metabolic pathways in the liver and frequently impaired in MASH [88].

290 The randomized clinical trial studies conducted in patients with fibrosing MASH (NCT02912260[j] and

291 NCT04197479[k]) [89,90], both men and women were enrolled, who were not stratified according to

292 sex. In the phase 2 study, resmetirom was more frequently effective than placebo in women even

293 at the lower dose [89], but no significant differences were reported in the phase 3 study. However,

294 studies reported an increased in circulating levels of liver sex hormone-binding globulin (SHBG),

295 which may bind two estrogen ligands stronger than that of albumin [91]. Interestingly, a very recent

296 paper [92] investigated resmetiron efficacy in a model originated from patient-derived primary cells

297 (liver acinus microphysiology system, LAMPS). The authors observed increased steatosis, immune

298 and stellate cells activation in models homozygous for the *PNPLA3* p.I148M variant compared to the

299 wild type and greater resmetiron efficacy in PNPLA3 wild type LAMPS compared to those

300 homozygous for the *PNPLA3* p.I148M variant.

301   Therefore, it would be interesting to analyse the effect of resmetirom on hepatic fat content

302 *in vivo* as well, especially in women carrying the *PNPLA3* p.I148M variant, considering also the

303 estradiol-mediated PNPLA3 protein expression ad described above.

304

Concluding remarks

306   While differences between sexes are evident in the occurrence, fibrosis progression, and

307 liver related events, our comprehension of sex differences in MASLD has remained relatively limited

308 so far, due to the complex interaction of different factors (Figure 2, Key Figure). To bridge this gap,

309 it will essential to collect more precise epidemiological and pathophysiological data through
310 extensive cohort studies. Furthermore, future research directions may involve the exploration of
311 the mechanisms by which estrogens modulate liver function, identifying novel estrogen receptor
312 targets, and developing targeted therapies for MASLD (see Outstanding questions). Investigating
313 the interplay between estrogen signaling and other metabolic pathways involved in MASLD could
314 offer valuable insights into the complexity of this condition. By further understanding estrogens'
315 role in MASLD, researchers may pave the way for more effective treatments in the future (see
316 Clinician's corner). Additionally, it is essential to promote clinical trials focusing on both sex and
317 gender differences in MASH treatment. This will enable a more precise identification of sex-specific
318 therapeutic targets helping in further advancing therapeutic options for men and women.

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Acknowledgments

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Declaration of interests

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Resources

336 [i] https://clinicaltrials.gov/study/NCT04821141

337 [j] https://classic.clinicaltrials.gov/ct2/show/NCT02912260

338 [k] https://clinicaltrials.gov/study/NCT04197479

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Box 1

MASLD, formerly known as non-alcoholic fatty liver disease (NAFLD), is globally the most common cause of liver-related morbidity and mortality, affecting more than 30% of individuals [1–3]. The global prevalence of MASLD has risen from 25% in 1990-2006 to 38% 2016-2019, and its incidence is expected to exceed 40% within 10 years, with a large increase in younger population. MASLD is defined by the presence of steatosis in ≥5% of hepatocytes together with at least one of the features of insulin resistance and when no other causes for secondary hepatic fat accumulation (e.g., excessive alcohol consumption) can be identified and represents the most common cause of steatotic liver disease (SLD). It is a spectrum of liver disease states ranging from uncomplicated and reversible steatosis to metabolic dysfunction-associated steatohepatitis (MASH), characterized by inflammation and hepatocyte ballooning, potentially leading to the deposition of fibrosis [4]. Persistence of fibrosing MASH could progress to cirrhosis and hepatocellular carcinoma (HCC), the second cause of cancer-related mortality worldwide [5,6], becoming the leading indication for liver transplant.

Box 2

Mouse (*Mus musculus*) is the most commonly used animal model in MASLD research because of its relative phylogenetic closeness and physiological similarity to our species [93]. Mouse model shows numerous biological characteristics, including short life cycle, gestation and lifespan, as well as its high fecundity and easier of breeding. Furthermore, compared to other mammals, mice are less expensive to maintain and manage, allowing for large-scale studies. Mice models can also exhibit features of metabolic syndrome, including obesity and disturbances in lipid, glucose, and insulin metabolism [94].

