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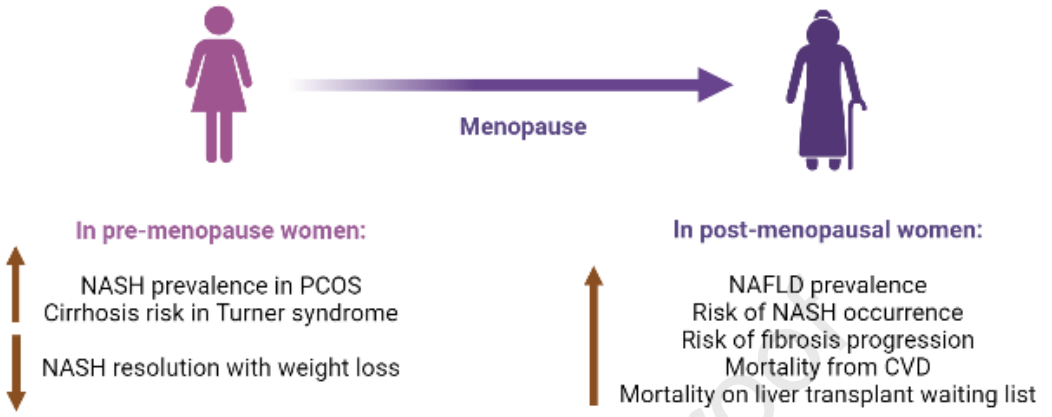

Title: Non-alcoholic fatty liver disease in Women – Current Knowledge and Emerging Concepts

Short title: NAFLD in Women

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Summary

Non-alcoholic fatty liver disease (NAFLD) is a major cause of liver disease worldwide, affecting up to 30% of adults. Progression to non-alcoholic steatohepatitis (NASH) is a key risk factor for cirrhosis, hepatocellular carcinoma and cardiovascular events. Alterations in reproductive hormones are linked to the development and/or progression of NAFLD/NASH in women. Women with Polycystic Ovary Syndrome (PCOS) and those with estrogen deficiency are at increased risk of NAFLD/NASH, with higher mortality rates in older women compared to men of similar ages. NAFLD/NASH is currently the leading indication for liver transplantation in women without hepatocellular carcinoma. Therefore, a better understanding of NAFLD in women is needed to improve outcomes. In this review, we discuss the hormonal and non-hormonal factors contributing to NAFLD development and progression in women. Furthermore, we highlight areas of focus for clinical practice and for future research.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is a chronic liver disease characterized by increased hepatic fat content (≥5%), which is diagnosed after exclusion of well-established causes of hepatic steatosis such as alcohol, steatogenic dugs and inherited errors of metabolism.[1] Hepatic triglyceride accumulation by itself is not hepatotoxic.[2] However, pathogenic processes such as adipose tissue dysfunction[3], gut microbiome dysbiosis[4], fructose-induced mitochondrial dysfunction and endoplasmic reticulum oxidative stress[5] may drive hepatic steatosis to hepatic inflammation and hepatocellular ballooning (non-alcoholic steatohepatitis or NASH) with or without fibrosis, leading to fibrosis and eventually cirrhosis.[6] Liver fibrosis represents the main predictor of liver and non-liver-related adverse clinical outcomes. Hepatocellular carcinoma (HCC) can occur in both cirrhotic and non-cirrhotic patients.

Globally, NAFLD has a prevalence of 30%[7] and this is projected to rise to 56%[8], paralleling the increased incidence of obesity and type 2 diabetes. In adults, up to a third of patients with NAFLD develop NASH over a period of ~7 years[9], and around 40% of the individuals who have histologically proven NASH progress to fibrosis.[10] NAFLD has a higher prevalence in men than in premenopausal women below the age of 50 years old.[9] However, in women, the prevalence of NAFLD increases after menopause with a rising trend observed after the age of 50 years, followed by a peak at 60 to 69 years, before declining after the age of 70 years.[10]

Recently, a panel of international experts proposed the redefinition of NAFLD to metabolic dysfunction fatty liver disease (MAFLD) based on the presence of hepatic steatosis and metabolic risk factors (overweight/obesity, type 2 diabetes and/or metabolic dysfunction).[11] The term MAFLD may include patients with concomitant causes of liver diseases and it may exclude those with steatosis but without the full

metabolic risk factor spectrum.[12] However, some studies suggest women with NAFLD may be less likely to be meet the criteria for diagnosis of MAFLD than men with NAFLD[13], which could have a detrimental effect on outcomes in women. Hence, we have elected to use the NAFLD nomenclature in this review.

