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Sex Differences in NAFLD: State of the Art and Identification of Research Gaps

Amedeo Lonardo[1], **Fabio Nascimbeni**[1,ǂ], **Stefano Ballestri**[2,ǂ], **DeLisa Fairweather**[3], **Sanda Win**[4], **Tin A. Than**[4], **Manal F. Abdelmalek**[5], **Ayako Suzuki**[5,6,*]

[1]Azienda Ospedaliero-Universitaria di Modena, Modena, Italy

[2]Azienda USL di Modena, Modena, Italy

[3]Department of Cardiovascular Medicine, Mayo Clinic, Jacksonville, Florida, USA

[4]University of Southern California Research Center for Liver Diseases, Keck School of Medicine, University of Southern California, Los Angeles, USA

[5]Division of Gastroenterology, Duke University Medical Center, Durham, North Carolina, USA

[6]Division of Gastroenterology, Durham VA Medical Center, Durham. North Carolina, USA

Abstract

In spite of tremendous research advancements in nonalcoholic fatty liver disease (NAFLD), our understanding of sex-differences in NAFLD remains insufficient. This review summarizes current knowledge on sex differences in NAFLD, identifies current gaps, and discusses important considerations for future research. The prevalence and severity of NAFLD are higher in men than in women during the reproductive age. However, after menopause, NAFLD occurs at a higher rate in women suggesting that estrogen is protective. Sex differences also exist for the major risk factors of NAFLD. In general, animal models of NAFLD recapitulate sex differences observed in patients with more severe steatosis and steatohepatitis, more pro-inflammatory/pro-fibrotic cytokines, and a higher incidence of hepatic tumors in males than females. Based on computer modeling, female and male livers are metabolically distinct with unique regulators modulating sex-specific metabolic outcomes. Analysis of the literature reveals that most published clinical and epidemiological studies fail to examine sex differences appropriately. Considering the paucity of data on sex differences and the knowledge that regulators of pathways relevant to current therapeutic targets for NAFLD differ by sex, clinical trials should be designed to test drug efficacy and safety according to sex, age, reproductive stage (i.e., menopause) and synthetic hormone use.

Conclusion: Sex differences do exist in the prevalence, risk factors, fibrosis, and clinical outcomes of NAFLD suggesting that, while not yet incorporated, sex will probably be considered in future practice guidelines. Adequate consideration of sex differences, sex hormones/menopause status, age, and other reproductive information in clinical investigation and gene association

[*]**Corresponding author:** Ayako Suzuki, MD, PhD, MSc, Gastroenterology, Duke University, 40 Duke Medicine Circle, Suite 03107, Durham, NC, 27710, TEL: 919-684-6211, FAX: 919-684-8857, ayako.suzuki@va.gov.
[ǂ]equally contributed

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studies of NAFLD are needed to fill current gaps and implement precision medicine for patients with NAFLD.

Keywords

gender difference; fibrosis; hepatocellular tumors; metabolic syndrome; menopause; molecular pathogenesis; nonalcoholic steatohepatitis

BACKGROUND

The nonalcoholic fatty liver disease (NAFLD) epidemic is a global public health concern with a heavy healthcare burden.(1) NAFLD is the fastest growing cause for orthotopic liver transplantation due to end-stage liver disease (ESLD) (2) and hepatocellular carcinoma (HCC).(3) Heterogeneity in NAFLD risk profiles and treatment responsiveness challenges accurate identification of high-risk individuals and personalized preventive/therapeutic strategies thus hampering attempts to decelerate this ever-increasing health threat.

The study of sex differences is a rapidly growing area of medicine. The 2014 NIH announcement prompting researchers to assess sex differences in preclinical NIH-funded studies facilitated a steady increase in the number of publications on sex differences.(4) (Figure 1) Biological *sex differences* in normal physiology and disease arise principally from sex chromosomes and sex hormones. Physiological levels of sex hormones vary significantly throughout the reproductive and menstrual cycle in premenopausal women and influence physiological functions and disease susceptibility. Thus, without considering sex and age, clinical and animal studies may fail to identify the influence of biological sex on study outcomes or arrive at erroneous conclusions.

Differences in men and women that are influenced by socio-cultural factors are termed *gender differences,* which should be distinguished from biological sex differences. Sex and gender differences undergird fundamental biological variation in disease as well as its progression. For this reason consideration of sex and age (e.g., puberty, menopause) is crucial in determining risk assessment, disease prevention, and treatment.(5) Most precision medicine approaches typically omit discussion of sex differences as they relate to disease susceptibility, phenotypes, and outcomes.(6) Sex differences have been extensively studied in recent years in fields relevant to NAFLD and its pathogenesis (Figure 1). Compared to other areas of study, fewer publications describe sex differences in NAFLD even though major risk factors for NAFLD (i.e., metabolic syndrome (MetS), regional adiposity, type 2 diabetes (T2D)) are known to display profound sex differences.

Differences in socio-cultural characteristics (i.e., *gender differences*), such as dietary patterns, exercise, and quality of life (7, 8), are as equally important to consider in NAFLD as sex differences. Due to space constraints, *gender differences* will not be discussed here. This review will rather focus on biological *sex differences* in primary NAFLD in adults and, to a lesser extent, children, identify important gaps in knowledge, and address basic and clinical unanswered research questions.