There are several murine models used to study MASLD, each with specific advantages:

- Dietary model. Mice fed with both high-fat and high-fructose diets develop obesity, insulin resistance, and hepatic steatosis, mimicking the progression of MASLD in humans [90,91].
- Genetic models. Through genetic manipulation mice models have been created to mimic a human polymorphism implicated in MASLD occurrence (such as *PNPLA3* p.I148M variant) or to study a particular stage of the MASLD spectrum [95].
- Chemical models. Mice treated with chemicals like thioacetamide and carbon tetrachloride can induce liver damage and inflammation, allowing the study of the transition from MASLD to MASH [97,98].

Despite numerous advantages, the use of mice as a model for MASLD presents some limitations:

- Metabolic differences. Some aspects of lipid metabolism and inflammatory response differ between mice and humans, potentially affecting the translation of results.
- Ethical aspects. Measures must be taken to minimize pain and distress, adhering to ethical guideline for the animal treatment.

Clinician's corner

Similarly to other metabolic-associated diseases, MASLD is characterized by crucial sex differences in disease development and risk of progression. It is known that male subjects are more prone to a severe form of the disease and that women after the menopause tend to lose the protection they display during fertile age. Part of the variation we observe between males and females is certainly associated to the role of sex hormones which contribute to the disease through several mechanisms (modulation of glycolipid metabolism, gut microbiota, oxidative stress, interaction with genetic factors involved in hepatic fat remodeling).

In this respect, one important message for the clinician is to consider for instance the higher risk of MASLD and MASLD progression in menopausal women, especially in the subgroup of subjects carrying unfavorable genetic profile, namely *PNPLA3* p.I148M variant carriers. These patients may be considered for a closer follow up or for personalized targeted treatments.

Sex differences moreover represent inspiration for providing new insights in the pathogenesis of MASLD, thus leading the search for novel therapeutic strategies that may impact on the prevention or future treatment of MASLD in both postmenopausal women and men.

Glossary

17β-estradiol: the most prevalent estrogens produced in female during the reproductive lifespan.

Androgens: hormones that help to develop sex organs in men; they also contribute to sexual function in men and women.

Androgen receptor (AR): nuclear receptor activated by the binding of androgens, especially testosterone and dihydrotestosterone.

Aromatase KO mice: cannot synthesize endogenous estrogens providing a useful model to examine the role that estrogens play in development and homeostasis in mammals.

Endocrine-disrupting Chemicals (EDCs): natural and man-made chemicals that can either mimic, block, or disrupt the action of hormones. EDCs are associated with numerous adverse human health issues, including reproductive health problems, obesity, diabetes, hormone-related cancers, neurological issues, and other disorders.

Estrogens: group of steroid compounds that are the primary female sex hormones. They promote the development of female secondary sex characteristics and control aspects of regulating the menstrual cycle.

Estrogen receptor alpha (ERα): the receptor for estrogens most expressed at the hepatic level.

Gender: it is a complex and multifaceted concept. It refers to the social and cultural roles, behaviors, expectations, and identities that societies assign to individuals based on their perceived sex.

Klinefelter syndrome: is a fairly common genetic condition found in males only by having one or more extra X chromosomes. Males with this disorder may exhibit enlarged breasts, reduced facial and body hair, a rounded body shape, and small testicles. Sometimes, boys with Klinefelter may learn to speak much later than other children with difficulty learning to read and write.

Metabolic dysfunction-associated fatty liver disease (MASLD): a pathological syndrome characterized by excessive fat deposition in liver cells; it is closely related to insulin resistance, genetic susceptibility, and hormonal imbalances.

Metabolic dysfunction-associated steatohepatitis (MASH): a stage of MASLD, characterized by liver damage and inflammation.

Menopause: permanent cessation of menses, clinically defined as 12 months after a woman's last menstrual cycle. It marks the end of normal ovarian functions.

Orchidectomy (ORX): surgical procedure to remove the testes.