Women aged ≥50 years with NAFLD are 1.2 times more likely to develop NASH compared to age-matched men and are more likely to progress to advanced fibrosis[14], with preliminary transcriptomic and plasma profiling studies suggesting that NAFLD may follow a distinct biological trajectory in women aged ≥50 years.[15,16] Liver fibrosis stage is associated with increased mortality from 0·32 deaths per 100 person-years at stages F0 to F2 to 1·76 deaths per 100 person-years at stage F4, resulting in an almost seven-fold increased predisposition to hepatic decompensation (hazard ratio of 6·8, 95% CI 2·2 to 2·13).[17] Predicting the presence of fibrosis with blood-based non-invasive markers, that may perform differently according to sex, may require dedicated cut-offs for women.[18] This may be due to the fact that women tend to have lower serum liver enzyme activities compared to age-matched men.[18] Nevertheless, there is no evidence that non-invasive markers of fibrosis, such as FIB-4 and NAFLD fibrosis score, which rely heavily on measurement of transaminase activities, may perform differently in women. Interestingly, a recently developed non-invasive marker, called the AGILE 3+, has demonstrated how integrating sex with other clinical parameters may improve the risk stratification of patients with NAFLD.[19] In addition, HCC occurs less frequently in women compared to men, in both cirrhotic and non-cirrhotic patients[20], suggesting that dedicated surveillance strategies may need to be explored.

NASH is the leading cause of end stage liver disease requiring transplantation in women who do not have HCC.[21] In women undergoing liver transplantation, long-term survival is higher compared to men.[22] However, women are more likely to die whilst

on the waiting list for liver transplantation due to NASH, partly due to underestimation of mortality in women using current stratification scores (i.e. the Model of Endstage Liver Disease or MELD score).[23] A sex- and sodium-adjusted MELD score for liver transplant allocation has recently been proposed[24], which may help to ensure more equitable access to liver transplantation.

Women with NAFLD have increased mortality rates from cardiovascular disease (CVD) compared to women without NAFLD.[25] This excess risk of CVD is also higher in women compared to age-matched men with NAFLD (e.g. 10% in a 40-year old woman with NAFLD vs 8% in a 40-year old man with NAFLD).[26] The excess CVD risk increases with age, and is exaggerated after menopause (e.g. in people with NAFLD aged 60 years, the CVD risk in women is 18% vs 9% in men).[26]

In this review, we summarize factors contributing to the development and progression of NAFLD in women and in specific population groups. We aim to raise awareness of NAFLD in women, highlight areas for future research to address gaps in knowledge of underlying pathophysiological mechanisms, and management of this complex condition.

Search strategy and selection criteria

A literature search was performed to identify studies investigating NAFLD/NASH in women, published up to November 2022. Original research and review articles were identified through searches in the PubMed database, Scopus database, Ovid Medline, Ovid EMBASE, limited to articles published in the English language. We included basic science studies, randomized controlled trials, reviews, original prospective studies, cross-sectional studies, retrospective studies and best practice guidelines using different combinations of the following search terms: “fatty liver” OR “non-alcoholic

fatty liver disease" OR "NAFLD" OR "steatohepatitis" OR "NASH" OR "liver fibrosis" OR "liver disease" OR "liver cancer" AND "women" OR "gender" OR "female" OR "sex difference" OR "reproductive age" OR "premenopausal women" OR "postmenopausal women". For effects of hormones on NAFLD, we used the search terms: "androgens" OR "estrogens" OR "oestrogens" OR "testosterone" OR "sex hormones" OR "sexual dimorphism" OR "menopause" OR "hormone replacement therapy" AND "NAFLD" OR "NASH" OR "steatohepatitis" OR "liver fibrosis". For effects of NAFLD in specific population groups, we use a combination of search terms including "NAFLD in Polycystic Ovary Syndrome", "NAFLD in Turner syndrome".

Reproductive hormones and NAFLD

ESTROGENS

Estrogens play important roles in regulating lipogenesis and fatty acid oxidation. Ovariectomised female rats had a 51% increase in hepatic lipogenesis and a 34% reduction in fatty acid oxidation[27] due to decreased synthesis of peroxisome proliferator-activated receptor α (PPARα, a regulator of fatty acid oxidation) and upregulation of the genes encoding sterol regulatory element-binding protein 1 (SREBP-1, a nuclear transcription factor that promotes lipid synthesis).[27] Additionally, stearoyl coenzyme A desaturase 1 (SCD1, the rate-limiting enzyme in triglyceride synthesis) is upregulated.[28]

The metabolic actions of estrogens are typically attributed to classical estrogen receptor-α (ERα) signalling.[29] Both male and female ERα knockout mice exhibit upregulation of lipogenic (SREBP-1 and fatty acid synthase or FAS) and adipogenic (PPARγ and lipoprotein lipase) genes, a process that is reversed by ERα agonist treatment.[27,28] Mice lacking G-protein coupled estrogen receptor (GPER) and mice with liver ERα-knockout (LERKO) exhibit similar metabolic phenotypes including higher body weight and increased visceral adiposity.[30,31] Female, but not male, GPER-knockout mice fed a high fat diet display lower levels of high-density lipoprotein (HDL)-cholesterol and greater liver fat accumulation compared to controls.[31] This suggests that both ERα and GPER pathways are important for hepatic and whole-body lipid homeostasis and contribute to sexual dimorphism in NAFLD.