OVERVIEW OF NAFLD PATHOBIOLOGYAND SEX DIFFERENCES

The pathobiology of NAFLD is complex and multi-phasic (Figure 2). Positive energy balance, dysfunctional adipose tissue, systemic inflammation, insulin resistance (IR), and hepatic lipid accumulation are all fundamental drivers of liver injury, while intestinal microbiota and bile acids (BA) interact with key players in the pathogenesis of NAFLD in a multiphasic manner. Nonalcoholic steatohepatitis (NASH) increases the risk for hepatic fibrosis, cirrhosis, ESLD and HCC.(9, 10) Known sex differences in key mechanisms are depicted in Figure 2.

SEX DIFFERENCES IN CLINICAL NAFLD

Disease Risk

Prevalence of NAFLD is globally 25.24% and varies among countries.(9) In general adult populations, overall NAFLD prevalence is higher in men than in women (Table 1). Menopause or age-specific sex difference was infrequently considered in published studies, but when examined, NAFLD prevalence and incidence are higher in men than in premenopausal women (or ≤ age 50–60 years) while they tend to become more common in women after menopause (or ≥ age 50–60 years) (11–15)(Table 1). Postmenopausal women on hormone replacement therapy (HRT) had a lower prevalence of NAFLD compared to postmenopausal women not on HRT (16). In a randomized clinical trial, combined HRT significantly decreased aminotransferase levels in postmenopausal women with T2D and presumed NAFLD compared to placebo controls.(17) Collectively, current evidence suggests that estrogen protects from NAFLD.

In pediatric populations, a meta-analytic study showed that the pooled NAFLD prevalence is higher in boys than in girls in general populations and obese clinical cohorts (18). The study also revealed significant variance across the published reports, which is partly explained by the technique used to diagnose NAFLD (ultrasound vs. aminotransferases) (18) and by failure to consider pubertal stages when sex hormone levels change dramatically in a sex-specific manner.

Several studies have non-invasively evaluated the risk of fibrosis/mortality in general populations, but the results are inconsistent in terms of sex differences (Table 1). An increased liver stiffness measured by transient elastography occurred more often in men and was associated with MetS features in a cohort without known liver conditions or other severe comorbidities.(19) The risk of liver fibrosis (Fibrosis-4 ≥ 2.67) in patients with NAFLD was lower in men than women after adjusting for other metabolic variables.(20) The risk of fibrosis progression assessed by the AST to Platelet Ratio Index in a NAFLD population was associated with obesity and weight gain, but not sex.(21) These studies did not consider menopause status or age-specific sex-difference, which may have confounded the results (Table 1). Lastly, a multi-national study with biopsy-confirmed NAFLD and advanced hepatic fibrosis showed that older age and male sex were associated with worse survival and greater incidence of HCC.(22)

Regional Adiposity and MetS

The risk of developing NAFLD increases after menopause owing to body fat distribution shifting to the abdominal position.(11, 23) Men and postmenopausal women are at a greater risk of MetS compared to premenopausal women.(24) Of note, premature ovarian insufficiency carries a higher risk of MetS and IR.(25) Menstrual irregularities in premenopausal women are a risk factor for developing T2D.(26) Sleep deprivation/ disorders, which are associated with obesity and MetS,(27) exert worse metabolic consequences in women compared to men.(28) Serum uric acid levels are closely linked to sex and menopause status (29) and are associated with NAFLD, but only in men with T2D. (30) These findings suggest that sex modulates the association between hyperuricemia and NAFLD. Sex differences in adiposity and other metabolic risk factors are likely to contribute to sex differences in the driving force of disease (Figure 2).

Histologic Features

Current evidence suggests that sex, puberty, and menopause significantly affect NAFLD histology. In a NAFLD registry study, Mallory-Denk bodies appeared only after puberty while severe portal inflammation was more prevalent before puberty.(31) In another NAFLD registry study of adults, premenopausal women had more severe lobular inflammation, hepatocyte ballooning and Mallory-Denk bodies than men or postmenopausal women after adjusting for variables defining hepatic metabolic stress.(32) This suggests that sex hormones modulate hepatic injury/inflammation for any given level of metabolic stress. Estrogen is known to increase T and T-regulatory cell numbers,(33) which may either enhance or reduce inflammation in the liver. Immune cells are well known to differ by sex(33). However, sex hormone effects on immune phenotypes (pro-inflammatory vs. regulatory) in NASH remain to be investigated.

Although premenopausal women have more severe hepatocyte injury and inflammation, they have less hepatic fibrosis compared to men and postmenopausal women,(32) suggesting multi-phasic effects of sex and hormones on NAFLD pathogenesis (Figure 2). The specific mechanisms underlying the incongruous data are currently unknown. Future studies characterizing the immune infiltrate in NASH human liver are needed to explore sex differences in inflammation and cytokine profiles which may promote fibrosis. Among postmenopausal women with NAFLD, premature menopause and a longer duration of estrogen deficiency are associated with more severe liver fibrosis.(34) Collectively, data indicate that estrogen protects the liver from fibrosis in NAFLD. Features of hepatic injury and inflammation in response to metabolic stress appear to be diverse and depend on sex, pubertal stage, and sex hormone levels. This area requires further research.