Ovariectomy (OVX): surgical procedure to remove the ovaries.

Sex: refers to a set of biological attributes, primarily associated with physical and physiological features including chromosomes, gene expression, hormone levels and function, and reproductive/sexual anatomy.
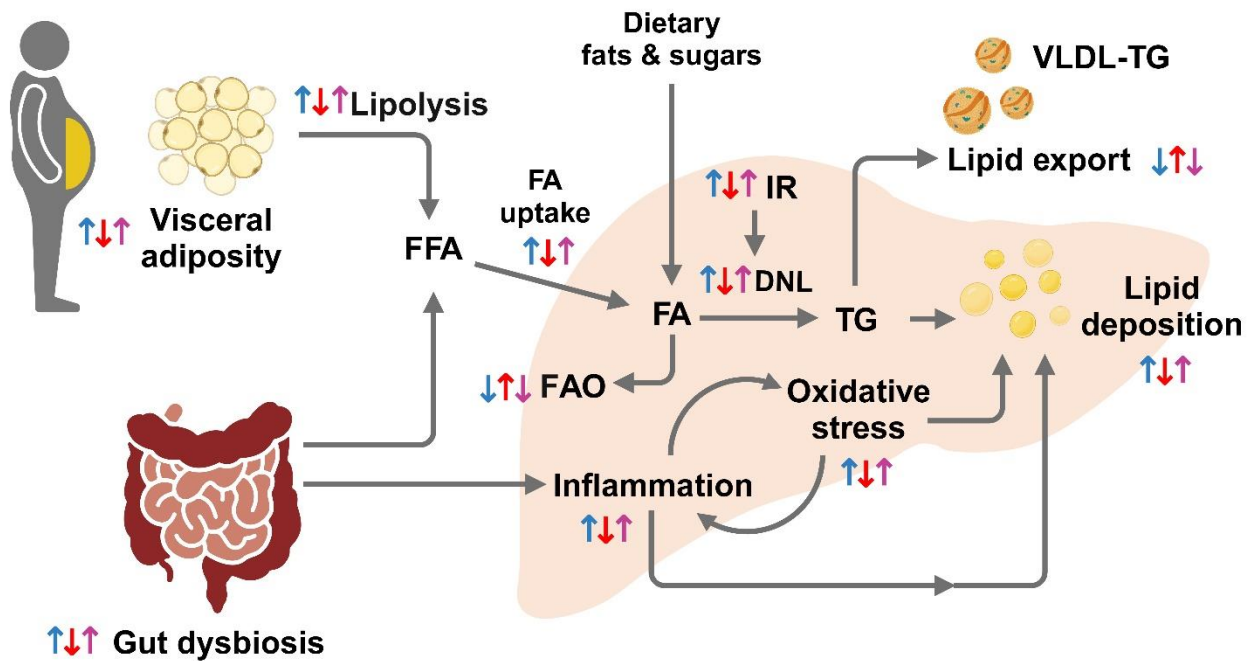
Turner syndrome: A genetic condition found in females characterized by a missing or abnormal X chromosome. Turner syndrome is characterized by shorter-than-average height, nonfunctional ovaries, and infertility.