Estrogens also influence reverse cholesterol export, i.e. the process by which peripheral cholesterol is returned to the liver.[32] In LERKO mice, hepatic low-density lipoprotein (LDL) receptors are reduced by ~18 to 22%[32] and hepatic expression of PDZK1 protein (which plays a role in HDL cholesterol uptake) is reduced by 22% and 33% in male

and female mice, respectively.[32] Loss of ERα reduces cholesterol efflux from foam cells into HDL particles in female and male LERKO mice.[32] Thus, estrogen deficiency disrupts the molecular machinery involved in hepatic lipogenesis and adipogenesis. Consistent with these findings, progression from pre- to post-menopause is independently associated with an increase in total cholesterol and LDL cholesterol in women aged between 47 and 55 years.[33] This may contribute to the higher prevalence of NAFLD in post-menopausal women.

Interactions between estrogens and glucagon may be important in the pathogenesis of NAFLD. Glucagon promotes hepatic lipolysis and suppresses de novo lipogenesis. Glucagon levels have been observed to be inversely associated to NAFLD progression.[34] Attenuation of glucagon receptor signalling is also proposed to increase the risk of NAFLD.[35] Furthermore, in NAFLD, expression of the glucagon receptor gene and the function of the glucagon protein may be impaired, resulting in glucagon resistance.[34,36] In vitro studies have shown that physiological levels of estrogen can inhibit glucagon secretion via binding to the GPR30 estrogen receptor[37], and estradiol-mediated inhibition of glucagon release is attenuated by deletion of GPR30 receptors.[38] Ovariectomy has also been shown to increase circulating glucagon in rodents[37,39] and glucagon levels are suppressed by estradiol treatment.[37,40] These data suggest estrogen deficiency would be predicted to have beneficial effects in NAFLD via increased glucagon levels. However, estrogen deficiency has detrimental effects as described above. Therefore, the roles of estrogen (and estrogen deficiency) in the development and progression of NAFLD require further study.

ANDROGENS

Prenatal exposure of female rodents to androgens disrupts the balance between enzymes involved in lipogenesis (SREBP, PPAR and carbohydrate-responsive element-binding protein or ChREBP) and lipolysis.[41] In young adult ewes, prenatal exposure to androgens downregulates hepatic PEPCK and causes hepatic insulin resistance.[42] Upregulation of expression of other hepatic metabolic genes including mitogen activated protein kinase 4 (a pro-inflammatory protein involved in ceramide signalling), UDP-glucose ceramide glucosyltransferase (involved in ceremide metabolism) and acyl-coenzyme A dehydrogenase (involved in lipid metabolism) also occurs, further exacerbating liver damage.[42]

The effects of androgens in animal models could be mediated by changes in body adiposity/composition exacerbated by a high fat diet[43] and/or via changes in transcriptional activity of gluconeogenic genes.[44] Postnatal exposure of female rodents to dihydrotestosterone (DHT) induces hepatic steatosis, insulin resistance and recapitulates the reproductive phenotype of PCOS.[45] In normal weight female mice, low dose DHT upregulates SREBP cleavage activating protein (SCAP) and SREBP-1, which promotes FAS and acetyl-CoA carboxylase expression, resulting in hepatic steatosis.[46] In DHT-exposed female rats, NASH may develop via activation of NF-κB signalling, enhanced expression of pro-inflammatory cytokines (IL-6, IL-1β, and TNFα) and an increase in pro-apoptotic markers.[47] Cumulatively, prenatal or postnatal androgen exposure appears to increase the risk of NAFLD development and progression by increasing lipogenesis and pro-inflammatory mediators.

Factors contributing to the development and progression of NAFLD in women

AGE OF MENARCHE

Earlier onset of menstruation (i.e. age of menarche <12 years) has been associated with increased risk of cardiometabolic disease in post-menopausal women.[48] In the CARDIA study, earlier menarche by 1 year conferred a 10% increased risk of NAFLD (diagnosed using CT scans) in adulthood independent of socio-economic factors and baseline BMI.[48] Early menarche is often preceded by rapid accumulation of fat during childhood, a physically less active lifestyle and/or behavioural factors that could also increase the risk of the metabolic syndrome.[49] Therefore, other factors such as obesity, insulin resistance or a hyperandrogenic phenotype (such as in PCOS)[50] may interact with early menarche to confer an additional risk of developing NAFLD (Figure 1).

