

Reproductive Endocrinology of Nonalcoholic Fatty Liver Disease

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ABSTRACT The liver and the reproductive system interact in a multifaceted bidirectional fashion. Sex steroid signaling influences hepatic endobiotic and xenobiotic metabolism and contributes to the pathogenesis of functional and structural disorders of the liver. In turn, liver function affects the reproductive axis via modulating sex steroid metabolism and transport to tissues via sex hormone-binding globulin (SHBG). The liver senses the body's metabolic status and adapts its energy homeostasis in a sex-dependent fashion, a dimorphism signaled by the sex steroid milieu and possibly related to the metabolic costs of reproduction. Sex steroids impact the pathogenesis of nonalcoholic fatty liver disease, including development of hepatic steatosis, fibrosis, and carcinogenesis. Preclinical studies in male rodents demonstrate that androgens protect against hepatic steatosis and insulin resistance both via androgen receptor signaling and, following aromatization to estradiol, estrogen receptor signaling, through regulating genes involved in hepatic lipogenesis and glucose metabolism. In female rodents in contrast to males, androgens promote hepatic steatosis and dysglycemia, whereas estradiol is similarly protective against liver disease. In men, hepatic steatosis is associated with modest reductions in circulating testosterone, in part consequent to a reduction in circulating SHBG. Testosterone treatment has not been demonstrated to improve hepatic steatosis in randomized controlled clinical trials. Consistent with sex-dimorphic preclinical findings, androgens promote hepatic steatosis and dysglycemia in women, whereas endogenous estradiol appears protective in both men and women. In both sexes, androgens promote hepatic fibrosis and the development of hepatocellular carcinoma, whereas estradiol is protective. (*Endocrine Reviews* 40: 417 – 446, 2019)

In this review, we examine the interaction between the liver, a key metabolic organ, and the reproductive system in both health and disease. We review the intricate bidirectional interaction between liver and the hypothalamic–pituitary–gonadal axis in normal physiology reflecting the close connection between energy homeostasis and reproduction, and we highlight sex-dimorphic aspects. Next, we examine how this bidirectional crosstalk shapes the pathogenesis of the most common hepatic pathology, nonalcoholic liver disease and its sequelae, including the development of hepatocellular carcinoma.

By receiving input from multiple metabolically active organs, the liver serves as a key integrator of the body's energy needs and metabolic balance as well as the major effector of endobiotic and xenobiotic

metabolism to protect and promote health. This central role of integrating metabolic pathways and energy balance in response to metabolic cues is served by supply to the bloodstream of energy-providing metabolic substrates as well as in detoxifying endogenous and xenobiotic substances. This gives the liver a pivotal role in the reversible regulation of reproductive function, which responds to the body's health status by suppressing reproductive activity when energy metabolism or general health is poor, allowing for an orderly revival of reproductive function when energy or health status recover, a mechanism termed ontogenic reversion.

In response to multiple afferent metabolic signals (pancreatic, enteric, and adipose hormones, as well as nutrients and neural inputs) (1), the liver controls

ISSN Print: 0163-769X

ISSN Online: 1945-7189

Printed in USA

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Endocrine Society

Received: 21 June 2018

Accepted: 19 November 2018

First Published Online:

30 November 2018

ESSENTIAL POINTS

- The liver and the reproductive system interact in a complex bidirectional fashion
- The liver, an organ with prominent functional sexual dimorphism, adapts energy metabolism to sex-specific reproductive needs
- The liver modulates the reproductive axis via regulating sex steroid metabolism and transport to tissues
- Mechanistic and clinical evidence demonstrates sex-specific roles for sex steroids in the pathogenesis of the full spectrum of nonalcoholic fatty liver disease

energy storage and transfer by its crucial role in regulating lipid and glucose metabolism. Additionally, it plays a major role as the central site of metabolism of endogenous bioactive molecules and exogenous drugs. In fulfilling both of these roles, the liver expresses sex steroid receptors with both androgens and estrogens having direct as well as indirect effects on hepatic function. Sex steroids directly impact hepatic metabolism and detoxification mechanisms as well as indirectly influence them in concert with sex steroid actions on other tissues such as brain, adipose tissue, and skeletal muscle to influence homeostasis of the entire body. Sex steroids also protect against or promote hepatic disease in both men and women, although with divergent mechanisms reflecting sex differences in the underlying hormonal milieu. In turn, the liver regulates biological actions of sex steroids via their hepatic metabolism and via production of the sex steroid transport protein, sex hormone-binding globulin (SHBG). Not surprisingly, in view of the tight link between energy homeostasis and reproduction, liver disease is associated with reproductive dysfunction in both sexes, which is largely ameliorated by successful liver transplantation (2–4). The bidirectional interactions between the liver and the reproductive system discussed in this review are summarized in Fig. 1.

In this review, we outline the principles of sex steroid signaling in the liver and of hepatic sex steroid

metabolism. Next, we review the biologic basis of hepatic sex dimorphism that has likely evolved to couple hepatic metabolism to sex-specific reproductive needs. We then provide an overview on the epidemiology and clinical spectrum of nonalcoholic fatty liver disease (NAFLD) and discuss the role of sex steroid signaling in hepatic lipid and glucose metabolism in both experimental and clinical studies. Lastly, we discuss the role of sex steroids in the progression across the NAFLD spectrum, including hepatic carcinogenesis.

The material reviewed was derived from a comprehensive literature search of the medical databases MEDLINE, EMBASE, and the Cochrane Central Register of controlled trials until 30 September 2017, with no start date restriction. The search was conducted with the help of an experienced medical librarian using the search terms sex/reproductive steroids [including testosterone, androgens, dihydrotestosterone, estradiol, estrogens] and hepatic/liver disease (including fatty liver/steatosis/nonalcoholic fatty liver disease/steatohepatitis/cirrhosis). This search retrieved 2199 citations. Articles were reviewed when they reported preclinical or clinical data pertaining to the interaction of sex steroids with hepatic physiology and metabolic liver disease. Additionally, the reviewed material was supplemented by a manual search of article references, as well as the authors own databases.

Background

Hepatic sex steroid expression and signaling

The liver is a major nonreproductive target organ of sex steroid action. Both androgen receptors (ARs) and estrogen receptors (ERs) are expressed in the rodent and human male and female liver (5, 6). Studies in rats have demonstrated that AR mRNA is expressed at 20-fold higher levels in male rodent liver compared with that in females (7). In male rats, hepatic AR expression levels are highest soon after sexual maturation and gradually decline with aging (8). The AR is also expressed in human liver in both sexes, although evidence largely stems from studies examining normal hepatic tissue surrounding diseased liver specimens (5, 6).

Androgens increase the synthesis of many hepatic proteins, notably the clotting factors, α_1 -antitrypsin and haptoglobin. Conversely, androgens decrease the hepatic production of proteins such as SHBG, thyroxine- and cortisol-binding globulins, transferrin, and fibrinogen (9). However, androgen-mediated suppression of SHBG only occurs as a consequence of hepatic exposure to supraphysiologic androgen concentrations as seen with oral androgens due to first pass effects (10), or transiently with high-dose injectable testosterone formulations, especially short-acting testosterone esters (11). As described in later sections, hepatic AR signaling plays many important roles in regulating the expression of hepatic proteins involved in energy homeostasis and the metabolism of

intermediary metabolites, steroid hormones, and drugs. AR signaling is also implicated in the progression of liver fibrosis and the pathogenesis of hepatocellular carcinoma (HCC).

Both rodent as well as human hepatocytes of both sexes also express ERs (5, 6). In the rat, levels of nuclear ER α are primarily age- rather than sex-dependent, with highest levels in the perinatal and postpubertal period (12), which contrasts with the much higher AR levels in the male liver than in the female liver across the lifespan. In the mature rodent liver of both sexes, ER α is the most abundant subtype, but ER β and the membrane-bound G protein-coupled receptor 30 (Gpr30), also known as G protein-coupled ER (GPER), are also expressed (13). In rodents, ER β is expressed predominantly in hepatic stellate cells (14). In the female rodent liver, hepatic ER α transcriptional activity, similar to ER α in reproductive organs, parallels circulating estradiol concentrations across the estrous cycle (15), in contrast to the nonphasic ER α transcriptional activity in other nonreproductive tissues (15).

In the fetal human liver, neither ER α nor ER β is expressed, although GPER is detected (16). In the adult human liver in both sexes, similar to rodents, ER α is most abundant, with lesser expression of ER β and GPER (13). As described in “Role of Sex Steroids in Hepatic Glucose and Lipid Metabolism: Preclinical Studies” and “Role of Sex Steroids in Hepatic Glucose and Lipid Metabolism: Clinical Studies” below, hepatic ER signaling plays roles in lipogenesis and lipid transport, as well as in glucose homeostasis and insulin sensitivity.

Hepatic sex steroid metabolism

The liver is the major site of sex steroid metabolism, thereby regulating their activity and clearance. Hepatic phase 1 metabolism of androgens includes hydroxylation by the P450/CYP3A group of enzymes (predominantly CYP3A4), 5 β reduction by the 5 β -reductase (AKR1D1), and oxidation by the hydroxysteroid dehydrogenases 17 and 11. Hepatic phase 2 metabolism involves mainly glucuronidation and sulfation by uridine 5'-diphospho-glucuronosyltransferases and sulfotransferases, respectively, to facilitate renal excretion of the more hydrophilic conjugates (17). Conjugated metabolites are subsequently secreted into the circulation by active transport and eliminated by urinary and/or biliary excretion. The metabolic clearance rate of testosterone is higher in women than in men owing to greater amounts of CYP3A4, the major phase I testosterone-metabolizing enzyme (18, 19), and is reduced by increases in circulating SHBG (20) such as occurs in liver disease or by reductions in liver blood flow and/or function (21).

Similarly, estradiol is hydroxylated in phase I metabolism by hepatic oxidoreductases including CYPs before passing on to phase II metabolism

involving conjugation into more hydrophilic sulfate and glucuronide metabolites that are subsequently excreted via the kidneys (22). Hepatic sex steroid metabolism is not simply an excretory function, however, but it is tightly regulated by multiple mechanisms, including the circulating sex steroid concentrations themselves. Therefore, dynamic changes in hepatic sex steroid metabolism make important contributions to their biological actions (23).

The original discovery in rodents that sex differences in hepatic cytochrome P450 metabolism of certain drugs were abolished by castration (or high-dose estrogen treatment) of males and restored by testosterone treatment was shown to require an intact pituitary gland (24, 25). Subsequent rodent studies showed that sex differences in phase I hepatic oxidoreductive metabolism were conditioned by the temporal patterns of pituitary GH secretion, whereby males exhibit more intermittent, pulsatile GH secretion and circulating concentrations whereas females display more continuous GH secretion and stable circulating GH concentrations (26–28). In turn, these durable patterns of GH secretion in adulthood were imprinted by sex steroid exposure during the early postnatal period (29). Humans also display sex differences dependent on sex steroid exposure in GH secretion patterns (30–32), although to a lesser extent than in rodents. Moreover, experimental studies in humans suggest that hepatic effects of androgens, especially those on glucose metabolism, are mediated, at least in part, through interactions with GH (33). Therefore, the impact of sexually dimorphic GH secretion and signaling on xenobiotic and endobiotic

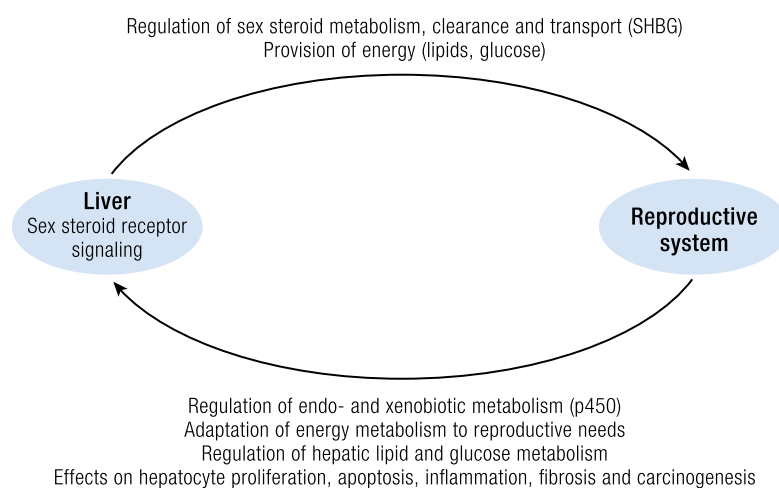


Figure 1. Interactions between the liver and the reproductive system. Depicted is an overview of the principal bidirectional interactions between the liver and the reproductive system. Sex steroids regulate hepatic lipid metabolism by modulating lipogenesis, fatty acid oxidation, and lipid export. Hepatic sex steroid signaling also relays information regarding the metabolic status of the body to the reproductive system. As detailed throughout the review, the effects of the reproductive system on liver physiology and liver disease with regard to sex dimorphism are illustrated.

metabolism, although less well studied, is likely also relevant in humans. Sex differences in drug handling in humans has multiple components, all related to long-term and prevailing exposure to sex steroids (34), but hepatic drug metabolism is among the most prominent (35).