Hepatocellular Tumors

Irrespective of its etiology, HCC is more common in men than women.(35) Hepatocellular adenoma (HCA) predominantly occurs in women, but men have a 10-fold increased risk of HCC.(36) Large cohort studies of patients with NASH-cirrhosis found that men had a 2- to 7-fold higher risk of developing HCC than women.(10, 22) Women have higher survival rates from HCC than men before age 55, but this advantage is reversed after this age.(37) Chronic injury and inflammation are well-established prerequisites for tumorigenesis, and,

in NASH, HCC risk parallels increasing hepatic fibrosis (Figure 2).(38) A cross-sectional study of 87 patients with NASH found that men developed HCC at earlier liver fibrosis stages than women.(38) Overall, data indicate that men are at a higher risk of NAFLD-HCC than women.

SEX DIFFERENCES IN EXPERIMENTAL NAFLD

Computational models

A recent mice computational model concluded that female and male livers are metabolically distinct organs.(39) Simulating transcriptional regulation of estradiol, androgen, and sex-specific patterns of growth hormone secretion (40), the modeling approach identified genes which regulate sex-specific effects on metabolism pertaining to hepatic triglyceride accumulation (e.g., triglyceride export, fatty acid (FA) oxidation): peroxisome proliferator-activated receptor (*PPPAR*)- γ coactivator 1-α co*PGC1a*), farnesoid X receptor (*FXR*), liver X receptor (*LXR*), and *PPAR*-α.(39) These regulators are currently being investigated as novel therapeutic targets in NASH (41) stressing the importance of examining sex differences in the efficacy and safety of drugs that target these genes.

Animal models

Most experimental NAFLD studies using genetically engineered mice and mice fed altered diets find that disease is more severe in males, recapitulating the main feature of clinical NAFLD. However, sex differences differ by model, mouse strain, and/or outcome criteria. *FXR* deficient male mice fed a Western diet had more severe steatohepatitis than females. (42) Another study examining the effect of a high-fat, high-fructose diet found that males had higher hepatic triglyceride levels and developed more severe steatosis compared to females for most (but not all) of the more than 100 inbred mouse strains used.(43) Studies with high-fat diets (HFD) found more severe liver histology changes in males than females. (44) Methionine-choline deficient diet-induced steatosis is more severe in male than female mice.(45) Contrarily, female C57BL/6 mice fed a high-fructose diet had similar liver steatosis to males but greater hepatic inflammation and decreased adiponectin in the visceral adipose tissue despite higher absolute weight gain in males.(46)

Sex hormones function as effect modifiers. Female rodents are protected from the adverse metabolic consequences of a high-fructose diet, but this protection is lost with ovariectomy. (47, 48) Importantly, this sex difference in the metabolic effects of fructose overfeeding has also been observed in humans, with triglyceride and alanine aminotransferase rising significantly in men but not women (49) although sex differences in histologic features remain unknown.

SEX DIFFERENCES IN NAFLD PATHOBIOLOGY

Adipose Tissue, Skeletal Muscle and Metabolism

Regional fat distribution is directly associated with the risk of metabolic disorders and NAFLD, with a lower risk resulting from gynoid gluteo-femoral subcutaneous distribution and a higher risk with android visceral adiposity.(50) Compared to abdominal adipose tissue,

gluteo-femoral adipoctyes have a lower lipolytic response to epinephrine and norepinephrine (51) and release fewer FA. Estradiol lowers lipolysis and improves adipose tissue insulin sensitivity (52, 53) which, in turn, reduces excess delivery of FA to the liver. Regardless of menopausal status, serum adiponectin is higher in women than men.(54) Collectively, women, especially premenopausal women, are protected from the adverse consequence of excess fat storage. Higher androgen levels in women increase abdominal adiposity and the risk of metabolic disorders while in men androgen reduces abdominal adiposity. (55)

Skeletal muscle, one of the major organs responsible for peripheral glucose disposal, is generally more insulin sensitive in women than men.(56) Sarcopenia, which is associated with IR, reduced physical activity, pro-inflammatory cytokines and the lack of anabolic hormones (57), has been associated with NAFLD independent of MetS features.(58) Sex-differences in muscle physiology, sarcopenia, and response to treatment have been extensively reviewed elsewhere (59). Skeletal muscle expression of estrogen receptor-α is markedly reduced in women with MetS (60) and, compared to placebo controls, HRT increases lean body mass while reducing fat mass in postmenopausal women.(61) Hepatic insulin sensitivity is also lower in obese men compared to BMI-matched women.(62)

Intestinal Microbiome and Bile Acids

Dysbiosis and BA play pivotal roles in the pathogenesis of NAFLD, NASH, and HCC. Recent advancements are summarized elsewhere.(63, 64) Gut microbiota modulates the ‘gut-liver axis’ via FXR signaling in the intestine, releasing fibroblast growth factor-15/19 (FGF-15/19) which regulates BA synthesis and lipid/glucose metabolism. Serum FGF-19 is decreased in patients with NASH (65) but increases after gastric bypass surgery.(66) A non-tumorigenic analogue of FGF-19 demonstrated a significant reduction in steatosis, serum aminotransferases, and fibrosis markers in patients with NASH, regardless of patient sex. (67) Various factors (e.g., age, sex, diet, physical activity)(63) influence gut microbiota composition and diversity. Recent clinical studies demonstrate that BMI-specific sex differences and variation by menopausal status occur in gut microbiota, (68, 69) possibly explaining sex differences observed in the adiposity and metabolism of NAFLD patients. (70) Serum and hepatic BA profiles in female mice are different from males in an age-specific manner due to sex-divergent expression of BA transporters Ntcp and Oatp1b2 and BA synthetic enzyme CYP7a1.(71) How sex differences in BA affect NAFLD risk and treatment response in humans remains to be investigated.