MENOPAUSAL STATUS

Estradiol, being the most abundant circulating female reproductive hormone, plays important roles in the regulation of lipid and glucose metabolism in hepatic and adipose tissues. In pre-menopausal women, estradiol is predominantly secreted by the ovaries.[51] However, after menopause[51], ovarian estrogen secretion ceases and circulating estradiol levels decline to a mean value of ~10pmol/L[52], but low quantities are still produced by non-ovarian tissues.[51,53] The decline of circulating estradiol during natural menopause is associated with increased risk of NAFLD, type 2 diabetes, central adiposity and hypertriglyceridemia.[54]

In a cross-sectional study involving 541 people with biopsy-proven NASH[55], advanced fibrosis was more prevalent in post-menopausal women (27·6%) compared to men (22·2%) and pre-menopausal women (14·4%).[55] Women over the age of 50 years have increased odds of advanced fibrosis (OR 1·8, 95% CI 1·2-2·7) even after adjustment for covariates (enrolling site, ethnicity, degrees of portal inflammation).[55] The risk of severe fibrosis remained elevated in lean post-menopausal women with NAFLD

compared to lean pre-menopausal women with NAFLD (OR 2·17, 95% CI 1·1-4·5).[56] This suggests that menopause is associated with severe fibrosis that is, in part, independent of age or body fat composition.

Women who have undergone oophorectomy have an increased risk of NAFLD compared to pre-menopausal women who have not undergone oophorectomy.[57] In fact, a stronger association was observed in women who underwent oophorectomy before the age of 45 years.[57] Similarly, women with premature menopause prior to the age of 40 years have a 90% increased risk of severe fibrosis on histology compared to women who went through menopause after 40 years.[58] Conceivably, the duration of estradiol deficiency contributes significantly to post-menopausal hepatic fibrosis risk.

HORMONE REPLACEMENT THERAPY

The role of hormone replacement therapy (HRT) in preventing the development and/or progression of NAFLD remains unclear. A randomised double-blind study comparing women with type 2 diabetes on oral HRT (1 mg estradiol plus 0·5 mg norethisterone) to those on placebo for 6 months showed that women on HRT (n=19) had reduced circulating concentrations of liver enzymes compared to the placebo group (n=23).[59] A South American study reported that post-menopausal women on HRT (dose and type of hormones not specified) for at least 6 months (n=14) had lower waist circumference, lower HOMA-IR index, lower ferritin levels (a surrogate marker of parenchymal inflammation) and lower γ-glutamyl transferase when compared with women not taking HRT (n=79).[60] However, improvement in liver biochemistry may not reflect improvement in liver histology. Thus, the same group of researchers assessed frequency of NAFLD diagnosed by abdominal ultrasound and reported a lower frequency of NAFLD in women taking HRT (14/53, 26·4%) compared with women not taking HRT

(79/198, 39·9%) irrespective of the type of HRT, duration of use and route of administration.[61]

However, other studies did not report reduction in the risk of NAFLD[62] or severe hepatic fibrosis amongst post-menopausal women taking HRT.[55] One study demonstrated an increased risk of severe lobular inflammation with HRT use in post-menopausal women and oral contraceptive use in pre-menopausal women.[63] Details of the types, routes of administration and doses of oestrogens (and progestins) and their differential effects on the risk of severe inflammation, were not reported. Future studies are indicated to investigate the impact of synthetic estrogens and progestins on the natural history of NAFLD and/or NASH in post-menopausal women.

SELECTIVE ESTROGEN RECEPTOR MODULATORS

Selective estrogen receptor modulators (SERMs, e.g. tamoxifen) are agents that elicit tissue-specific estrogen receptor agonist or antagonist activity. Women treated with tamoxifen have a higher prevalence of NAFLD and an increased risk of progression to NASH and advanced fibrosis.[64] The mechanisms by which tamoxifen influences NAFLD risk remain unclear. In vitro, genes involved in lipogenesis and fatty acid synthesis (e.g. SREBP-1c, FAS, SCD1 and acetyl coenzyme A carboxylase) are upregulated after treating HepG2 cells with tamoxifen.[65] Obese female Wistar rats who were fed a high-fat diet for 15 weeks and then given tamoxifen for 2 weeks were observed to have increased hepatic lipid synthesis and decreased triglyceride export.[66] This was associated with a marked downregulation of salient information regulator 1 (SIRT1) and upregulation of p-FoxO1/LXRα-SREBP1c signalling leading to increased hepatic steatosis.[66] Administration of a SIRT1 agonist inhibited the promotion of

tamoxifen-induced lipid synthesis, suggesting that SIRT1 is a regulator of tamoxifen-induced fatty liver disease.[66]

In addition, tamoxifen-treated ovariectomized C57BL6/J female mice are protected from HFD-induced steatosis via selective activation of ERα-activating factor1 (ERα-AF1).[67] This contradicts findings from a previous study indicating that protective metabolic actions of estradiol are mediated mostly via ERα-AF2.[67] It is likely that there is redundancy in the ERα-AF1 and ERα-AF2 systems or the effects of tamoxifen may differ depending on the tissue type.[67] More mechanistic studies are needed to elucidate the influence of SERMs on NAFLD. More importantly, targeting liver ERα-AF1 or SIRT1 are potential future strategies to mitigate against the development and progression of NAFLD.