The liver as a sexually dimorphic organ

Beyond the categorical differences between male and female reproductive systems, sex differences in many physiological functions of the liver are among the most conspicuous of any nonreproductive organ. The biological basis of these sex differences include both genetic and epigenetic hormonal (sex steroids, puberty, pregnancy, menopause, hormonal contraceptives, gonadal dysfunction) and environmental factors (e.g., diet, drugs, chemicals, diseases) as well as age. The rodent liver expresses an estimated 72% of genes in a sexually dimorphic fashion compared with 14% in the brain (36). A transcriptosomal profiling analysis reported that among 1249 sex-biased genes expressed in nontumorous human liver specimens (obtained from 224 patients undergoing liver resection for various reasons), >70% demonstrated higher expression in females compared with males with a pronounced diversity in the genes of energy metabolism (37).

In the liver, sex steroid receptors sense the body's metabolic status and play a role in adapting hepatic energy homeostasis to changing energy requirements by regulating genes involved in a broad range of metabolic processes. In studies predominantly conducted in rodents, the hepatic expression of energy metabolism regulatory genes differs between male and female livers, a dimorphism possibly related to different metabolic costs of reproduction (38). A primitive evolutionary mechanism reduces reproductive success in a nutrition-scarce environment by preferentially assigning egg protein synthesis to the organ responsible for breakdown and partitioning nutrients, that is, the female liver or its ancestral equivalents (39–41). In turn, hepatic ER signaling in female mice modulates synthesis of molecules required for reproductive cycle progression and egg maturation (42). In mammals, complex functional crosstalk between the female liver and ovary serves to optimize energy utilization (predominantly lipids) during ovulation, pregnancy, and lactation (43). This hepatic–ovarian crosstalk may play a role in the decreased susceptibility of premenopausal women to metabolic diseases such as NAFLD compared with men, a protection that is lost with age or menopause (43, 44) (see “Estrogens” under “Women” in “Role of Sex Steroids in Hepatic Glucose and Lipid Metabolism: Clinical Studies”).

An analogous mechanism also occurs in the male reproductive system under adverse environmental conditions (including disease states) whereby reproductive function in men regresses in a reversible fashion to a prepubertal state in an orderly withdrawal

of steroidogenesis and spermatogenesis that facilitates their later recrudescence when environmental conditions ameliorate (45, 46). This reversal of pubertal development, termed ontogenic reversion, is analogous to the seasonality of certain mammalian species that is now, however, vestigial in nonseasonal humans but is operative under pathological conditions.

Consistent with sex-dimorphic differences in energy requirements, sex steroids differentially regulate genes involved in hepatic lipid metabolism. For example, in HepG2 cells, a human liver cancer cell line, AR agonists attenuate the estrogen-induced upregulation of low-density lipoprotein receptor expression (47). Additionally, hepatic microsomal triglyceride transfer protein (MTP) expression is sex differentiated in rats and regulated by the sexually dimorphic secretory pattern of GH at the level of mRNA (48). There is also evidence in both humans and rodents for sex dimorphism in expression of hepatic cytochrome P450 genes that contributes to sex differences in sex steroid hormone metabolism (35).

The liver also exhibits sexual dimorphism in its capacity to regenerate. This is linked to dynamic changes in hepatic sex steroid receptor expression and circulating sex steroid concentrations in rodents and humans (49). Androgenic and estrogenic signaling via their receptors differentially regulate liver cell proliferation and apoptosis (50, 51). Differential hepatic sex steroid signaling also contributes to hepatic proinflammatory, profibrotic and procarcinogenic pathways (52) (“Role of Sex Steroids in Hepatic Fibrogenesis and Carcinogenesis: Preclinical Studies”). Furthermore, differences in hepatic sex steroid signaling between males and females may explain the sex dimorphism in the prevalence and severity of many hepatic diseases, including fatty liver, cirrhosis and cancer (see “Role of Sex Steroids in Hepatic Glucose and Lipid Metabolism: Clinical Studies” and “Role of Sex Steroids in Hepatic Fibrogenesis and Carcinogenesis: Preclinical Studies”).

NAFLD

NAFLD is defined as the presence of $\geq 5\%$ hepatic steatosis in the absence of any other cause of hepatic fat accumulation, such as heavy alcohol consumption (53), although whether there is any fundamental difference between these different metabolic triggers remains uncertain. NAFLD constitutes a spectrum of diseases of progressive severity: nonalcoholic fatty liver (NAFL) is defined as hepatic steatosis in the absence of liver inflammation, and termed nonalcoholic steatohepatitis (NASH) when inflammation is present. Hepatic inflammation in NASH is associated with hepatocellular injury (histologically characterized by ballooning) and predisposes to the development of liver fibrosis (scarring), the most important histological feature associated with an increased risk of liver disease–related death (54). Fibrosis is histologically

graded in five stages: F0, no fibrosis; F1, portal fibrosis without septa; F2, portal fibrosis with few septa; F3, bridging septa between central and portal veins; and F4, cirrhosis (53). Histologically, cirrhosis is characterized by the formation of regenerative nodules (55). Cardinal manifestations of liver cirrhosis include impaired hepatocellular function causing liver failure and increased intrahepatic resistance causing portal hypertension.

Epidemiological evidence suggests that NAFLD, which is driven by metabolic diseases such as obesity and diabetes, is the most common contemporary cause of chronic liver disease among adults worldwide. Prevalence estimates, based largely on ultrasound imaging or other indirect methods rather than gold standard liver biopsy, are 24% in the United States, 24% in Europe, 30% in South America, and 32% and in the Middle East. Data from the Asia-Pacific region and Africa are less comprehensive and vary widely depending on economic, political, and educational development (56). NASH is less common with the prevalence in the United States estimated to be ~5% (56).

Across the spectrum of NAFLD, progression of fibrosis is highly variable and depends on the stage of the disease. In a meta-analysis of 366 patients with NAFLD and serial liver biopsies, including 2145 person-years of follow-up evaluation, fibrosis progressed in 36%, remained stable in 46%, and improved in 21% of patients (57).

Overall, it appears that simple hepatic steatosis (NAFL) is relatively benign. In the aforementioned meta-analysis, among 133 patients with NAFL who had serial liver biopsies, the fibrosis progression rate was 0.07 stages per year, corresponding to 14 years per stage of fibrosis (57). In contrast, the fibrosis progression rate among 116 patients with NASH was about twice as fast, progressing by 0.14 stages per year (7 years per stage of fibrosis) (57). A systematic review of 187 patients with paired liver biopsies reported that the median time to develop advanced fibrosis (defined as stage F3 or greater) was 4.2 years among patients with evidence of inflammation on the initial liver biopsy, compared with 13.4 years in those without inflammation (58). Overall, an estimated 20% of patients with NASH progress to liver cirrhosis. Clinical risk factors implicated in disease progression include older age, type 2 diabetes, serum transaminases more than twofold the upper limit of normal, and a body mass index (BMI) >28 kg/m² (2, 59, 60). A systematic review of 61 studies concluded that in patients with established NAFLD-related cirrhosis the risk of developing HCC varied considerably, ranging from 2.4% over 7 years to 12.8% over 3 years (61). Among patients with NAFLD but without cirrhosis followed for up to 20 years, the risk of dying from HCC was relatively low, ranging from 0% to 3% (61). However, studies have also reported that up to 45% of HCC in patients with NAFLD develops in the absence of cirrhosis (62).

NASH-related cirrhosis is the fastest growing and currently second most common indication for liver transplants in the United States, after hepatitis C-related cirrhosis (63). Recent estimates suggest that the lifetime risk of developing NASH-related liver cirrhosis for all US adults is 2% (53). With the rise of obesity and diabetes, as well as the increasing availability of effective treatments for some forms of viral hepatitis, NAFLD is predicted to become the leading indication for liver transplantation by 2020 (63). Although patients with NASH are at increased risk of liver-related deaths, the most common cause of death among patients with NAFLD is cardiovascular and cerebrovascular disease in 31% of patients, followed by malignancy (24%) rather than hepatic disease, which is responsible for death in only 13% of patients (64).

NAFLD overlaps so closely with features of the metabolic syndrome, sharing many underlying risk factors, that it may be considered its hepatic manifestation (56). In a global meta-analysis of studies among NAFLD patients, an estimated 51% had obesity, 22% diabetes, 70% dyslipidemia, 39% hypertension, and 43% met the definition of the metabolic syndrome (65). Similar to the metabolic syndrome, NAFLD is a strong predictor of type 2 diabetes and is associated with increased diabetes risk independently of age and BMI (66). Hepatic glucose production accounts for 90% of endogenous glucose production so that hepatic insulin resistance, closely associated with intrahepatic triglyceride content, is central in the development of type 2 diabetes (67). One relatively small but intensive study subjecting 40 participants with obesity (including 30 women and 10 men) to euglycemic-hyperinsulinemic clamps, body composition analysis by magnetic resonance imaging, and detailed lipid kinetic studies reported that intrahepatic triglyceride content was more closely correlated with obesity-related metabolic dysfunction than even visceral adiposity (68). Hence, hepatic fat accumulation and insulin resistance are closely associated with systemic dysmetabolism and diabetes risk; however, the causality remains speculative as to whether the liver abnormalities are a cause or effect of the systemic metabolic disorder. In “Role of Sex Steroids in Hepatic Glucose and Lipid Metabolism: Preclinical Studies” and “Role of Sex Steroids in Hepatic Glucose and Lipid Metabolism: Clinical Studies” we discuss the importance of sex steroid signaling in hepatic metabolism and its possible roles in the development of, or protection against, NAFLD.

Role of Sex Steroids in Hepatic Glucose and Lipid Metabolism: Preclinical Studies

In this section, we review the insights gained from preclinical studies based primarily on experimental rodent data. In general, similar findings have been

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Table 1. Hepatic Metabolic Effects of Altered Sex Steroid Signaling in Male Rodent Models

Study (Reference)	Study Design	Effects on Lipid Accumulation	Effects on Glucose Metabolism	Other Effects
Nikolaenko <i>et al.</i> , 2014 (69)	Orchidectomy in rats ^a	Increased microvesicular and macrovesicular hepatic fat content	No change serum glucose, serum insulin, or in insulin-regulated hepatic protein expression	Reduced body weight Increased body fat percentage
Jia <i>et al.</i> , 2017 (70)		Reduced hepatic lipid export proteins (ApoB, MTP)	Increased adiponectin	Increased liver inflammation and hepatocyte apoptosis Activation of the ER stress pathway (JNK, NF- κ B)
Senmaru <i>et al.</i> , 2013 (71)	Orchidectomy in mice ^a	Increased hepatic triglyceride and cholesterol content Increased lipogenesis (SREBP-1c, FAS) Reduced fatty acid oxidation (PPAR- α , PCG1- α , AMPK) Reduced hepatic lipid export (MTP)	No effect on serum glucose	
Lin <i>et al.</i> , 2005 (72)	Global AR deletion (GARKO, exon 2 frameshift)	Increased hepatic triglyceride content Reduced PPAR- α	Impaired glucose tolerance (GTT) Increased insulin resistance (ITT) Reduced hepatic PIK3 activity Reduced adiponectin	Increased body weight and fat mass
Fan <i>et al.</i> , 2005 (73)	Global AR deletion (GARKO, exon 1 frameshift)	No evidence of fatty liver (no data shown)	No change in glucose metabolism (GTT, ITT) Increased adiponectin	Increased body weight and total and visceral fat mass
Rana <i>et al.</i> , 2011 (74)	Global AR deletion (GARKO, exon 3 in frame retaining non-DNA binding-dependent actions)	No change in liver fat droplets (no data shown)	No change in insulin sensitivity (ITT) Increased adiponectin	Decreased body weight, increased fat mass
Lin <i>et al.</i> , 2008 (75)	Liver-targeted deletion of the AR (LARKO)	Increased macrovesicular steatosis and liver weight Increased lipogenesis (SREBP-1c, ACC) Reduced fatty acid oxidation (PPAR- α)	Impaired glucose tolerance (GTT) Increased insulin resistance (ITT) Reduced hepatic PIK3 activity, increased PTP1B, PEPCCK	Increased body weight and fat mass
Jones <i>et al.</i> , 2001 (76)	AromKO ^b	Increased hepatic triglyceride content, lipid droplets, and liver weight	Impaired glucose tolerance (GTT) and increased insulin resistance (ITT) at 3 months of age	No change in body weight Increased adiposity
Hewitt <i>et al.</i> , 2004 (77)		Increased lipogenesis (FAS, ACC, SCD1)		Increased extrahepatic insulin resistance (>6 months of age)
Nemoto <i>et al.</i> , 2000 (78)		Reduced fatty acid oxidation (catalase, MCAD)	Increased G6Pase, PEPCCK	
Van Sinderen <i>et al.</i> , 2014 (79)		Increased hepatic mitochondrial apoptosis and permeability	Reduced adiponectin	
Moro <i>et al.</i> , 2010 (80)				