Innate Immune Response

The innate immune response to damaged hepatocytes is a key component of the pathogenesis of NASH, given that the damaged hepatocyte recruits inflammation which promotes remodeling and fibrosis. A HFD induces steatohepatitis and inflammasome activation only in male mice.(44) Key cells involved in the innate immune response in the liver are Kupffer cells and neutrophils. Most immune cells express multiple sex hormone receptors which drive immune responses in a sex-specific manner (reviewed in (33)). Briefly, Kupffer cells from female mice express higher levels of the TLR-associated transcription factor MyD88, greater p38 MAP kinase phosphorylation and, therefore, greater activation following lipopolysaccharide challenge than macrophages from male mice.(72)

Macrophages from males express higher levels of TLR4 than females. TLR4 increases the pro-inflammatory and pro-fibrotic cytokine IL-1β, which leads to increased IL-6. Inflammatory chemokine/cytokine production also differs by sex in NAFLD animal models with higher production of the chemokine CXCL10 and the pro-inflammatory cytokines, TNFα, IL-1β, and IL-6, in macrophages from males while macrophages from females produce higher anti-inflammatory prostanoids.(72) Thus, animal models demonstrate that innate immune cells from male mice are activated in such a manner as to promote liver inflammation and fibrosis, while macrophages from female mice display either a regulatory or a more protective, anti-fibrotic phenotype.

Remodeling and Fibrosis

Remodeling is a dynamic process cycling between the breakdown of extracellular matrix components and the build-up of scar tissue. Matrix remodeling is driven by matrix metalloproteinases (MMPs) that differ by sex.(73)

Hepatocyte apoptosis, activation of Kupffer and other innate immune cells, and pro-fibrotic cytokines are necessary for fibrogenesis. The most characteristic fibrosis pattern in the liver from patients with NASH is pericellular fibrosis in zone 3 (i.e. chicken-wire appearance). Animal models have shown that estradiol inhibits stellate cell activation and liver fibrosis via estrogen receptor-β in both sexes.(74, 75) Stellate cells express progesterone receptors in both sexes and, in culture, progesterone activates stellate cells by inducing ROS generation, MAPK pathway activation, and TGFβ1 expression, all of which are inhibited by estrogen.(76) Whether synthetic progesterone use in oral contraceptives, which alters the estrogen to progesterone ratio, is detrimental to NAFLD progression in premenopausal women remains to be investigated.

Another fibrosis pattern seen in the liver of patients with NASH is periportal/portal fibrosis, which is associated with activated hepatic progenitor cells and portal inflammation.(77) Since different mechanisms are involved in these fibrosis patterns, whether or not estrogen and progesterone have similar effects on periportal/portal fibrosis vs. pericellular fibrosis remains unknown.

Tumorigenesis

Hepatic tumorigenesis is driven by chronic liver inflammation, persistent tissue injury, and subsequent compensatory hepatocyte proliferation. Male animals develop hepatic tumors more often than females, which is consistent with the clinical notion of increased NAFLD-HCC in men. This is explained, at least in part, by a higher production of IL-6 by Kupffer cells in males and inhibitory effects of estrogen on IL-6 production in females, which reduces hepatic injury and compensatory proliferation of hepatocytes.(78) Sex difference in IL-6 levels has been attributed to TLR4-induced MyD88 activation.(78) Western diet-fed *FXR* deficient mice develop fatty adenoma only in males.(42) Phosphatase and tensin homolog (*PTEN*) deficient mice develop steatosis, NASH, and HCC in a male-dominant manner.(79) Transgenic Mito-Ob obese mice that over-express prohibitin in adipocytes develop IR, NASH, and HCC with age in a male-specific manner.(80)

Men are over-represented in the molecular subgroup of HCA harboring mutations of beta-catenin in exon 3, which is strongly associated with androgen exposure and HCC development. This subgroup of HCA is also found more often in women who have a low lifetime estrogen exposure.(81) Estrogen protects from liver tumorigenesis (78) and, theoretically, prolonged estrogen depletion (i.e., premature menopause) may lead to increased HCC risk, which needs to be investigated in women with NAFLD.

CONSIDERATION OF SEX DIFFERENCES IN NAFLD RESEARCH

Our review has critically discussed sex differences in clinical and experimental NAFLD and has highlighted that many clinical/epidemiological publications on NAFLD do not properly analyze such sex differences. Indeed most studies ‘assume’ sex and age as independent variables, without assessing their interaction and without considering menopause status in the study design/analysis. Sex differences affect various physiological functions and act as effect modifiers (Figure 3). Thus, analyzing a population without considering potential sex-specific effects or hormonal effects, probably masks important observations. How reproductive status and synthetic hormone use impact disease risk in women with NAFLD deserves further investigation.