TURNER SYNDROME

Turner syndrome (TS) is a sex-chromosome disorder in females caused by an abnormal or absent X chromosome.[68] Women with TS have a 4·4-fold increased risk of type 2 diabetes[69], and a 5.5-fold increased risk of developing liver cirrhosis.[69] Histological evidence of nodular hyperplasia, NAFLD and cirrhosis have been described in women with TS.[70] Elevated liver enzymes were found in ~50% of women with TS (n=125).[71] Of the 21 women who had Fibroscans, liver stiffness measurements suggestive of fibrosis were reported in 38%[71] and liver architecture changes were found in the 11 women who consented for biopsy.[71] Compared to age-matched eugonadal women or estradiol-treated women with premature ovarian insufficiency, women with TS have higher waist circumference, elevated BMI, increased IL-6 and triglyceride levels.[72] Women with TS also have increased intrahepatocellular lipid content, which is correlated to duration of estrogen deficiency.[73] Although larger studies are needed to

explore the relationship between estradiol and metabolic risk, these data suggests a role for estrogen deficiency in promoting hepatic steatosis and insulin resistance in this context.

It is difficult to disentangle the contributions of gonadal hormones from that of sex chromosomes in patients with Turner syndrome. In the four core genotype model (FCG mice in which sex chromosomes are unrelated to gonadal sex), mice with one X chromosome had reduced body weight compared to XX mice.[74] By contrast, women with one X chromosome have higher body weight and increased risks of developing metabolic disease than women with two X chromosomes.[75] Although low levels of sex hormones contribute to the increased risk of developing metabolic disease, imprinting of X-linked genes may also contribute to metabolic dysregulation in Turner syndrome.[76] Depending on the parental origin of the X chromosome, imprinting of maternally transmitted X-linked genes in patients with TS has been shown to prevent visceral fat accumulation whereas imprinting of paternally transmitted X-linked genes promoted higher triglyceride and lipid levels.[76] The rarity of sex chromosome aneuploidies presents challenges in determining the relative contributions of reduced numbers of sex chromosomes and hypogonadism in the development of NAFLD in women with TS. However, the FCG mouse model may help advance our understanding of these two contributing factors.

POLYCYSTIC OVARY SYNDROME (PCOS)

PCOS affects up to 13% of women of reproductive age and is characterised by ovulatory dysfunction, hyperandrogenism and/or polycystic ovarian morphology.[77] Women with PCOS have increased prevalence of NAFLD compared to age-, BMI- and waist circumference-matched women without PCOS.[78] This excess risk is also present

in lean women (BMI <25kg/m^2) with PCOS.[79] A concerning finding is the higher prevalence of biopsy-proven NASH in women with PCOS younger than 40 years.[80] Hyperandrogenism is associated with increased NAFLD risk in women with PCOS. In a retrospective study involving 63,210 women with PCOS, serum testosterone levels >3·0 nmol/L were associated with an increased risk of NAFLD (HR 2·30, 95% CI 1·16–4·53).[79] Liver fat is greater in hyperandrogenic women with PCOS compared to normo-androgenic women with PCOS after correcting for visceral adiposity and BMI.[81] Consistent with these findings, a cross-sectional study of 400 Chinese women with PCOS concluded that the risk of NAFLD increases with free androgen index, which is a surrogate measure of androgen bioavailability.[82] Notably, excess androgens are associated with increased risk of developing NAFLD in women, independent of obesity and insulin resistance.[82] Women with hyperandrogenic PCOS also had higher circulating levels of glycerophospholipids and lysoglycerophospholipids which are potential biomarkers of NASH.[83] Intra-adipose androgen generation by enzyme aldo-ketoreductase type 1C3 was increased in subcutaneous adipose tissue (SAT) of women with PCOS resulting in lipotoxicity and predisposing women with hyperandrogenic PCOS to liver injury.[84] Although a causative role for androgens has not been proven, these association studies suggest a potential use for anti-androgens in treating women with PCOS and NAFLD.