(Continued)

Table 1. Continued

Study (Reference)	Study Design	Effects on Lipid Accumulation	Effects on Glucose Metabolism	Other Effects
Chow <i>et al.</i> , 2011 (81)				
Heine <i>et al.</i> , 2000 (82)	α ERKO	Increased hepatic triglyceride and cholesterol content	Impaired glucose tolerance (GTT), increased serum insulin	No change in body weight Increased adiposity
Lemieux <i>et al.</i> , 2005 (83)				
Bryzgalova <i>et al.</i> , 2006 (84)				
Matic <i>et al.</i> , 2013 (85)	LERKO	No change in gross liver structure and hepatic lipid content No change in <i>SREBP-1c</i>	No change in glucose metabolism (GTT, ITT)	No change in body weight Increased adiposity
Zhu <i>et al.</i> , 2014 (86)	LERKO	Increased hepatic triglyceride and diacylglycerol content Increased lipogenesis (ACC) Reduced hepatic lipid export (ApoB, MTP)	Increased hepatic insulin resistance (clamp) Reduced Akt phosphorylation	No change in muscle glucose disposal
Qiu <i>et al.</i> , 2017 (87)	Liver-targeted knockdown of ER α	Increased hepatic lipid droplets and triglyceride content Increased lipogenesis (ACC, FAS)	Increased hepatic gluconeogenesis (<i>PEPCK</i> , <i>G6Pase</i>)	No change in body weight

Italic type denotes mRNA changes. Studies using the same design with consistent findings are summarized, whereas studies using the same design with conflicting findings are presented separately.

Abbreviations: Akt, protein kinase B; AMPK, adenosine monophosphate-activated protein kinase C; ApoB, apolipoprotein B; clamp, hyperglycemic-euglycemic clamp; FAS, fatty acid synthase; GTT, glucose tolerance test; G6Pase, glucose-6-phosphatase; ITT, insulin tolerance test; PTP1B, protein tyrosine phosphatase 1B.

^aEffects reversed by testosterone administration.

^bEffects reversed by estradiol administration.

reported in other species. We concentrate on metabolic actions of sex steroids directed at the liver.

Males

Androgens

To examine the role of androgens in liver metabolism, several models have been used, including hormonal ablation and replacement of testosterone or silencing of the ligand or the receptor. Key studies, discussed further below, are summarized in Table 1 (69–87) and an overview of different models is provided in Fig. 2.

Orchidectomy. Surgical removal of the testes produces hepatic steatosis that is reversed by testosterone administration in a variety of species, including rats (88), capons (89), pigs (90), and mice (91), fed standard (88, 89, 91) or high-fat diets (90). In rats, orchidectomy was associated with the development of insulin resistance at both hepatic (increased hepatic glucose production) and extrahepatic (decreased

skeletal muscle glucose uptake) levels that were reversed by testosterone administration (92). In orchidectomized mice, insulin resistance was also associated with upregulation of glucose-6-phosphatase (91), a key hepatic gluconeogenesis gene.

Hepatic steatosis in rats induced by orchidectomy in conjunction with a high-fat diet also resulted in liver inflammation and hepatocyte apoptosis, which were reversed by testosterone administration (69). Additional features of this rat model of hepatic steatosis include the appearance of increased hepatic lipid droplet formation and suppression of apolipoprotein B-100- and MTP-mediated hepatic lipid export (70). These findings were confirmed in an analogous mouse model (71) that also showed reduced hepatic expression of MTP as well as reduced expression of genes involved in fatty acid oxidation, including lipin-1, peroxisome proliferator-activated receptor (PPAR)- α , PPAR- γ , and adenosine monophosphate-activated protein kinase C (71). Lipogenic genes, including fatty acid synthase

(FAS) and sterol regulatory element-binding protein 1c (SREBP-1c), were upregulated (71). In concert, these findings suggest that testosterone prevents hepatic steatosis by reducing *de novo* hepatic lipid synthesis as well as promoting fatty acid oxidation and fat export from the liver. Evidence for an anti-inflammatory role for androgens is established by studies showing that orchidectomy induced and testosterone reversed activation of the hepatic endocytosomal reticulum stress pathway with activation of proinflammatory nuclear factor κ B (NF- κ B) signaling (70). A role for androgens in suppressing hepatic inflammation is also supported by observations in an inflammation-prone mouse line in which hepatic steatosis associated with T cell activation and proinflammatory IL-17 signaling developed much more frequently in females than in males, a sex difference abolished by orchidectomy or by testosterone treatment of females (93).

The findings in rodent models have been confirmed in a larger mammalian species with high fat-fed pigs displaying that orchidectomy increased, and testosterone treatment reversed, hepatic gene expression of multiple genes of lipid and glucose metabolism, inflammation, oxidative stress, and apoptosis (90). Taken together, these findings suggest a multifaceted role for testosterone in preventing experimental hepatic steatosis in rodents, including insulin-sensitizing, antilipogenic, and anti-inflammatory actions.

Testosterone vs dihydrotestosterone. A model using orchidectomy establishes a role for testosterone in preventing hepatic steatosis, but it cannot determine whether these effects are mediated by testosterone acting as an androgen either directly or via conversion by SRD5A1 (5 α reductase) and SRD5A2 to the more potent, pure androgen dihydrotestosterone (DHT) via AR signaling and/or by

indirect effects mediated by conversion by aromatase to estradiol and consequent ER signaling. Male mice with a genetic deletion of 5 α -reductase type 1 develop hepatic steatosis and insulin resistance (94, 95). Treatment of obese male rats with the 5 α reductase inhibitor finasteride (a selective type 2 inhibitor in humans, but less selective in rodents) (95) or with the AR antagonist flutamide (96) promotes hepatic lipid accumulation and insulin resistance consistent with a protective antisteatosis role of DHT.

Thus, these studies suggest that AR-mediated signaling (via testosterone and/or DHT) is important to prevent experimental hepatic steatosis in male rodents. However, aromatization of testosterone to estradiol is also important, and the preclinical evidence regarding protective effects of ER signaling on male hepatic glucose and lipid metabolism is discussed in "Estrogens" under "Males" in "Role of Sex Steroids in Hepatic Glucose and Lipid Metabolism: Preclinical Studies".

AR knockout mice. Studies using targeted AR inactivation have interrogated the involvement of AR as the defining characteristic of pure androgen action. Findings among independently generated mice with a global deletion of the AR (ARKO) have not been consistent, possibly related to differences in the AR deletion strategy and genetic background of these models. In one model, global AR deletion resulted in liver fat accumulation and hepatic steatosis especially at older age and was associated with late-onset visceral obesity (97). These male ARKO mice also developed hyperinsulinemia, impaired glucose tolerance at advanced age, and reduced hepatic expression of phosphoinositide 3-kinase (PI3K), a kinase mediating many metabolic effects of insulin (72). However, hepatic steatosis and insulin resistance were not present in two other independently generated ARKO mouse

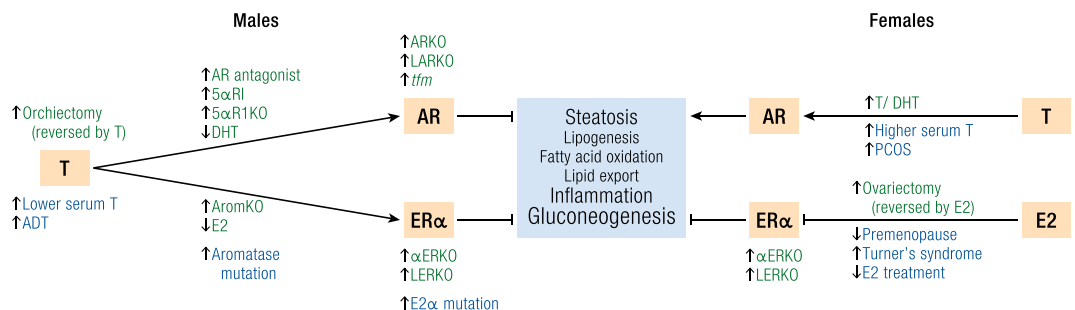


Figure 2. Effects of sex steroid signaling on metabolic liver disease. Presented is an overview of the effects of sex steroid signaling on hepatic steatosis and glucose metabolism in preclinical and clinical models of both males and females. In males (left panel), testosterone (T) signaling, both via the AR and, after aromatization to estradiol (E2), predominantly via ER α , inhibits the development of hepatic steatosis by reducing lipogenesis and by increasing fatty acid oxidation and lipid export. Both via AR and ER α signaling, T also reduces hepatic inflammation and gluconeogenesis. In females (right panel), T, in contrast to males, promotes hepatic steatosis, inflammation, and gluconeogenesis via AR signaling, whereas similar to males, E2, largely via ER α , is protective against hepatic steatosis, inflammation, and gluconeogenesis. Shown are supportive preclinical models (green) and clinical models (blue), with upward arrows denoting stimulation and downward arrows denoting inhibition of hepatic steatosis, inflammation, and gluconeogenesis. Caveats and limitations of various models are discussed in the text. 5 α RI, 5 α reductase inhibitor; 5 α R1KO, 5 α reductase type 1 knockout.

models with different AR targeting (73, 74), with both models demonstrating increased circulating adiponectin levels despite adiposity (73, 74). The dissociation between adiposity and insulin resistance in association with increased adiponectin levels is consistent with findings in orchidectomized rats (69), as well as with cognate clinical evidence reporting that androgen deprivation increases circulating adiponectin in men (98).

The role of direct androgen action in the liver has been further investigated by analysis of a liver-targeted deletion of the AR (LARKO). Male LARKO mice maintain normal reproductive function, including circulating testosterone concentrations (75), but, unlike wild-type mice, they develop hepatic lipid accumulation even when fed normal chow, and this was aggravated by a high-fat diet. This development of hepatic steatosis was associated with enhanced *de novo* lipogenesis with increased hepatic expression of lipogenic genes with essential roles in cholesterol fatty acid biosynthesis such as SREBP-1c and acetyl-coenzyme A carboxylase (ACC). Furthermore, fatty acid degradation via β -oxidation was also decreased by reduced expression of PPAR- α (75). Hence, the presence of a functional hepatic AR is required to prevent experimental hepatic steatosis in male mice, and both increased fatty acid synthesis and reduced fatty acid degradation contribute to the development of experimental hepatic steatosis. Male LARKO mice also exhibited increased hepatic insulin resistance (75), associated with increased hepatic expression of protein tyrosine phosphatase 1B, a negative regulator of insulin signaling. The activity of PI3K, a kinase mediating many metabolic effects of insulin, was reduced in the liver, but not in skeletal muscle (75).

Overall these experiments suggest that, in males, testosterone prevents hepatic steatosis by reducing lipid accumulation through reduced lipid synthesis, by promoting fatty acid oxidation and hepatic lipid export, and by suppressing inflammation and oxidative stress. In some, but not all, studies, hepatic steatosis is accompanied by increased insulin resistance (Table 1; Fig. 2).

Estrogens

In male mice, estrogen action mediated via ER α is relevant to the regulation of lipid metabolism and glucose homeostasis in the prevention of hepatic steatosis (Table 1; Fig. 2).

Aromatase knockout mice. Aromatase knockout (AromKO) mice, lacking endogenous estradiol production by deletion of the aromatase gene (76), display increased adiposity, glucose intolerance, and insulin resistance in both sexes. Hepatic steatosis, however, is only observed in male AromKO mice (77) and is prevented by estradiol treatment (77, 78). During the first 6 months of life, increased insulin resistance in male AromKO mice was primarily due to increased

hepatic gluconeogenesis (79) without impairment of insulin sensitivity in adipose tissue and muscle, suggesting that systemic insulin resistance is triggered by hepatic insulin insensitivity (79). These findings are reminiscent of LARKO mice in which hepatic insulin resistance preceded that of other tissues (75). The precise mechanisms of estrogen action in lipid metabolism that prevents hepatic steatosis in males have not been fully elucidated. In male AromKO mice, estradiol preserves hepatic mitochondrial function, including regulation of mitochondrial apoptosis and permeability, respectively (80).