Figure 1. Main sex differences in the regulation of metabolic and inflammatory signaling pathways accounting for sex differences in MASLD susceptibility. Arrows represent the relative regulation between men (blue), pre-menopausal women (red), and post-menopausal women (violet). With respect to men and post-menopausal women, pre-menopausal women display decreased visceral adiposity and adipose tissue lipolysis, limited FA uptake and DNL, reduced lipid storage, restrained gut dysbiosis and inflammation, and enhanced FAO and lipid secretion.
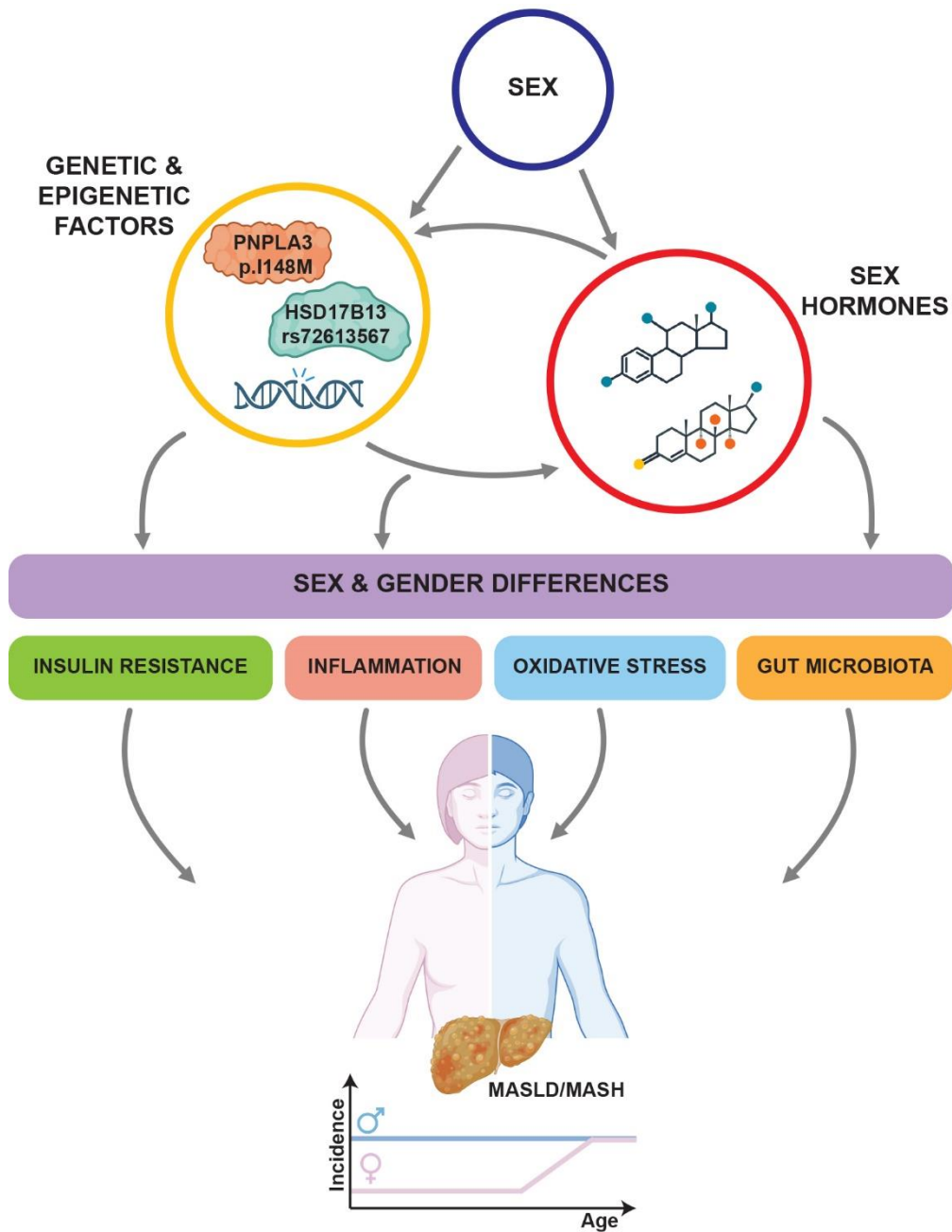
Abbreviations: DNL, de novo lipogenesis; FA, fatty acids; FAO, fatty acids oxidation; FFA, free fatty acids; IR, insulin resistance; TG, triglycerides; VLDL-TG, very-low density lipoproteins-triglycerides.

662   Figure2

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Figure 2. Key figure. Interaction of specific factors linked to MASLD sex disparity incidence. Sex hormones interact with a multitude of MASLD factors including genetic variants, epigenetic factors, insulin resistance, oxidative stress, inflammation, and gut microbiota and alter the risk profiles and phenotypes of MASLD. This figure was created with Biorender (www.biorender.com). Abbreviations: HSD17B13, Hydroxysteroid 17-Beta Dehydrogenase 13; PNPLA3, Patatin Like Phospholipase Domain Containing 3.