BODY FAT DISTRIBUTION

Sex-specific body fat distribution influences an individual's predisposition to cardiometabolic complications independent of body weight or body fat percentage.[85] Compared to age- and BMI-matched men, pre-menopausal women typically have greater SAT mass in the abdominal[86] and femoral-gluteal areas.[87] By contrast, men have

a higher percentage of visceral adipose tissue (VAT), 10-20% in men vs 5-8% in women.[88] Given the higher VAT in men, men have a greater ability to dispose meal-derived free fatty acids (FFA) in VAT which results in higher liver fat disposal (Figure 2).[89] Excess FFA released into the bloodstream predisposes to lipotoxicity and increased lipid uptake by liver, pancreas or muscle.[90] This overflow of FFA to liver could lead to increased cellular levels of ceramides, long chain fatty acyl-coenzyme A and pro-inflammatory processes causing chronic low-grade inflammation.[90,91] Unsurprisingly, people with increased VAT mass are more insulin resistant, have impaired glucose metabolism and are more likely to develop NAFLD.[92] Indeed, a prospective study showed rising incidence of NAFLD based on ultrasound and CT imaging with increasing quartiles of VAT (17·1%, 18·1%, 25·2% and 34·4%, respectively) in both men and women after a median follow-up of 4·4 years.[93] By contrast, individuals with the highest quartile of SAT are more likely to be at lower risk of developing NAFLD (HR 2·30, 95%CI 1·28–4·12) compared to individuals with the lowest quartile of SAT.[93]

Prior to menopause, women accrue more fat in SAT, which protects them from the negative consequences of the metabolic syndrome.[94] As women transition through menopause, both SAT and VAT increase but VAT expands more at the onset of menopause and then plateaus at a higher set-point after menopause.[94]

During menopause, changes in SAT and VAT metabolism also results in alterations in in body fat distribution.[95] Although premenopausal and postmenopausal women retain similar sensitivity and responsiveness to sympathetic activation by beta-adrenergic agonists, adipose tissue basal lipolysis rate is reduced and lipoprotein lipase activity (which promotes hydrolysis of circulating TG to FFA) is enhanced in the gluteal and abdominal adipose tissues of postmenopausal women.[96] Compared to premenopausal

358 women, expression of FAS is reduced in the SAT of postmenopausal women by 61%[95],
359 whereas PPARγ expression is increased in VAT by 83%.[95] The increased PPARγ
360 expression in VAT may reflect a compensatory attempt to curtail the need for increased
361 lipid storage, as VAT accumulation correlates with features of insulin resistance.[95]
362 Interestingly, thiazolidinediones (PPARγ agonists used to treat type 2 diabetes), may
363 promote a redistribution of SAT and a lower expression of transcriptional genes for
364 VAT, suggesting an effect on adipose tissue depot-specific regulation.[97] However, their
365 unfavourable safety profile (e.g. increased risks of atypical humeral fracture and
366 bladder cancer) limits their use in clinical practice. Changes in adipose tissue
367 metabolism, coupled with preferential fat accumulation in VAT during menopause
368 (Figure 1) predispose women to increased cardiometabolic risk.[98]
369 Estrogen levels correlate positively with percentage of SAT and negatively with
370 visceral fat accumulation in pre-menopausal women.[98] Estrogen treatment decreases
371 insulin resistance by ~50% and decreases abdominal visceral adiposity in post-
372 menopausal women and ovariectomized female animal models.[99,100] Estrogen also
373 reverses the increase in hepatic triglyceride content caused by diet-induced obesity in
374 LERKO mice.[101] Evidently, estrogens play a role in insulin sensitivity and glucose
375 homeostasis in women in addition to promoting fat accumulation in SAT and modifies
376 the risks of NAFLD progression.

377 ***MUSCLE QUALITY AND QUANTITY***

378 Sarcopenia is defined as generalised progressive loss of skeletal muscle mass, muscle
379 function and muscle strength. Meta-analyses have shown that the risks of NAFLD and
380 NASH are increased by 1·5 to 2·5-fold among individuals with sarcopenia.[102,103]
381 Furthermore, among individuals with NAFLD, sarcopenia is independently associated

with hepatic fibrosis after adjusting for obesity and insulin resistance (OR 2·59, 95% CI 1·22-5·48).[104] Coexistence of sarcopenia and NAFLD doubles mortality risk, independent of fibrosis stage.[105] It remains unclear if NAFLD directly contributes to sarcopenia or sarcopenia causes NAFLD.