In genome-wide association studies (GWAS) on NAFLD, sex is generally considered only as a co-variate, but sex-gene and gene-sex hormone interactions are neglected despite the fact that both experimental and clinical studies have documented the importance of sex as an effect modifier in gene-association studies.(43, 82) Immune-regulating genes on X-chromosomes (83) are typically excluded from GWAS studies owing to their complex regulation. A new mathematical model should promote research in this area.(84)

Although sex differences do exist in NASH mechanisms, current evidence is insufficient to allow sex-specific personalized therapies. Further investigations are needed with proper consideration of sex and reproductive status in the study design, from pre-clinical to epidemiological studies, and clinical trials. Currently, there is no specific regulatory guideline for sex/gender consideration by the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use. Global regulatory recommendations should be enacted to institutionalize sex/gender consideration in future drug development.

Summary

Given the heterogeneity in NAFLD, evidence-based, tailored clinical care is crucial to reduce the burden of the NAFLD epidemic. Sex and sex hormones are one of the largest influencers of biological variance in human diseases. A proper consideration of sex, age, hormonal status, and socio-cultural gender differences will lead to a better understanding of sex and gender differences in NAFLD risk, therapeutic targets, and treatment responses and aids to achieve precision medicine.

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ABBREVIATIONS

BA	bile acids
BMI	body mass index
CXCL10	C-X-C motif chemokine 10
ESLD	end-stage liver disease
FA	fatty acids
FGF	fibroblast growth factor
FIB-4	fibrosis-4 index
FXR	farnesoid X receptor
GWAS	genome-wide association mapping studies
HCA	hepatocellular adenoma
HCC	hepatocellular carcinoma
HFD	high-fat diet
HRT	hormone replacement therapy
IL	interleukin
IR	insulin resistance
LXR	liver X receptor
MetS	metabolic syndrome
MMPs	metalloproteineases
NAFLD	nonalcoholic fatty liver disease
NASH	nonalcoholic steatohepatitis
PGC1	peroxisome proliferator-activated receptor-γ coactivator 1
PPAR	peroxisome proliferator-activated receptor
PTEN	phosphatase and tensin homolog
TGF	transforming growth factor
TLR	Toll-like receptor
TN	tumor necrosis factor

T2D type 2 diabetes

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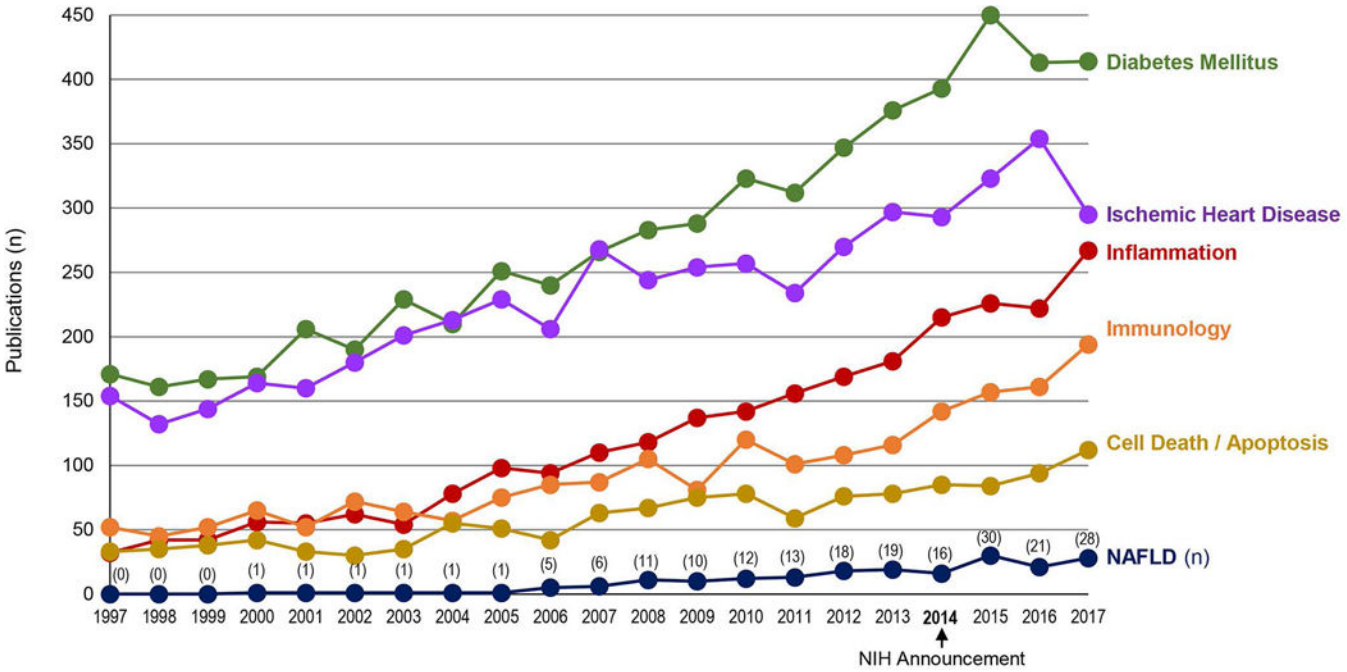

Figure. 1. Number of annual publications on sex differences in NAFLD and related fields.

Data were obtained from PubMed using the keyword *'sex difference'* combined with 'inflammation', 'immunology', 'cell death or apoptosis', 'diabetes mellitus', or 'ischemic heart disease'. Research on sex differences in NAFLD has apparently lagged behind other areas.