Indirect evidence for the importance of estrogen action in the origins of male hepatic steatosis arises from PPAR- α ^{-/-} mice (99). When PPAR- α ^{-/-} mice are treated with etoxomir, an inhibitor of hepatocellular fatty acid flux, all male mice but only 25% of female mice die with massive hepatic steatosis. Estradiol pretreatment of male PPAR- α ^{-/-} mice reduced etoxomir-induced mortality to 20% (99). Consistent with a role for estradiol-mediated PPAR- α signaling in protecting against hepatic steatosis, treatment of male AromKO mice with bezafibrate, a potent PPAR- α agonist, markedly regressed hepatic steatosis (100). In summary, the data are consistent with an important role for estrogens in preventing hepatic steatosis and insulin resistance in males.

ER knockouts. Similar to females (see “Estrogens” under “Females” in “Role of Sex Steroids in Hepatic Glucose and Lipid Metabolism: Preclinical Studies”), male mice with global ER α knockout (α ERKO) develop insulin resistance and increased adiposity (82, 84), but this does not occur in global ER β knockout mice of either sex (84). Male α ERKO mice have marked hepatic steatosis and demonstrate impaired glucose tolerance (82–84). Treatment of male AromKO mice with the ER α -specific agonist 16 α -LE2 reduced hepatic steatosis whereas the ER β -specific agonist 8 β -VE2 had no effect (81), consistent with a dominant role for ER α . Male mice with inactivation of the putative cell membrane ER Gpr30 did not display hepatic steatosis, suggesting no role for nonnuclear ER signaling in an androgen-replete environment in preventing hepatic steatosis (101).

Liver-targeted deletion of ER α (LERKO) in male mice enhanced diet-induced hepatic steatosis (86) with evidence of hepatic, but not skeletal, muscle insulin resistance in hyperglycemic-euglycemic clamp studies (86). This was associated with reduced insulin-mediated phosphorylation of its major downstream kinase protein kinase B in the liver, leading to nuclear accumulation of forkhead box protein O1 (FoxO1) and consequent failure to suppress hepatic glucose production (86). A subsequent study using viral CRE-Lox to acutely silence ER α in the adult liver confirmed these developmental findings (100). In contrast, other investigators did not find evidence of

abnormal glucose metabolism in male α ERKO mice, although clamp studies were not performed (85).

In summary, these findings suggest that in males, the liver is an important target of estrogen action in preventing hepatic steatosis (Table 1; Fig. 2). Mechanistically, hepatic estradiol signaling reduces hepatic triglyceride accumulation by decreasing *de novo* hepatic lipogenesis and increases lipid metabolism via fatty acid oxidation, as well as modifying insulin resistance and suppressing hepatic gluconeogenesis.

SHBG. Given the inverse association of circulating SHBG with hepatic steatosis and insulin resistance in both men and women (see “Role of Sex Steroids in Hepatic Glucose and Lipid Metabolism: Clinical Studies”), preclinical studies have been designed in an attempt to determine whether reduced SHBG is a cause or a consequence of the metabolic dysregulation. In mice expressing human SHBG transgenes or in HepG2 hepatoblastoma cells, monosaccharides such as glucose or fructose effectively decrease hepatic SHBG expression by inducing lipogenesis in the liver (102). These data provide a potential mechanism by which fatty liver causes a reduction in SHBG. The authors concluded that SHBG may be a biomarker, rather than a mediator, of metabolic dysregulation (102). However, the same group recently reported that obesity-prone db/db mice with a transgenic overexpression of SHBG were protected against high-fat diet–induced hepatic steatosis, even following orchidectomy (103). Therefore, SHBG may contribute to rather than be solely a consequence of hepatic fat accumulation.

Females

Androgens

The role of androgens in females has been less extensively investigated than in males. In females, pharmacological androgen effects may not always be consonant with physiological effects given the low concentrations of endogenous testosterone in the circulation. Key studies discussed further below are summarized in Table 2 (82–86, 104–115), and Fig. 2 provides an overview of the evidence derived from the various experimental models.

Prenatal exposure. Prenatal exposure of female fetuses to androgens induces postnatal hepatic steatosis and insulin resistance in a variety of animal species, including mice (104), rats (105), and sheep (116, 117). This was associated with subsequent downregulation of phosphoenolpyruvate carboxykinase (PEPCK) in the adult liver of prenatally androgen-exposed female sheep (116), an enzyme that catalyzes the rate-limiting step of hepatic gluconeogenesis. Mice with a liver-targeted deletion of PEPCK also develop hepatic steatosis (118). Moreover, insulin-stimulated phosphorylation of hepatic protein kinase B was downregulated in adult livers from androgen-

exposed sheep, suggesting hepatic insulin resistance (117). Prenatal androgen exposure was also associated with hepatic upregulation of proinflammatory MAPK kinase 4 (116), a kinase associated with the development of NAFLD in humans (119).

Postnatal exposure. Postnatal androgen exposure of female rodents using DHT also induces development of hepatic steatosis and insulin resistance in conjunction with reproductive phenotypes replicating some features of polycystic ovarian syndrome (PCOS) (104). In such models, hepatic steatosis is exacerbated by a high-fat diet (120, 121). Whether androgen effects on the liver are direct, which may be dose related (106), or indirect, such as mediated by whole-body adiposity, remains unclear. In lean mice, low-dose DHT treatment not affecting body fat mass resulted in hepatic insulin resistance that was associated with disrupted insulin signaling in the liver and subsequent increased hepatic gluconeogenesis (106).

In contrast to male mice, female mice with a global (ARKO) (97) or liver-targeted (LARKO) (75) deletion of the AR do not develop obvious metabolic disturbances, but careful metabolic characterizations have not been reported. In summary, the preclinical evidence in females is consistent with a role for excess androgens in promoting hepatic steatosis and the development of insulin resistance.

Estrogens

Ovariectomy. In female mice, ovariectomy leads to hepatic steatosis and insulin resistance, regardless of energy intake, which is reversed by estradiol add-back (107, 110). Estradiol treatment reverses the ovariectomy-associated decrease in the hepatic expression of senescence marker protein-30 (SMP30) (109). Given that SMP30 knockout mice exhibit hepatic steatosis with associated inflammation and apoptosis (122), upregulation of hepatic SMP30 by estradiol may be one mechanism by which estrogens confer resistance to hepatic cell injury. Further evidence for an anti-inflammatory action of estradiol stems from a study exposing ovariectomized mice to a high-fat diet. These mice developed liver injury associated with hypercholesterolemia, increased liver macrophage infiltration, and increased hepatic expression of proinflammatory genes, including hepatocyte monocyte chemoattractant protein-1 and monocyte chemokine receptor 2, changes that improved with physiologic estradiol treatment (108).

ER knockout mice. Although findings are consistent with estrogen action preventing hepatic steatosis in female mice, further analyses have examined the relevant ER subtype and tissue-specific estrogen signaling. Transcriptome analysis in female mice implicates hepatic ER α expression in the regulation of lipid and glucose metabolism genes (123). Global deletion of ER α (α ERKO) induces hepatic steatosis associated with upregulation of hepatic

lipogenesis genes and downregulation of lipid transport genes, as well as increased insulin resistance associated with increased hepatic inflammatory signaling (83, 84, 111).

In female mice, hepatic ER α transcriptional activity reflects reciprocal changes in circulating estradiol concentrations across the estrous cycle, leading to changes in the expression of key enzymes of lipogenesis and cholesterol biosynthesis (124). The cycle-dependent regulation of lipid metabolism suggests that hepatic ERs may sense changes in circulating estradiol, as it does for food intake (125), thereby facilitating the adaptation of liver metabolism to reproductive energy requirements and further supporting a role of hepatic ER α as a peripheral integrator of diverse systemic metabolic functions.

Hepatic steatosis has also been reported in transgenic mice designed to limit ER α signaling to the cytoplasm (126) and in mice with a targeted deletion of Gpr30, a functional ER located at the cell and endoplasmic reticulum membranes (101). Moreover, a knock-in of a mutant ER α with disrupted ER-response element signaling rescued the metabolic phenotype of female α ERKO mice (127). Collectively, these findings suggest that the protective effects of estradiol on liver steatosis are mediated by both classical and non-nuclear (or extrahepatic) ER α signaling.

The impact of liver-specific inactivation of ER α has been reported by generating LERKO mice. In this model, hepatic steatosis was evident (113) and estradiol treatment failed to prevent diet-induced hepatic steatosis and insulin resistance, in contrast to wild-type ovariectomized littermates (110). Additional studies using short hairpin RNA-mediated knockdown of the ER α in adult mice have confirmed the role of ER α in preventing hepatic steatosis by showing that acute downregulation of the hepatic ER α is sufficient to induce hepatic steatosis, confirming the development effects of genetic ER α knockouts. Mechanistically this was linked to ER α -mediated upregulation of small heterodimer partner (114), a transcription factor important in the regulation of hepatic metabolic processes and in the protection against hepatic inflammation (128). Similarly, liver-specific knockdown of ER α increased susceptibility to high-fat diet-induced hepatic steatosis (115). In this model, upregulation of the microRNA miR-125b by estradiol conferred resistance to hepatic steatosis (115). Deletion of the hepatocyte ER α impaired hepatic blood cholesterol clearance and reversed cholesterol transport, leading to cholesterol accumulation in the circulation and increased susceptibility to atherosclerosis (129).

However, not all studies agree. In other studies, LERKO mice were less susceptible than α ERKO mice to high-fat diet-induced hepatic fat accumulation (112), and when subjected to intraperitoneal glucose and insulin tolerance tests did not demonstrate evidence of disturbed glucose homeostasis (85). Collectively, these

studies suggest that hepatic ER α signaling has a role in preventing fatty liver and insulin resistance in most but not all studies.

In contrast, female mice with a global deletion of ER β , which is expressed at lower levels in liver compared with ER α (12), independently generated by two teams of investigators demonstrated no evidence of hepatic steatosis or insulin resistance (84, 130), even after introducing a high-fat diet that increased adiposity (131).

In summary, the preclinical data in female rodent models demonstrate a role for liver-specific and systemic estradiol actions in the prevention of hepatic steatosis and the regulation of glucose metabolism predominantly mediated by ER α signaling (Table 2; Fig. 2).

Role of Sex Steroids in Hepatic Glucose and Lipid Metabolism: Clinical Studies

In this section, we focus on clinical studies that have reported interactions between sex steroids and NAFLD as well as disturbances in hepatic glucose and lipid metabolism.

Men

Androgens

Observational studies. Several observational studies have shown relationships between hepatic steatosis and lower circulating testosterone concentrations in community-dwelling men [Table 3 (132–138); Fig. 2]. In a cross-sectional study of 1912 German men, men with low circulating testosterone concentrations [defined as <346 ng/dL (<12 nmol/L)] had a higher prevalence of hepatic steatosis, defined by sonographic criteria, independent of age, alcohol consumption, and BMI (OR, 2.36; 95% CI, 1.04 to 2.43) (132). Similar findings were subsequently reported in non-white men (134, 139) and in men with type 2 diabetes (140). A meta-analysis of 16 studies comprising 13,721 men reported modestly lower testosterone [mean difference, –80 ng/dL; 95% CI, –98 to –62 (–2.78 nmol/L; 95% CI, –3.40 to –2.15)] in men with NAFLD compared with controls (133). In another cross-sectional study, Chinese men with NAFLD had not only lower testosterone concentrations, but also reduced sperm concentration, count, and motility compared with men without fatty liver (141).

In addition to these findings in community-dwelling men, in a case-control study, 22 of 63 Chinese men with organic idiopathic hypogonadotropic hypogonadism [mean testosterone, 27 ng/dL (0.95 nmol/L)] had ultrasonographic evidence of hepatic steatosis and higher transaminase concentrations, compared with 61 of 336 healthy controls [mean testosterone, 527 ng/dL (18.3 nmol/L)] (142).

"The...modest reductions in circulating testosterone observed in...community-dwelling men may largely be a consequence of reduced SHBG."