Skeletal muscle is a major site of insulin-stimulated glucose uptake.[106] Ageing results in loss of muscle mass and reduction in type 2 (fast-twitch) muscle fibres (by ~10 to 14% per decade).[106] Fast-twitch muscles depend on glycolysis for energy production[107], and the gradual reduction in fast-twitch muscle during ageing results in reduced dependence on cytosolic glycolytic processes for glucose disposal.[106] Mitochondrial bioenergetics are also altered with ageing. Reduced expression of gene regulators, such as PPARγ coactivator (PGC)-1α in aged skeletal muscles suppresses AMP-activated protein kinase, SIRT1 and mitogen-associated protein kinase (p38 MAPK).[108] Suppression of SIRT1 limits oxidative capacity and lipid metabolism leading to hyperlipidaemia, dysregulated glucose metabolism, hyperinsulinemia and insulin resistance.[109]

Ectopic fat accumulation in the muscles (myosteatosis) can be a consequence of insulin resistance and perpetuate NAFLD. Severe myosteatosis is associated with a 2- to 3-fold increased risk of early NASH in patients with NAFLD.[110] In a recent study, the fat content in psoas skeletal muscle (measured by a parameter known as skeletal muscle fat index) was observed to be higher in individuals with NASH and advanced fibrosis (≥F3) than in those with NASH and early stages of fibrosis (F1 to F2).[111] Myosteatosis promotes endoplasmic reticulum stress, which in turn impairs mitochondrial function.[112] Furthermore, myosteatosis contributes to reduced skeletal muscle protein synthesis stimulated by anabolic hormones (insulin, estradiol and testosterone).[112]

Estradiol reduction during menopause further promotes proteolysis, reduction in lean mass, and increased fat mass.[112]

Mechanisms underlying the manifestation of sarcopenia are likely to be multifactorial. Although low estradiol levels may play a potential role in decline in muscle mass in women after the age of 50 years old, evidence elucidating the contribution of menopause to sarcopenia remains unclear. Some studies have reported an accelerated decline in muscle mass in women during menopausal transition.[113,114] Samson et al. observed a decline in isometric knee extensor strength (IKES) and handgrip strength (HGS) by 40.2% and 28% in elderly women 55 to 80 years old whereas the decrease in IKES and HGS was 10.3% and 8.2% in women 20 to 55 years old.[113] By contrast, the decline in IKES and HGS was 23% and 17.4% in men 55 to 80 years old but in the younger men between age 20 to 55 years old, decline in IKES and HGS were 24% and 19.6%, respectively.[113] A 20% reduction in maximum voluntary force of the adductor pollicis (by ~20%) has also been seen around the time of menopause in women followed by little change after that, whereas in men (n=176), muscle force was maintained before weakness started at age of 60 years.[114] In the same study, women receiving HRT had attenuated loss of muscle force, suggesting a possible role of estrogens in preventing loss of muscle strength and weakness.[114] However, other studies did not find any differences in the rate of decline of height adjusted appendicular skeletal muscle mass between males and females before the age of 60 years old.[115]

The fluctuation of estradiol during the menstrual cycle (estrus cycle in rodents) also does not seem to affect the muscle strength, fatiguability or power performance of young female athletes (n=29)[116] or rodents.[117] Evidence to support the impact of menopause on muscle strength and muscle mass independent of ageing are equivocal and further research is needed to specify the contribution of menopause to sarcopenia.

Nevertheless, sarcopenia and NAFLD remain closely linked with each entity increasing the risk of the other (Figure 2), resulting in cardiometabolic complications and the effects of the menopause could potentially increase this risk.

Areas of Focus in Clinical Practice

DIAGNOSIS

Despite the high prevalence of NAFLD, diagnostic and management approaches in clinical practice are variable. This is partly due to low rate of recognition of NAFLD among non-hepatology specialists[118] and delayed referral of patients at risk of advanced liver disease to specialists for evaluation and care.[118] Even more worryingly, data collected from 102 countries revealed that at least 31% of the countries surveyed do not have any national guidance, strategies or action plans in place to address the increasing prevalence of NAFLD.[119]

Due to the lack of data on cost-effectiveness and value of non-invasive liver tests, screening for NAFLD in the general population is currently not recommended.[120–124] American and Asia-Pacific guidelines advise adopting a high index of suspicion to investigate for presence of NAFLD in high-risk individuals.[122,124,125] European and Latin-American guidelines offer more specific recommendations and suggest screening in patients with persistently elevated liver enzymes, in people with metabolic syndrome, in type 2 diabetes and/or obesity (BMI $\geq$30kg/m^2).[6,120] Risk prediction tools such as the Fibrosis-4 score, NAFLD fibrosis score or Enhanced Liver Fibrosis score, and transient elastography are recommended as next step in identifying patients at risk of advanced fibrosis and cirrhosis, as these patients should be referred to a hepatologist for specialist management.[126] However, these prediction tools do not consider the effects of sex, ethnic heritage and hormonal status on liver-related outcomes.

Reassuringly, sex does not influence the likelihood of unreliable liver stiffness measurements assessed by vibration-controlled transient elastography.[127]

LIFESTYLE INTERVENTIONS

Current management is focused on optimising associated co-morbidities including diabetes, hypertension, hyperlipidaemia, and reducing cardiovascular risk by encouraging smoking cessation and prescribing lipid lowering medication. Data from Korea suggest that women (but not men) with NAFLD have an increased risk of cardiovascular and liver-related mortality.[128] By contrast, data from America indicate that men with NAFLD have an increased risk of death from cancer and cardiovascular causes compared to women.[129,130] Therefore, more data are required before recommending sex-specific risk factor reduction.