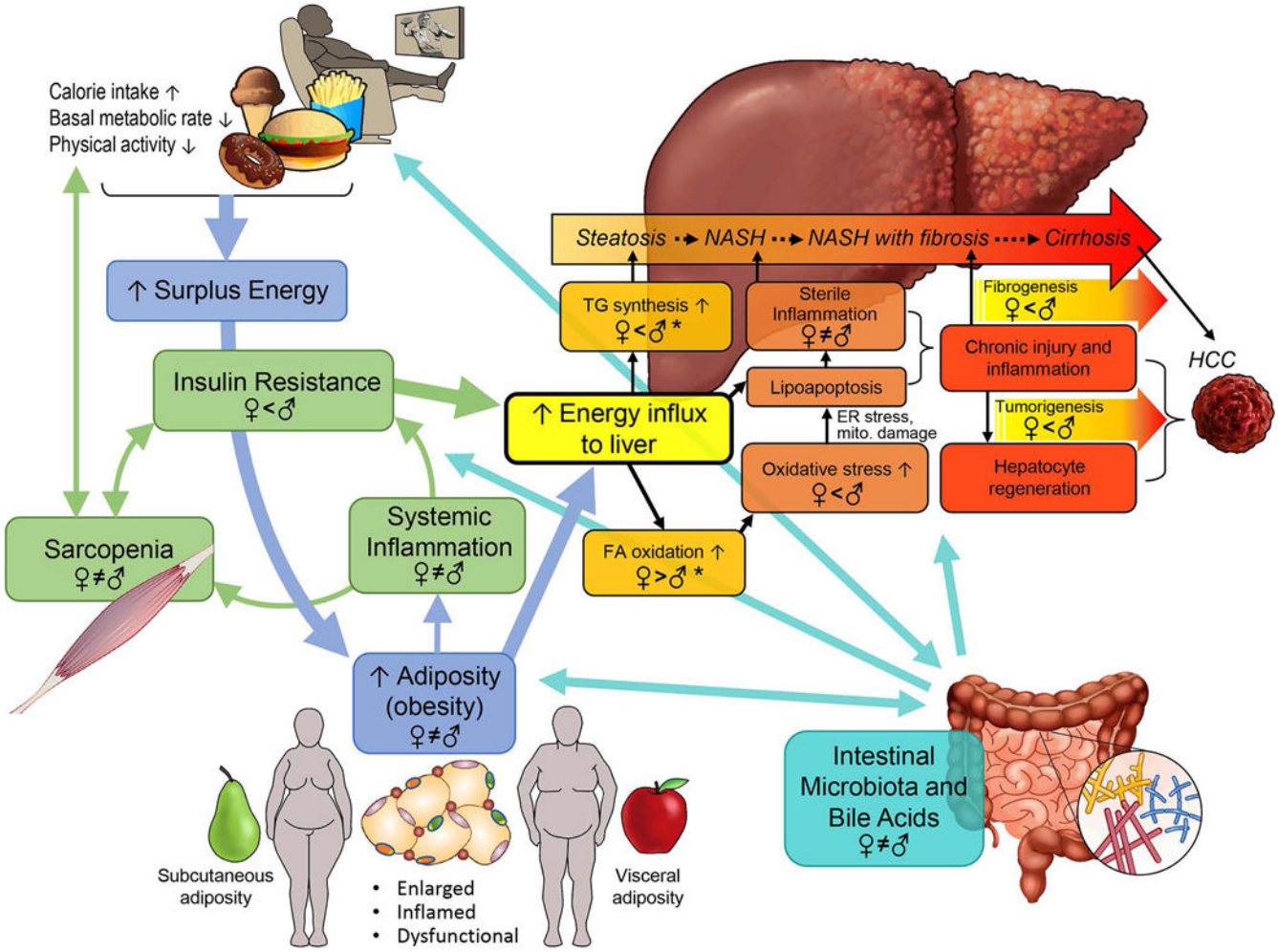

Figure. 2. Overview of NAFLD pathogenesis and sex differences.

TG: triglycerides. Women and men store surplus calories differently: gluteo-femoral subcutaneous in women vs. visceral adiposity in men. Enlarged, dysfunctional adipose tissue, especially visceral adiposity, leads to systemic inflammation and insulin resistance, which facilitates energy influx to the liver and increases metabolic stress in hepatocytes. Sarcopenia exacerbates these changes, by generating a vicious cycle. When hepatocytes fail to adapt, the increased metabolic stress triggers oxidative stress or direct toxic effect of free FA on hepatocytes, and induces lipoapoptosis, which, in turn, leads to sterile inflammation. Chronic inflammation promotes fibrosis, cirrhosis, and tumorigenesis. Intestinal microbiota and BA play pivotal roles in regulating NAFLD pathogenesis in a multi-phasic manner while interacting with key players. Known sex differences and hormonal effects are depicted in a mechanism-specific way for further discussion in this review. Sex differences in TG synthesis, FA oxidation, and oxidative stress are not covered in this review. Sex difference in oxidative stress is well accepted. * see reference (85)