Table 2. Hepatic Metabolic Effects of Altered Sex Steroid Signaling in Female Rodent Models

Study (Reference)	Methodology	Effects on Hepatic Lipid Accumulation	Effects on Glucose Metabolism	Other Effects
Caldwell <i>et al.</i> , 2014 (104)	Offspring of female mice receiving 250 μ g of DHT on days 16 to 18 of gestation	Increased hepatic steatosis (histomorphometry)	No change in insulin sensitivity (ITT)	No change in body fat composition
Abruzzese <i>et al.</i> , 2016 (105)	Offspring of female rats receiving 1 mg of testosterone on days 16 to 19 of gestation	No change in hepatic lipid droplets or triglyceride content Increased lipogenesis (<i>SREBP-1c</i> , <i>FAS</i>) Reduced fatty acid oxidation (<i>PPAR-α</i>)	Decreased glucose tolerance (GTT)	No change in body weight
Caldwell <i>et al.</i> , 2014 (104)	DHT (10-mg implant) or DHEA (7.5-mg pellet) treatment in mice from 21 d of age for 90 d	Increased hepatic steatosis (histomorphometry)	No change in insulin sensitivity (ITT)	Increased body weight and fat mass
Andrisse <i>et al.</i> , 2017 (106)	Monthly DHT (2-mg pellet) in mice from 8 wk of age for 90 d	No change in hepatic lipid content	Impaired glucose tolerance (GTT) Increased insulin resistance (ITT) Reduced Akt, PI3K Increased hepatic gluconeogenesis (<i>Pck1</i> , <i>G6P</i> , <i>Foxo1</i> , <i>PEPCK</i> , <i>G6Pase</i> , <i>FOXO1</i>)	No change in body weight or composition Reduced hepatic <i>IL-6</i>
D'Eon <i>et al.</i> , 2005 (107)	Ovariectomy ^a in mice	Increased liver weight, triglyceride, diacylglycerol, and cholesterol content	Increased hepatic insulin resistance (clamp studies)	Increased body weight and fat mass
Kamada <i>et al.</i> , 2011 (108)		Increased lipogenesis (<i>SREBP-1c</i> , <i>ACC</i> , <i>FAS</i>)		Increased hepatic inflammation (<i>TNFα</i> , <i>MCP1</i> , <i>CCR2</i>) and fibrosis (<i>TGF-β1</i>)
Fukui <i>et al.</i> , 2011 (109)		Reduced hepatic lipid export (<i>ApoB</i>)		Increased hepatic ER stress and apoptosis (<i>CHOP</i> , <i>GRP78</i> , <i>caspase-3</i>)
Zhu <i>et al.</i> , 2013 (110)				
Heine <i>et al.</i> , 2000 (82)	α ERKO	Increased liver weight triglyceride content and macrovesicular and microvesicular steatosis	Increased hepatic insulin resistance (clamp)	Increased body weight and fat mass Increased hepatic inflammation (<i>IKKβ</i>)
Lemieux <i>et al.</i> , 2005 (83)		Increased lipogenesis (<i>Scd-1</i>)	Increased insulin resistance (PI3K)	
Bryzgalova <i>et al.</i> , 2006 (84)			Reduced adiponectin	
Ribas <i>et al.</i> , 2010 (111)				
Hart-Unger <i>et al.</i> , 2017 (112)				
Zhu <i>et al.</i> , 2014 (86)	LERKO	Increased hepatic triglyceride and diacylglycerol content, reduced hepatic triglyceride secretion	Reduced estradiol treatment-mediated reduction in insulin sensitivity after ovariectomy (clamp)	No change in body weight

(Continued)

Table 2. Continued

Study (Reference)	Methodology	Effects on Hepatic Lipid Accumulation	Effects on Glucose Metabolism	Other Effects
Matic <i>et al.</i> , 2013 (85)	LERKO	No change in gross liver structure and hepatic lipid content No change in <i>SREBP-1c</i>	No change in glucose metabolism (GTT, ITT)	No change in body weight Increased adiposity
Della Torre <i>et al.</i> , 2016 (113)	LERKO	Increased hepatic lipid content Altered hepatic expression of genes involved in lipid uptake and reverse cholesterol transport		Liver ER α -mediated coupling of hepatic lipid metabolism to the reproductive cycle
Hart-Unger <i>et al.</i> , 2017 (112)	LERKO	No change in liver weight and in macrovascular or microvascular steatosis	No change in glucose metabolism (GTT, ITT)	No change in body weight
Wang <i>et al.</i> , 2015 (114)	Liver-targeted knockdown of ER α	Increased hepatic triglyceride content	Increased insulin resistance (ITT)	No change in body weight or fat mass
Zhang <i>et al.</i> , 2015 (115)		Increased lipogenesis (<i>SREBP-1c</i> , <i>Scd-1</i> , <i>FAS</i>)		

Abbreviations: Akt, protein kinase B; ApoB, apolipoprotein B; CCR2, CCR2, monocyte chemokine receptor 2; clamp, hyperglycemic-euglycemic clamp; DHEA, dehydroepiandrosterone; FAS, fatty acid synthase; GTT, glucose tolerance test; G6Pase, glucose-6-phosphatase; ITT, insulin tolerance test; MCP1, MCP1, monocyte chemoattractant protein-1.

^aEffects reversed by estradiol administration. Studies using the same design with consistent findings are summarized, whereas studies using the same design with conflicting findings are presented separately.

An increased risk of NAFLD has also been reported in men with prostate cancer receiving androgen deprivation therapy (ADT), which reduces circulating testosterone to castrate concentrations. In a Surveillance, Epidemiology and End Results (SEER)–Medicare database analysis in 82,938 men aged 66 years and older with localized prostate cancer, men receiving ADT were more likely to be diagnosed with NAFLD [hazards ratio (HR), 1.54; 95% CI, 1.40 to 1.68] (135). Of note, men receiving ADT have circulating testosterone concentrations in the castrate range, significantly lower than the reductions associated with the increased incidence of hepatic steatosis in community-dwelling men.

SHBG. The role of SHBG, which may confound the testosterone–NAFLD relationship due to its ecological association with insulin resistance, was assessed in subsequent studies (Table 3) noting that direct biochemical/physiological analysis indicates the effect is confounded by direct effects of monosaccharides on the liver (102). In the previously considered meta-analysis of 13,721 men (133), those with NAFLD had lower circulating SHBG (mean difference, -8.72 nmol/L; 95% CI, -16.7 to -0.5), compared with controls (133).

Among 2899 men of the Multi-Ethnic Study of Atherosclerosis (MESA) cohort (136), high SHBG was associated with a reduced prevalence of fatty liver by CT attenuation (OR, 0.50; 95% CI, 0.3 to 0.84) after multivariable adjustment including homeostasis model of insulin resistance. In contrast to the previous studies (132, 139), bioavailable testosterone (calculated from immunoassay-measured testosterone) was not

associated with fatty liver prevalence (136); however, relationships between fatty liver and the directly measured testosterone were not reported (136), and, in general, the biological significance of derived testosterone fractions (“free,” “bioavailable”) remains uncertain (143). Similarly, among 2700 community-dwelling Chinese men, low SHBG but not testosterone was associated with NAFLD independently of several risk factors (137). Consistent with no established role for testosterone in the pathogenesis of NAFLD, in a longitudinal analysis of 1944 Korean men with a median follow-up of 4.2 years who underwent repeated liver ultrasonography, baseline testosterone concentrations did not predict the subsequent development of fatty liver (138); however circulating SHBG concentrations were not measured (138). Of note, in a prospective study of 90 men exposed to a 9-month lifestyle intervention, subjects with the largest decrease in liver fat had the largest increase in SHBG concentrations during the intervention. This was independent of changes in total body adiposity and visceral fat (144). This study emphasizes the strong association of SHBG with liver fat.

Overall, these studies suggest that the association of fatty liver with low SHBG is stronger than with low testosterone.

In summary, in line with mechanistic studies (see “Androgens” under “Males” in “Role of Sex Steroids in Hepatic Glucose and Lipid Metabolism: Preclinical Studies”), limited clinical evidence suggests that severe sex steroid deprivation [e.g., ADT in prostate cancer (135), organic HH (142)] is associated with fatty liver in men. However, the more modest reductions in

Table 3. Observational Studies Associating Hepatic Steatosis With Serum Testosterone and SHBG in Men

Study (Reference)	Design	Population	Definition of Hepatic Steatosis	Key Findings	Remarks
Völzke <i>et al.</i> , 2010 (132)	Cross-sectional	1912 Community-dwelling German men Median age 49 y; serum T 496 ng/dL	Ultrasound Present in 38% of total	aOR ^a 2.36 (95% CI, 1.66 to 3.37) in men with serum T <346 ng/dL vs >611 ng/dL	T measured by immunoassay SHBG not reported
Jaruvongvanich <i>et al.</i> , 2017 (133)	Meta-analysis of 11 observational studies	13,721 Men	Ultrasound	Lower serum T [mean difference, -80 ng/dL (95% CI, -98 to -63)] and lower SHBG [-8.72 nmol/L (95% CI, -16.70 to -0.75)] in men with hepatic steatosis vs without	T measured by immunoassay
Yim <i>et al.</i> , 2018 (134)	Cross-sectional	2352 Men (NHANES) Mean age 47 y	ALT >30 IU/L Present in 28% of total	aOR ^b 1.72 (95% CI, 1.05 to 2.82) in men with serum T in lowest quartile (mean, 209 ng/dL) vs highest quartile (641 ng/dL)	T measured by LC-MS SHBG not reported
Gild <i>et al.</i> , 2018 (135)	Longitudinal	82,938 Men (SEER database) aged ≥66 y with localized prostate cancer; 37.5% received ADT	Database codes	aHR ^c 1.54 (95% CI, 1.40 to 16.8) in men receiving ADT vs men not receiving ADT	T or SHBG not reported
Lazo <i>et al.</i> , 2015 (136)	Cross-sectional	2899 Men (MESA)	CT	aOR ^d 0.50 (95% CI, 0.3 to 0.84) in men with serum SHBG in highest tertile (median, 60.7 nmol/L) vs lowest tertile (58.0 nmol/L)	No association between hepatic steatosis and bioavailable T, but measured T (immunoassay) not reported
Wang <i>et al.</i> , 2016 (137)	Cross-sectional	2700 Chinese men Mean age 53 y	Ultrasound Present in 32% of total	aOR ^e 1.73 (95% CI, 1.23 to 2.45) in men with serum SHBG in lowest tertile (≤31.8 nmol/L) vs highest tertile (≥50.2 nmol/L)	No association between hepatic steatosis and serum T (immunoassay)
Seo <i>et al.</i> , 2015 (138)	Cross-sectional and longitudinal	1944 Chinese men Mean age 44 y	Ultrasound Present in 44% of total	Baseline aOR ^f 0.69 (95% CI, 0.61 to 0.78) per SD increase of T	No association between baseline serum T (immunoassay) and hepatic steatosis at follow-up (median, 4.2 y) in fully adjusted model

To convert serum testosterone to nmol/L, multiply by 0.0347.

Abbreviation: T, testosterone.

^aOR adjusted for age, alcohol consumption, smoking, diabetes, physical activity, and BMI.

^bOR adjusted for age, BMI, ethnicity, education level, marital and socioeconomic status, smoking, hypertension, diabetes, and total and HDL cholesterol.

^cHR adjusted for age, ethnicity, education level, marital and socioeconomic status, Charlson comorbidity index, and prostate cancer stage.

^dOR adjusted for age, BMI, ethnicity, smoking, hypertension, total and HDL cholesterol, and measures of insulin resistance.

^eOR adjusted for age, serum testosterone, waist circumference, systolic blood pressure, total and HDL cholesterol, and diabetes status.

^fOR adjusted for age, smoking, exercise, systolic blood pressure, waist circumference, glucose, HDL cholesterol, and triglycerides.

circulating testosterone observed in unselected community dwelling men may largely be a consequence of reduced SHBG.

Clinical trials. Ultimately, cause and consequence are best discriminated by adequately designed and powered clinical trials of testosterone treatment, with the caveat that randomized clinical trials (RCTs) do not resolve whether effects are physiological or pharmacological, or whether these are dose-dependent (including overdose) effects. However, definitive RCTs do not exist, and published clinical trials assessing the effect of testosterone treatment on hepatic steatosis have been inconclusive. In a cohort study of 117 men with the metabolic syndrome and baseline serum

testosterone from 170 to 349 ng/dL (5.9 to 12.1 nmol/L), intramuscular testosterone undecanoate treatment in doses recommended for hypogonadal men was associated with a reduction in liver enzymes and CRP, but importantly this study lacked a control group (145). Only two RCTs assessing hepatic steatosis have been published, and liver fat was not the primary outcome in either study. In an RCT of middle-aged men with obstructive sleep apnea and baseline mean serum testosterone concentrations of 380 ng/dL (13.2 nmol/L), intramuscular testosterone undecanoate treatment in doses recommended for hypogonadal men reduced liver fat modestly (0.09 CT Hounsfield attenuation ratio; 95% CI, 0.009 to 0.17) (146). In contrast, in a

secondary analysis of a 6-month RCT of 73 older men [baseline testosterone 251 ng/dL (8.7 nmol/L)], changes in liver fat, determined by MRI, did not differ between testosterone (10-g testosterone gel daily) and placebo-treated men. This was irrespective of the amount of liver fat at baseline (147).

Although the effects of conventionally dosed testosterone treatment on liver fat require further study, exposure to supraphysiological doses of synthetic androgens has been associated with an increased risk of hepatic steatosis in body builders (148). Currently available testosterone preparations used to treat hypogonadal men are devoid of hepatic toxicity as they lack 17 α -alkylation responsible for hepatic injury caused by compounds such as oral 17 α -methyl testosterone or 17 α -ethyl testosterone (149). These synthetic androgens are no longer in clinical use, but they continue to be widely abused without medical prescription.