Lifestyle modification remains the initial step in the management of NAFLD. Physical activity exceeding 150 minutes/week decreases serum aminotransferase levels.[122–124,131] Reducing calories by 750-1000kcal/day improves insulin resistance and hepatic steatosis.[122–124,131] Weight loss of at least 5% of body weight reduces hepatic steatosis but greater weight loss of ≥7%-10% improves NASH.[122–124,131] However, in women, ≥7-10% weight loss has a lower probability of NASH resolution, highlighting a need for sex-specific weight loss targets.[132] Additionally, the optimal amount of weight loss required to produce beneficial effects in NAFLD in post-menopausal women is not known. Furthermore, weight loss interventions that preserve or increase muscle mass[133] may have added benefits.

THERAPEUTICS

There are currently no licensed medications for the treatment of NAFLD. Vitamin E and pioglitazone have been recommended in some guidelines.[122–124] Vitamin E has been

demonstrated to have beneficial effects on liver transaminases, hepatic steatosis, lobular inflammation and hepatocellular ballooning.[134] However, sex-specific outcomes were not reported in this meta-analysis[134], nor in the individual studies included in the meta-analysis.[135,136] Furthermore, long-term high-dose Vitamin E use may increase the risk of heart failure[137] and prostate cancer.[138] Therefore, sex-specific analyses of treatment responses and adverse events are required as the risk-benefit ratio of Vitamin E use in NAFLD may differ between men and women.

Pioglitazone, a PPARγ activator, improves insulin sensitivity and attenuates inflammation and fibrosis in patients with and without diabetes with biopsy-proven NASH, but weight gain, fluid retention and increased risk of bone fractures are commonly-occurring adverse effects that limit its use.[122–124] Interestingly, women with NAFLD and pre-diabetes or type 2 diabetes treated with pioglitazone have greater reductions in liver fat content than men with similar co-morbidities.[139] This may be due to a greater reduction in insulin resistance by pioglitazone in women compared to men.[139] Until further data are available, both Vitamin E and pioglitazone are not recommended for patients without biopsy-proven NASH. [122–124]

Reproductive hormones impact the risk of NAFLD development and progression in women. However, current evidence is insufficient to recommend HRT as a treatment for NAFLD in post-menopausal women. In a small study that included men and women with NAFLD, combination treatment with spironolactone (which has anti-androgenic effects) and Vitamin E reduced hepatic fat scores after 52 weeks of treatment.[140] Sub-analyses by sex were not reported in this study. In women with PCOS, spironolactone use has been shown to improve insulin resistance and lipid levels.[141] Whether the anti-androgen effect of spironolactone would modify the risk of developing NASH in women with PCOS remains to be explored. As current management options for

NAFLD are limited, patients should be offered the opportunity to participate in research as they may benefit from early access to emerging therapies.

Future Directions and Conclusions

While several medications have failed to demonstrate an improvement in clinical trials endpoints, there are still promising agents in the pipeline for the treatment of NAFLD[142]. In addition, reproductive hormone receptor agonists involved in hepatic steatosis, inflammation and/or fibrosis, such as the kisspeptin receptor[143] and estrogen-related receptor α[144], are being developed as potential therapeutic agents. Data from large-scale studies like DAISY-PCOS (Dissecting Androgen excess and metabolic dysfunction – an Integrated Systems approach to PCOS) may advance our understanding of the influence of androgens on NAFLD and offer tailored management strategies in women.

In conclusion, management of women with NAFLD should take into consideration their risk profiles, hormonal status, age and metabolic factors. Evidence-based data on the influence of sex on biomarker sensitivity and/or sex-specific prediction models are needed. A better understanding of the influence of reproductive hormones on NAFLD and reporting of sex-based responses to therapeutic interventions could lead to the development of beneficial personalised management approaches in women.

Figure legends

Figure 1: Changes occur in adipose tissue, liver and skeletal muscle during the menopause that have detrimental metabolic effects. These may contribute to the increased prevalence of metabolic conditions in postmenopausal women.

Figure 2: Interactions between adipose tissue, muscle and liver contribute to the development and progression of Non-alcoholic Fatty Liver Disease (NAFLD) in women. Adipokines and myokines (such as myostatin) mediate adipose tissue-muscle interactions. Ageing and the menopause (i.e. estrogen deficiency) increase visceral adipose tissue (VAT) depots and reduce muscle mass and quality. Expanded VAT depots increase free fatty acid (FFA) delivery to the liver, which has detrimental effects. These alterations in body composition contribute to insulin resistance, hyperglycaemia and/or hyperlipidaemia, with consequent development and progression of NAFLD.

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Conflict Of Interest Statement

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