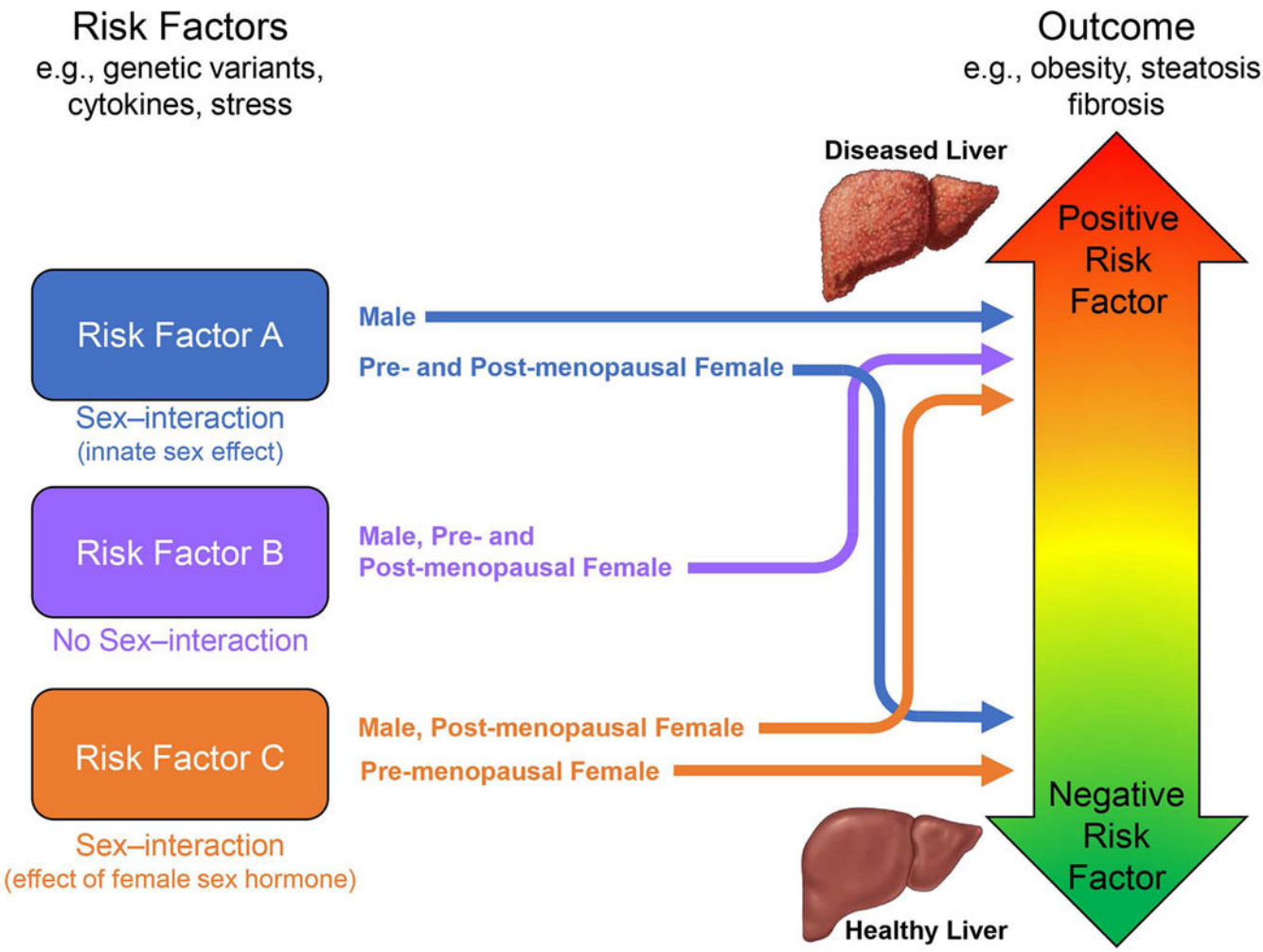

Figure. 3. Sex difference considerations in NAFLD research.

Risk factor A- A factor whose effect is significantly modified by sex or innate sex-related attributes (i.e., chromosomes) so that the outcome will significantly differ between women and men. **Risk factor B**- A factor whose effect is modified by neither sex nor sex hormones, resulting in an outcome which is invariably consistent among premenopausal women, postmenopausal women and men. **Risk factor C**- A factor whose effect is significantly modified by female sex hormones. In this case, the association between risk factor C and the outcome will significantly differ among premenopausal, postmenopausal women, and men. In the analysis of risk factors A and C, the lack of proper cohort classification (i.e., subgroup analysis) would either result in a spurious conclusion or mask important sex-specific effects. Both sex and sex hormones interact with numerous NAFLD risk factors and alter the risk profiles and phenotypes of NAFLD in individuals.

Table 1.