Estrogens

Observational studies. Only two epidemiological studies have reported the association of circulating estrogens with fatty liver in men. In the MESA cohort, men in the highest tertile of serum estradiol unexpectedly had a greater OR for fatty liver disease prevalence than those in the lowest estradiol tertile (1.96; 95% CI, 1.12 to 3.18) (136). Cause and consequence cannot be clarified in this cross-sectional study, and the higher estradiol concentrations may reflect more advanced liver disease, where circulating estradiol is increased (150). In contrast, among 1882 Chinese men, higher estradiol concentrations were weakly associated with a reduced prevalence of fatty liver (OR, 0.95; 95% CI, 0.94 to 0.97) (151). However, both studies measured estradiol concentrations by immunoassay rather than liquid chromatography–mass spectrometry (LC-MS), therefore allowing no firm conclusions to be drawn.

Aromatase and ER mutations. A protective role of endogenous estrogens in the prevention of hepatic steatosis is suggested by rare case reports of men with severe estradiol deprivation inactivating mutations of the aromatase gene. These men, analogous to the phenotype reported in male AromKO mice (see “Estrogens” under “Males” in “Role of Sex Steroids in Hepatic Glucose and Lipid Metabolism: Preclinical Studies”) usually demonstrate steatohepatitis and elevated liver enzymes. This is reversed by estradiol replacement (152). In one case report of an aromatase-deficient man, improvement of steatohepatitis with estradiol treatment was documented by serial liver biopsies (153). The experience with these rare case reports demonstrates that in extreme models, estradiol treatment of aromatase-deficient men improves insulin resistance and dyslipidemia. However, it is unknown whether the improvement in steatohepatitis was a direct effect of estradiol on the liver or due

to systemic improvements in metabolism. A recent hyperinsulinemic-euglycemic clamp study in healthy men reported that although drug-induced aromatase inhibition reduced insulin sensitivity, this was due to impairment of insulin-stimulated peripheral glucose disposal without affecting hepatic glucose production or lipolysis (154). Conversely, in an elegant study of men with obesity with induced hypogonadism given testosterone add-back with or without dutasteride (to block DHT production) or the aromatase inhibitor anastrozole (to suppress estradiol production), clamp dynamics suggested that the beneficial metabolic effects of estradiol were predominantly mediated by a reduction of hepatic insulin resistance (155). These data suggest that impairment of estradiol action both at the level of the liver and at the skeletal muscle is responsible for the increased insulin resistance. Although men with aromatase deficiency have high circulating testosterone concentrations, it appears unlikely that high testosterone contributes to insulin resistance given that insulin resistance was also reported in a man with an inactivating mutation of the ER α gene, despite normal circulating testosterone (156). The importance of ER α signaling in mediating the effects of estradiol on glucose metabolism is further underscored by the fact that in the man with an inactivating mutation of the ER α gene, insulin resistance did not improve despite high-dose estradiol treatment (156). The central importance of ER α in men is consistent with the data obtained in preclinical models (Fig. 2) (see “Estrogens” under “Males” in “Role of Sex Steroids in Hepatic Glucose and Lipid Metabolism: Preclinical Studies”).

Women

Androgens

Observational studies. Several large observational studies have reported that increased circulating androgens are associated with fatty liver (by imaging rather than liver biopsy) in community-dwelling premenopausal and postmenopausal women, opposite to findings in men [Table 4 (133, 134, 136, 137, 157–159)]. In the large MESA study, there was greater fatty liver prevalence in postmenopausal women in the highest tertile vs lowest tertile of circulating bioavailable testosterone, independent of confounders, including age, BMI, lipids insulin sensitivity, and use of menopausal hormone therapy (OR, 1.73; 95% CI, 1.05 to 2.87) (136). However, the study did not report on associations with directly measured testosterone concentrations (136). In a longitudinal study of 1052 premenopausal women with a mean age of 26 participating in the Coronary Artery Risk Development in Young Adults study, increasing quintiles of free testosterone (calculated from total testosterone by immunoassay) at baseline were associated with prevalent NAFLD at follow-up 23 years later (multivariable

Table 4. Observational Studies Associating Hepatic Steatosis With Serum Testosterone and SHBG in Women

Study (Reference)	Design	Population	Definition of Hepatic Steatosis	Key Findings	Remarks
Lazo <i>et al.</i> , 2015 (136)	Cross-sectional	2835P women (MESA)	CT	aOR ^a 1.73 (95% CI, 1.05 to 2.87) in women with serum bioavailable T in highest tertile (median, 61.4 ng/dL) vs lowest tertile (59.2 ng/dL)	Bioavailable T calculated from measured T (by immunoassay), but measured T not reported No association between hepatic steatosis and serum SHBG
Yim <i>et al.</i> , 2018 (134)	Cross-sectional	2406 Women (NHANES)	ALT >19 IU/L	aOR ^b 1.49 (95% CI, 1.06 to 2.11) in women with serum T in lowest quartile (mean, 9.4 ng/dL) vs highest quartile (43.0 ng/dL)	T measured by LC-MS
		Mean age 47 y	Present in 38% of total		aOR ^b significant in postmenopausal women [2.20 (95% CI, 1.36 to 3.57); n = 1017], but not in premenopausal women [0.97 (95% CI, 0.65 to 1.46); n = 1389]
					SHBG not reported
Sarkar <i>et al.</i> , 2017 (157)	Prospective 23-y follow-up	1052 Premenopausal women (CARDIA)	CT	aOR ^c 1.24 (95% CI, 1.04 to 1.49) with increasing free T quintiles	Free T calculated from measured T (by immunoassay), but measured T not reported
		Mean age 26 y	Present in 8.5% of total at follow-up		SHBG not reported
Jaruvongvanich <i>et al.</i> , 2017 (133)	Meta-analysis of 7 observational studies	5840 Women	Ultrasound	Pooled OR of 1.40 (95% CI, 1.11 to 1.77) in women with higher vs lower serum T Lower SHBG [−17.05 nmol/L (95% CI, −23.58 to −10.53)] in women with hepatic steatosis vs without	T measured by immunoassay
Rocha <i>et al.</i> , 2017 (158)	Meta-analysis of 17 observational studies	2734 Women with PCOS and 2561 controls	Ultrasound (15 studies)	OR 2.54 (95% CI, 2.19 to 2.95) in women with PCOS vs controls Higher serum T [mean difference, 11.5 ng/dL (95% CI, 8.4 to 14.4)] and higher free androgen index [4.46 (95% CI, 3.53 to 5.39)] in PCOS women with hepatic steatosis vs without	T measured by immunoassay in most studies
					SHBG not reported
Kumarendran <i>et al.</i> , 2018 (159)	Retrospective longitudinal cohort study	63,120 Women with PCOS and 121,064 age- and BMI-matched controls (United Kingdom primary care database)	Database codes	Incidence of hepatic steatosis, 9.2 per 10,000 person-years in women with PCOS vs 3.9 in women without	T measured by immunoassay (n = 71,016)
	3.5-y follow-up	Median age 30 y		aHR ^d 2.23 (95% CI, 1.86 to 2.66)	SHBG measured in n = 49,625
				aHR ^d 2.30 (95% CI, 1.16 to 4.53) in women with serum T 86.5 to 100.6 ng/dL and 3.40 (95% CI, 1.24 to 4.66) in women with T >100.6 ng/dL compared with women with T <86.5 ng/dL	
				aHR ^d 4.98 (95% CI, 2.45 to 10.11) in women with SHBG <30 nmol/L	

(Continued)

Table 4. Continued

Study (Reference)	Design	Population	Definition of Hepatic Steatosis	Key Findings	Remarks
Wang <i>et al.</i> , 2016 (137)	Cross-sectional	1461 South Chinese women >55 y of age	Ultrasound	Lower SHBG concentrations in women with mild [OR, 2.16 (95% CI, 1.48 to 3.14)] and moderate-severe NAFLD [OR, 6.84 (95% CI, 4.31 to 10.84)] compared with women without NAFLD	T not reported

To convert serum testosterone to nmol/L, multiply by 0.0347.

Abbreviations: CARDIA, Coronary Artery Risk Development in Young Adults; T, testosterone.

^aOR adjusted for age, BMI, ethnicity, smoking, hypertension, menopausal hormone therapy, total and HDL cholesterol, and measures of insulin resistance.

^bOR adjusted for age, BMI, ethnicity, education level, marital and socioeconomic status, smoking, hypertension, diabetes, and total and HDL cholesterol.

^cOR adjusted for age, BMI, ethnicity, waist circumference, homeostasis model of insulin resistance, HDL cholesterol, and triglycerides.

^dHR adjusted for age, BMI, socioeconomic status, diabetes, prediabetes, and hypothyroidism status.

adjusted OR, 1.24; 95% CI, 1.04 to 1.49). This association was partially mediated by visceral adipose tissue (157) and was apparent even in women without androgen excess defined as concentrations below the 95% percentile values for nonoligomenorrheic non-hirsute women (157).

A meta-analysis of 5840 women (including both premenopausal and postmenopausal women) reported that women with higher measured circulating testosterone had a higher OR of fatty liver prevalence (OR, 1.40; 95% CI, 1.11 to 1.77). This was in an opposite direction to men (133).

At odds with these findings is a recent cross-sectional survey in 1017 postmenopausal US National Health and Nutrition Examination Survey (NHANES) participants, reporting increased NAFLD ORs ranging from 1.50 to 2.20 in women with the lowest testosterone quartile vs the highest quartile (134). In this study, testosterone was measured by isotope dilution LC-MS. However, NAFLD was defined as serum ALT >19 IU/L rather than by imaging or biopsy, leaving potential for misclassification (134).

In summary, the large majority of observational studies in community-dwelling women have reported an association of higher circulating testosterone with hepatic steatosis. Shortcomings of these studies include the use of immunoassays with limited precision in quantifying the low testosterone circulations in women, especially after menopause, and reliance on derived testosterone fractions (free, bioavailable) with uncertain biological significance (143).

SHBG. In contrast to directly measured testosterone, calculated bioavailable or free testosterone relies heavily on circulating SHBG, suggesting that associations with total testosterone may be confounded by SHBG. Indeed, several cross-sectional studies among postmenopausal women of different ethnic background reported an inverse association of circulating SHBG with fatty liver (137). In a meta-

analysis (133), women with fatty liver had lower SHBG concentrations compared with women without (mean difference, -17.0 nmol/L; 95% CI, -23.6 to -10.5) (133).

Collectively, these results suggest that in women, low SHBG is associated with an increased prevalence of fatty liver (Table 4), as it is reported in men (see “Androgens” under “Men” in “Role of Sex Steroids in Hepatic Glucose and Lipid Metabolism: Clinical Studies”). The strong inverse association of SHBG with hepatic steatosis is supported by evidence that low SHBG is more strongly associated with the presence of hepatic steatosis than with components of the metabolic syndrome as defined by International Diabetes Foundation criteria (160). Moreover, in middle-aged women participating in the Study of Women’s Health Across the Nation, those with both highest hepatic fat content and lowest circulating SHBG had the most severe insulin resistance (161). Mechanistically, this close association between low SHBG and NAFLD could reflect the fact that SHBG is a (confounded) marker of insulin resistance reflecting more direct monosaccharide hepatic effects and the metabolic syndrome, but it does not exclude a possible role of SHBG as a pathogenic factor in NAFLD development (see “Androgens” under “Males” in “Role of Sex Steroids in Hepatic Glucose and Lipid Metabolism: Preclinical Studies”).

PCOS. The association of increased androgen concentrations with increased prevalence of NAFLD reported in women of the general population has also been documented in women with PCOS (Table 4). Multiple studies have associated increased circulating androgen (including testosterone and the proandrogens androstenedione and dehydroepiandrosterone) concentrations with an increased risk of fatty liver in women with PCOS, although the measurements of such low circulating sex steroid concentrations by immunoassay indicates caution about the

interpretations. A systematic review and meta-analysis of 17 studies including 2734 women with PCOS (defined by Rotterdam criteria in 13 of the 17 studies) and 2561 age- and BMI-matched healthy controls reported that women with PCOS had an increased prevalence of NAFLD (OR, 2.54; 95% CI, 2.19 to 2.95) compared with women who did not meet the diagnostic criteria for PCOS (158). Moreover, women with PCOS had higher serum testosterone [measured by immunoassay in most studies; mean difference, 11.5 ng/dL (95% CI, 8.4 to 14.4) (0.40 nmol/L; 95% CI, 0.29 to 0.50)] and a markedly higher free androgen index (mean difference, 4.46; 95% CI, 3.53 to 5.39) (158), although the latter largely represents simply the inverse of serum SHBG. A recent retrospective longitudinal cohort study evaluated NAFLD rates in 63,120 women with PCOS and 121,064 age- and BMI-matched controls registered in a United Kingdom primary care database. Multivariable analysis demonstrated an increased rate of NAFLD in women with PCOS (defined by Read codes, a hierarchical coding system for structured storage of information) compared with controls (HR, 2.23; 95% CI, 1.86 to 2.66; $P < 0.001$) after adjustment for BMI, diabetes, and prediabetes (impaired fasting glucose and/or impaired glucose tolerance) status (159). Among those with serum testosterone measurements by immunoassay ($n = 71,061$), a serum testosterone >86.5 ng/dL (>3 nmol/L) was associated with an increase in the incidence of NAFLD (HR, 2.30; 95% CI, 1.16 to 4.53; $P < 0.017$). Similarly, among those with SHBG measurements ($n = 49,625$), a SHBG <30 nmol/L was associated with an increase in NAFLD (HR, 4.98; 95% CI, 2.45 to 10.11; $P < 0.001$) (159).