Population-based studies: sex-differences in frequency of NAFLD, fatty liver, and NAFLD fibrosis

Author, year (Ref)	Country	M (n)/ F (n)	Age, yrs	Consideration of Menopausal status	Ethnicity	Main findings
Prevalence and incidence of NAFLD in adults						
Zelber-Sagi S *et al.*, 2006(86)	Israel	174/152	50.5 ± 10.3	No	Not specified	Overall NAFLD^{US} prevalence higher in men than in women (38% vs 21%). Male gender and metabolic factors independently predicted NAFLD.
Park *et al.*, 2006(11)	Korea	3,530/3,118	48.0 ± 11.8	1273 post-menopausal women	Asian	Menopause was an independent risk factor for prevalent NAFLD^{US} (OR 1.71) among women.
Caballeria et al., 2010(87)	Spain	323/443	53±14	No	Not specified	NAFLD^{US} prevalence significantly higher in men (33%) than women (20%). Male sex, age, MetS, IR and alanine aminotransferase independently predicted NAFLD.
Wong VW *et al.*, 2012(12)	Hong Kong	389/533	48 ± 11	Age 50 years cut-off	Asian	NAFLD^{MRS} prevalence higher in men than in women (37% vs 23%) overall. It peaked at 40 years and remained constant after in men while it increased steadily after 50 years in women.
Eguchi Y *et al.*, 2012(13)	Japan	2,627/2,448	50.0 ± 9.5	No	Asian	NAFLD^{US} prevalence higher in men than in women at all ages (overall: 41% vs 18%) and increases with age in women (31% after 60 years).
Lazo M *et al.*, 2013(88)	USA	Total 12,454	20–74	No	European descent	Computed NAFLD^{US} prevalence after adjustment for other confounders was higher in men (20.2%) than in women (15.8%).
Li Z *et al.*, 2014(89)	China	201,481/ 152,124 from 48 Chinese studies	Weighted average: 40.32 for men, 34.8 for women	No	Asian	This *meta-analysis* of Chinese studies found a higher prevalence of NAFLD^{US} in men (24.8%) than in women (13.2%).
Wang Z *et al.*, 2014(14)	China	15,551/9,481	44.1 ± 15.0	Age 60 years cut-off	Asian	NAFLD^{US} prevalence was higher in men than women (32% vs 13%) but comparable in women > 60 years (27.6%).
van den Berg et al., 2017(90)	Netherlands	14,226/23,270	44 (36–51)$^{\$}$	No	European	Male sex was independently associated with NAFLD prevalence (FLI ≥ 60).
Long MT *et al.*, 2018(15)	USA	364/321	45.0 ± 6.2	Yes (N not reported)	Largely European descent	Incidence of fatty liver ^{CT} was similar between postmenopausal women and men (19% vs 22%) and was lower in premenopausal women (9%).
Prevalence of fatty liver$^{\#}$ in adults						
Browning JD *et al.*, 2004(91)	USA	2240/1194	46 ± 9	No	Whites 38%, Blacks 43%, Hispanics 17.5%	Fatty liver ^{MRS} prevalence was higher in men than in women (42% vs 24%) among whites, but not in other ethnic groups.

Author, year (Ref)	Country	M (n)/ F (n)	Age, yrs	Consideration of Menopausal status	Ethnicity	Main findings
Zhou YJ *et al.*, 2007(92)	China	1311/2232	7– 70+	No	Asian	Fatty liver[US] prevalence significantly higher in men (13.8%) than women (7.1%) before 50 years; the opposite after 50 years.
Prevalence of NAFLD and fatty liver in children						
Anderson *et al.*, 2015(18)	Europe, America, Asia	Total 23,208 from 20 general populations and 23,892 from 56 obese clinical populations	2−19	-	European, American, Asian	This *meta-analysis* of children found NAFLD [US, LBx, MRI] prevalence higher in male than female adolescents both in general (9% vs 6%, based on 15 studies) and obese populations (30% vs 22%, based on 27 studies).
Prevalence of NAFLD fibrosis						
Kim et al., 2013(93)	USA	5,296/5,858	20−74	No	Non-Hispanic white, non-Hispanic black, Mexican American	4,083 of 11,154 had NAFLD[US], of them 173 (3.2%) had elevated NFS (>0.676) with no sex difference.
Hartleb et al., 2017(94)	Poland	1405/1598	79.6±8.3	No	European	NAFLD (FLI ≥ 60) with fibrosis was more prevalent in males when defined by Fib-4 (≥ 1.45) but not NFS (>0.676).
Caballeria et al., 2018(19)	Spain	1289/1725	54±12	No	European	Higher liver stiffness (TE) was independently associated with male sex.
Tanaka et al. 2018(20)	Japan	3316/3611	50 (42−57) [$]	No	Asian	Among 1935 patients with NAFLD[US], 0.9% men and 1.0% women had advanced fibrosis (FIB-4 ≥ 2.67).

[#]: more than minimal alcohol use and/or viral hepatitis infection were not excluded.

[$]: median and interquartile range

NAFLD definition - US: ultrasonography; MRS: Magnetic resonance spectroscopy; CT: computerized tomography; MRI: Magnetic resonance imaging; LBx: liver biopsy

Abbreviations: ALP, alkaline phosphatase; AST, aspartate aminotransferases; AUC, area under the curve; FL, fatty liver; FLI: fatty liver index; HDL-C, high density lipoprotein cholesterol; IR,insulin resistance; hsCRP, high sensitivity C reactive protein; LDL-C, low density lipoprotein cholesterol; MetS, metabolic syndrome; MVA, multivariable analysis; NA, not assessed; NASH, nonalcoholic steatohepatitis; NFS: NAFLD fibrosis score; SUA, serum uric acid; T2D, type 2 diabetes; TC, total cholesterol; TE: transient elastography; USA, United States of America; VAT, visceral adipose tissue; VFA, visceral fat area; WC, waist circumference