A case-control study conducted detailed body compositional analyses with proton magnetic resonance spectrometry and whole-body MRI in 29 obese women with PCOS, aged 28 years, and in 22 age- and BMI-matched healthy controls. The study demonstrated that hyperandrogenic women with PCOS had higher liver fat compared with both normoandrogenic women with PCOS and controls, after adjustment for insulin resistance and internal and visceral adipose tissue volumes (162). In a case-control study of premenopausal women who had androgen measurements by LC-MS, PCOS patients had significantly higher circulating concentrations of free testosterone and DHT compared with matched controls. In women with PCOS, a high testosterone/DHT ratio was associated increased hepatic transaminases and other parameters suggesting an adverse metabolic phenotype (163).

Overall, these studies suggest, consistent with preclinical investigations (see “Androgens” under “Females” in “Role of Sex Steroids in Hepatic Glucose and Lipid Metabolism: Preclinical Studies”), a role for increased circulating androgens in the promotion of fatty liver in women with (or without) PCOS.

Estrogens

Observational studies. Several epidemiologic studies have inferred a protective effect of estrogens against hepatic steatosis in women, consistent with the preclinical data in rodents (see “Females” in “Role of Sex Steroids in Hepatic Glucose and Lipid Metabolism: Preclinical Studies”). A cross-sectional study among 12,241 adults participating in NHANES III reported that NAFLD was twice as common in postmenopausal women as in premenopausal women (OR, 2.05; 95% CI, 1.43 to 2.94). Postmenopausal women who received hormonal therapy were significantly less likely to have NAFLD than postmenopausal women who did not (OR, 0.69; 95% CI, 0.48 to 0.99) (164). A more recent observational study of 4338 premenopausal women enrolled in NHANES III reported that current users of the oral contraceptive pill (OCP) had a lower risk of NAFLD (by ultrasound criteria) compared with never users, after adjustment for age, ethnicity, smoking, diabetes, hypertension, and education (OR, 0.50; 95% CI, 0.26 to 0.98). However, this relationship was attenuated after adjustment for adiposity, suggesting it was either mediated or confounded by increased body fat (165). In a cross-sectional survey of 3118 Korean women, postmenopausal status was independently associated with increased prevalence of hepatic steatosis, independent of several confounders, including BMI, dyslipidemia, and insulin resistance (166).

Of note, circulating estradiol concentrations were not measured in these studies, and evidence linking circulating estradiol concentrations to fatty liver in women are limited and inconclusive, especially in their reliance on estradiol immunoassays to measure low circulating concentrations after menopause. In a cross-sectional study among 197 Mexican women, the prevalence of NAFLD was 32% in premenopausal women and 62% in postmenopausal women. Premenopausal women without NAFLD had higher circulating estradiol (measured by immunoassay) compared with premenopausal women with NAFLD (167). The cross-sectional MESA study included 2835 postmenopausal women who had sex steroid measurements by immunoassay and liver fat quantitation by CT. There was unexpectedly a greater OR of fatty liver prevalence in those in the highest estradiol tertile compared with the lowest. This persisted after adjustment for multiple potential confounders, including age, BMI, lipids, insulin sensitivity, and use of menopausal hormone therapy (OR, 2.42; 95% CI, 1.37 to 4.29) (136). Findings in a sensitivity analysis excluding self-reported heavy alcohol users remained unchanged (136). Measurement of estradiol concentrations by immunoassay, however, is a major limitation of this study.

Turner syndrome. Women with Turner syndrome, a model of premature ovarian insufficiency and severe early estrogen deficiency, commonly [36%–44%

(168, 169)] have elevated liver enzymes, and the prevalence of elevated liver enzymes increases with follow-up (168). In a study of 27 patients with Turner syndrome with unexplained persistent liver test abnormalities who underwent a liver biopsy, 15 had histological evidence of fibrosis and/or NAFLD, and 12 had nodular hyperplasia and/or evidence of cirrhosis (170). In a Danish registry study, women with Turner syndrome had an increased risk of metabolic disorders, including a 4.4-fold increased risk of type 2 diabetes and a 5.7-fold increased risk of liver cirrhosis (171). In a small case-control study of women undergoing detailed phenotyping, women with Turner syndrome had increased intrahepatocellular lipid content compared with age- and BMI-matched controls. Intrahepatocellular lipid content was strongly correlated to cumulative estrogen-deficient years ($r = 0.93$, $P = 0.008$) (172). Thus, the increased risk of NAFLD in women with Turner syndrome may at least in part be mediated by estradiol deficiency, although these may be confounded by increased adiposity commonly prevalent in such women. Overall, the data are consistent with a protective role of estrogen treatment against hepatic steatosis and insulin resistance, similar to studies in men and rodents.

Clinical trials. Limited evidence from intervention studies are consistent with a positive effect of estrogen treatment on hepatic metabolism, although such studies do not clarify whether this effect is physiological or pharmacological.

In an RCT of 40 postmenopausal diabetic women, oral treatment with 6 weeks of micronized estradiol at supraphysiologic doses of 2 mg daily lowered HbA_{1c} levels. Hyperglycemic-euglycemic clamp studies suggested that improved hepatic insulin sensitivity, rather than improved skeletal muscle or adipose tissue insulin resistance, was the major determinant of improved glycemic control (173). Consistent with these findings, continuous administration of low-dose transdermal estradiol (50 µg/d) for 3-months in postmenopausal women was associated with an improvement of glucose metabolism by increasing hepatic insulin clearance (174). In a 6-month RCT among 50 diabetic women, low-dose continuous combined menopausal hormone therapy significantly improved hepatic enzymes compared with placebo (175). A prospective study of patients with Turner syndrome ($n = 20$) reported that hormone therapy with estradiol valerate at 2 mg/levonorgestrel at 75 µg was associated with an improvement of circulating liver enzyme concentrations (169).

In aggregate, these observational and interventional data suggest a protective effect of estrogen treatments against NAFLD, consistent with preclinical studies (see “Estrogens” under “Females” in “Role of Sex Steroids in Hepatic Glucose and Lipid Metabolism: Preclinical Studies”). Similar findings have

been reported in males, both in preclinical and clinical studies (see above).

Role of Sex Steroids in Hepatic Fibrogenesis and Carcinogenesis: Preclinical Studies

Progressive hepatic fibrosis markedly increases the risk of developing liver cirrhosis and HCC, and it is the only histological measure that predicts liver transplantation and liver-related death (53). A number of preclinical and epidemiological studies have suggested a role for sex steroids in modulating the progression of hepatic fibrosis and carcinogenesis (Fig. 3). Overall, these studies suggest that androgens are profibrotic and carcinogenic, at least in the early stages of HCC development. In contrast, AR signaling may exert antiproliferative and antimetastatic effects in advanced HCC (176). Estrogens may be protective against hepatic fibrogenesis. Overall, although factors others than sex steroids play a role, the experimental studies are consistent with clinical studies reporting that men are at high risk of developing severe hepatic fibrosis and HCC compared with women (see “Role of Sex Steroids in Hepatic Fibrogenesis and Carcinogenesis: Clinical Studies”).

Males

Androgens

Few experimental/preclinical studies have reported potential effects of androgens on liver fibrosis and carcinogenesis despite well-established differences in sex ratio. Experimentally, bone marrow mesenchymal stem cells migrate to the liver and promote hepatic repair (177). Furthermore, in mouse models of liver cirrhosis induced by carbon tetrachloride, deletion of the AR in bone marrow mesenchymal stem cells increased migration potential and increased their anti-inflammatory and antifibrotic actions (178). In this study, AR deletion enhanced liver repair through suppression of hepatic macrophage infiltration and hepatic stellate cell activation (178).

AR signaling may also be procarcinogenic, at least in early stage HCC. Several rodent models report an increased HCC susceptibility in males compared with females (179, 180) using diethylnitrosamine (DEN) (181) or ethylnitrosourea (182) to induce HCC in mice. Conversely, orchidectomy reduced tumor development (183). Moreover, in a model of TGF- α -driven hepatocarcinogenesis, orchidectomy also reduced HCC development, an effect reversed by DHT treatment (184). In DEN-induced HCC, LARKO mice developed HCC later and less frequently than did their wild-type littermates (185). Treatment of wild-type mice with AR-degrading compounds or small interference RNAs also reduced HCC development (185). Mechanistically, loss of AR reduced

“In aggregate...observational and interventional data suggest a protective effect of estrogen treatments against NAFLD.”

reactive oxygen species and promoted p53-mediated DNA damage repair and apoptosis (185). A study using LARKO mice reported that the AR also promoted hepatitis B virus–induced HCC development (186). AR activation has been reported to stimulate pro-oncogenic TGF- β 1 signaling, and AR-mediated TGF- β 1 signaling was associated with HCC development in nude mice (187). In human HCC cell lines, DHT stimulates cell proliferation in an AR-dependent manner (188). Male mice lacking 5 α reductase type 1 were protected against HCC development (94). Collectively, these preclinical studies suggest that androgens stimulate hepatic fibrinogenesis and HCC development.

In contrast to profibrotic and oncogenic effects in the early development of HCC, AR signaling may suppress late-stage HCC-associated metastasis. Although LARKO mice developed HCC less frequently and later than did control wild-type mice, LARKO mice that did develop HCC had increased frequency of lung metastasis and shorter survival than did wild-type littermates (189). Mechanistically, AR activation suppressed pro-oncogenic p38 MAPK signaling thought to play a role in late-stage HCC. Consistent with these rodent findings, AR is highly expressed in human primary HCC but not in HCC metastasis, and AR expression is inversely correlated with p38 MAPK activity in the human tissue samples (189). Overall, the data suggest that AR-mediated androgen signaling promotes HCC development in early stages, but suppresses progression in late-stage HCC.

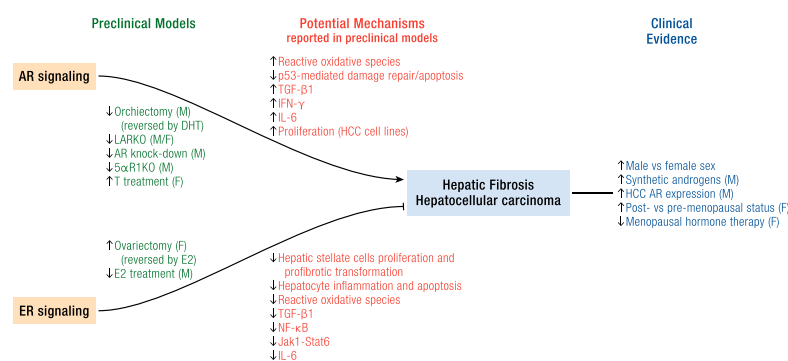


Figure 3. Effects of sex steroid signaling on the hepatic fibrosis and development of hepatic carcinoma. Presented is an overview of the effects of AR and ER signaling on profibrogenic and procarcinogenic pathways in the liver in both females (F) and males (M). In the left panel, preclinical models are shown in green. M/F denotes that the evidence from males and females in that model is consistent. Potential mechanisms identified in preclinical models of either sex are depicted in red text. In the right panel, supportive clinical evidence is presented in blue. Where applicable, M denotes that the evidence stems predominantly or exclusively from men, and F, from women. Overall the preclinical data demonstrate that interruption of AR signaling promotes, whereas interruption of ER signaling inhibits, hepatic fibrocarcinogenesis; clinical data are consistent. Note that there is some evidence that in late-stage HCC (see “Males” in “Role of Sex Steroids in Hepatic Fibrogenesis and Carcinogenesis: Preclinical Studies”), AR signaling may suppress HCC metastasis by suppression of p38 MAPK, at least in males. Caveats and limitations of various models are discussed in the text. 5 α R1KO, 5 α reductase type 1 knockout.

Estrogens

Several experimental studies provided mechanistic support for antifibrotic actions of estrogens. In male rats with DEN-induced hepatic fibrosis, estradiol treatment reduced collagen deposition in association with a reduction of hepatic mRNA for type I and III procollagens and the tissue inhibitor of metalloproteinase-1 (190). Estradiol treatment of male rats potentially inhibited profibrotic hepatic stellate cell transformation and inhibited collagen deposition in the liver (191). Hepatic stellate cells are considered the key cell type mediating hepatic fibrosis. Consistent with this, in cultured rat hepatic stellate cells, estradiol treatment had an antioxidant effect, suppressing the generation of reactive oxygen species and MAPK pathway signaling. This in turn reduced profibrotic TGF- β 1 expression and led to inactivation of the hepatic stellate cells (192). Moreover, estradiol treatment upregulated the expression of the antifibrotic microRNAs miR29a and miR29b through suppression of the NF- κ B signaling pathway in male mouse liver. This provided an additional potential mechanism by which endogenous and exogenous estrogens may protect against the development of hepatic fibrosis (193). Additionally, estrogens may exert antifibrotic effects by suppressing inflammation and hepatocyte apoptosis, the latter by upregulation of the survival factor Bcl-2 (194).

Estrogen signaling may also protect against HCC development. In DEN-induced HCC, high-dose estradiol treatment, using monthly pellets of 0.5 mg of estradiol, reduced HCC development in orchidectomized mice (181). Interestingly, the increased male susceptibility for HCC development has been linked to estradiol-mediated effects on proinflammatory signaling. In male mice, DEN administration led to more marked increases in circulating serum levels of the proinflammatory cytokine IL-6 compared with female mice. Ablation of IL-6 abolished sex differences in HCC development (195). Moreover, estradiol treatment inhibited IL-6 secretion from hepatic Kupffer cells *in vitro* and reduced circulating IL-6 and decreased liver injury in male mice *in vivo* (195). Thus, estradiol may protect against HCC development at least in part by suppressing oncogenic IL-6 signaling. Another potential mechanism by which estradiol may inhibit HCC progression is the suppression of proinflammatory Jak1-Stat6 signaling in hepatic macrophages (196). In summary, the available data consistently demonstrate that estrogens have antifibrotic and anticarcinogenic actions in the male rodent liver.

Females

Androgens

There are limited preclinical studies in females on the role of androgenic signaling in hepatic fibrotic and

carcinogenesis. In female mice, supraphysiologic testosterone treatment (using monthly, 1-mg pellets) promoted HCC development (181). Moreover, female LARKO mice developed HCC later and less frequently than did their wild-type littermates. These limited studies suggest that AR signaling may promote HCC development, similar to males (185).

Estrogens

Several experimental studies provided mechanistic support for antifibrotic actions of estrogens, similar to studies in males. In female rats with DEN-induced hepatic fibrosis, estradiol treatment reduced collagen deposition, inhibited the proliferation and profibrotic transformation of hepatic stellate cells (190), and suppressed the generation of reactive oxygen species and MAPK pathway signaling in cultured hepatic stellate cells (192).

Estradiol signaling may protect against HCC development, as female mice are less susceptible to DEN-induced HCC than are males (181). Consistent with this, ovariectomy promoted tumor development in mice (181). As discussed above, the protective effects of estradiol may in part be due to estradiol-mediated inhibition of proinflammatory cytokine IL-6 signaling (195). Consistent with this hypothesis, estradiol treatment of ovariectomized rats reduced HCC metastases in association with reduced intratumoral and circulating IL-6 levels (197). Whether these are physiological effects or pharmacological estrogen effects remains to be further clarified.

Role of Sex Steroids in Hepatic Fibrogenesis and Carcinogenesis: Clinical Studies

Men

Androgens

Observational studies. Epidemiological studies have reported that in patients with NAFLD, male sex is associated with increased severity of hepatic fibrosis, at least compared with premenopausal women (198, 199). Moreover, men are ~3.7-fold more likely to develop HCC than are women (200) and have worse survival (201). The reasons for this sex disparity in hepatic fibrosis and HCC prevalence and prognosis are not fully understood. Possible factors include differences in hepatitis carrier frequency or in environmental toxin exposure, as well as sex steroid-mediated effects. These observational studies are consistent with either a detrimental role for androgens or a protective role of estradiol.

Although serum testosterone has been reported to be higher in men with advanced fibrosis and more severe hepatic inflammation compared with those with lesser degrees of liver damage in a cross-sectional study of 308 men (202), circulating testosterone

concentrations have not been conclusively shown to predict HCC risk in men (203, 204). Very high doses of synthetic androgens, especially oral alkylated synthetic androgens with high first-pass effect in the liver, have been associated with the development of hepatocellular adenoma and HCC in body builders (205) or in patients with Fanconi anemia (206). With respect to the AR, most studies have reported that AR expression is increased in human HCC compared with normal liver (185). In a longitudinal study of 78 HCC patients who underwent HCC resection with curative intent, the 5-year recurrence-free survival was 55% for AR-negative tumors, but 0% for AR positive tumors (207). A Spanish study reported that AR positivity in the surrounding liver, rather than in the tumor tissue itself, was associated with increased HCC recurrence (208). A recent large study of 142 clinical HCC tissue samples reported that AR was overexpressed in 37% of tumors, compared with adjacent noncancerous livers and to normal liver tissue from non-HCC controls. Overexpression was associated with advanced disease stage and, during a median follow-up of 48 months, with increased mortality independent of confounders (209).

Clinical trials. Given the evidence discussed above regarding potential HCC-promoting effects of androgenic signaling, it is important to note that clinical trials of testosterone replacement in hypogonadal men or of testosterone treatment in older men dosed to increase circulating testosterone concentrations into the mid-normal young male reference range have not shown evidence of hepatotoxicity (210). Hepatotoxicity is limited to oral alkylated synthetic androgens with high first-pass effect in the liver (205, 206). Although hepatotoxic effects of testosterone therapy might be more evident in men with established chronic liver disease, in one RCT of testosterone treatment (oral micronized testosterone at a total daily dose of 600 mg) for 30 months of 126 men with biopsy-proven chronic liver disease, testosterone had no significant effect on the prevalence of any morphological changes compared with placebo-treated men (211). Moreover, in a recent 12-month RCT in men with established hepatic cirrhosis, testosterone treatment was associated with increased lean mass (mean adjusted difference, +4.74 kg; 95% CI, +1.75 to +7.74 kg; $P = 0.008$), reduced fat mass (−4.34 kg; 95% CI, −6.65 to −2.04; $P < 0.001$), and an improvement in glucose metabolism (mean adjusted difference in HbA1c, −0.35%; 95% CI, −0.05 to −0.54; $P = 0.028$) (212). However, these short-term trials were not designed or powered to HCC risks, and testosterone treatment should be used with caution in men with chronic liver disease.

Antiandrogen or GnRH agonists treatment has been evaluated in only a few small studies, mostly in patients with late-stage HCC. These studies, reviewed in Ma *et al.* (176), have not shown evidence of benefit.

Estrogens

We found no clinical studies reporting on the effects of estradiol on hepatic fibrogenesis or HCC development in men.

Women

Androgens

Although androgens have been associated with an increased risk of NAFLD, we found no clinical studies specifically reporting on effects of androgens on hepatic fibrogenesis or HCC development in women (see “Androgens” under “Women” in “Role of Sex Steroids in Hepatic Glucose and Lipid Metabolism: Clinical Studies”).

Estrogens

Observational studies. Evidence for a protective role of endogenous estradiol against hepatic fibrosis in women comes from epidemiological studies reporting that in premenopausal but not postmenopausal women with NAFLD, the severity of hepatic fibrosis is decreased compared with men. Using premenopausal women as a reference, Yang *et al.* (198) reported that among 541 US adults with a histological diagnosis of NASH, the adjusted cumulative OR for greater fibrosis severity was 1.4 (95% CI, 0.9 to 2.1) for postmenopausal women and 1.6 (95% CI, 1.0 to 2.5) for men. Among those <50 years of age, fibrosis severity was reduced in women compared with men, whereas those ≥50 years of age, this sex difference was not present (198). Similar findings were reported in nonobese Japanese women, demonstrating more severe hepatic fibrosis in postmenopausal compared with premenopausal women, after adjustment for confounders (OR, 2.2; 95% CI, 1.1 to 4.5) (199). Consistent with a protective effect of estrogens, a longer duration of estrogen deficiency is associated with an increased fibrosis risk in women. In women with nonalcoholic steatohepatitis, after multivariable adjustment, both premature menopause (OR, 1.9; 95% CI, 1.3 to 2.7) and time from menopause (OR for 5-year unit, 1.2; 95% CI, 1.1 to 1.3) were associated with more severe hepatic fibrosis (213). Overall, these epidemiological studies suggest that estradiol protects against progression of hepatic fibrosis in women.

With respect to HCC, a SEER database analysis of 39,345 patients diagnosed with HCC reported that, among patients aged <55 years, women had better overall survival compared with men. The protective effect of female sex was the greatest among patients aged 18 to 44 years (HR, 0.75; 95% CI, 0.65 to 0.85). Although the use of the OCP has been associated with increased HCC risk in small studies (214, 215), a meta-analysis of 12 case-control studies concluded that the evidence regarding a link between OCP and increased HCC risk is inconclusive (216). A recent case-control study has suggested that menopausal hormone therapy

is associated with a reduced risk of HCC (217). Thus, although exposure to physiological estradiol concentrations may be protective, the effects of OCPs are not conclusive.

In summary, the evidence from observational and preclinical studies suggest that androgens may promote hepatic fibrosis and HCC development at least in early stages, with more evidence from studies in males. In contrast, estrogens appear to protect against hepatic fibrosis in females, and possibly in males. With respect to HCC, physiologic estradiol exposure may be protective (Fig. 3).

Overall Summary

In this section we summarize the interactions between the liver and the reproductive system, as well as the roles of sex steroid signaling in the pathogenesis of NAFLD, highlighting similarities and differences in the preclinical and clinical data, and sex-dimorphic effects. We also provide an assessment as to the extent to which these relationships specifically pertain to hepatic effects, rather than being a manifestation of general insulin resistance and/or the metabolic syndrome.

Interactions between the liver and the reproductive system: evidence of sex dimorphism

As outlined in “Background” above, the liver is not only the major site of sex steroid metabolism, but it is also a major nonreproductive target organ of sex steroid action. Both ARs and ERs are expressed in rodent and human liver of either sex, and sex steroids regulate hepatic protein synthesis (such as clotting factors, hormone transport proteins), hepatic energy homeostasis, hepatic genes involved in lipid and glucose metabolism, endobiotic and xenobiotic metabolism, and liver cell proliferation and apoptosis.

For many of these biological sex steroid actions there is evidence for sexual dimorphism, mostly from rodent studies, such as pertaining to hepatic cytochrome P450 metabolism, expression of hepatic genes involved in the regulation of energy and lipid metabolism, and liver cell proliferation and apoptosis. Overall, in the rodent liver, 72% of genes are expressed in a sex-dimorphic fashion (36). In female rodents in particular, there is evidence of hepatic–gonadal cross-talk linking energy utilization to reproduction. For example, hepatic ER α transcriptional activity parallels circulating estradiol across the estrous cycle, resembling the pattern seen in reproductive organs, and distinct from the nonphasic ER α transcriptional activity seen in other nonreproductive organs (15).

Roles of sex steroids in the pathogenesis of NAFLD and its sequelae

Males

These links between sex steroid actions and hepatic metabolism raise the question as to the role of sex

steroids in the pathogenesis of metabolic liver disease. As discussed in “Role of Sex Steroids in Hepatic Glucose and Lipid Metabolism: Preclinical Studies” and “Role of Sex Steroids in Hepatic Glucose and Lipid Metabolism: Clinical Studies”, in males, there are multiple lines of evidence from nonhuman species, and consistent but comparatively more limited data from men, showing that severe global (both testosterone and estradiol) sex steroid deprivation leads to hepatic steatosis. Orchidectomy in animal studies consistently leads to hepatic steatosis, and in observational studies, severe sex steroid deficiency [ADT given to men with prostate cancer (135), organic HH (142)] is associated with hepatic steatosis. Rodent experiments (using aromatase and global or hepatic ER α knockout mice) consistently demonstrate an important role of estradiol (signaling via ER α) in the prevention of hepatic steatosis, and likewise men with inactivating mutations of the aromatase or ER α gene also have severe hepatic steatosis.

Although severe estradiol deficiency therefore promotes hepatic steatosis in males, evidence for deleterious effects of severe androgen deficiency is limited to preclinical data, and no human studies are available that definitively dissect differential effects of androgens vs estrogens. Rodent experiments demonstrate that AR antagonist treatment, genetic deletion of 5 α -reductase type 1, or LARKO promotes hepatic steatosis, suggesting a role of androgens independently of estradiol. Consistent with a role for estrogen and androgen actions in preventing hepatic steatosis, combined DHT/estradiol treatment was more effective in reversing hepatic steatosis in orchidectomized rats than either alone (218).

Whether the more modest reductions in circulating sex steroids seen in some community-dwelling middle-aged and older men with medical comorbidities and/or obesity promote hepatic steatosis is less certain. Although population-based studies (which include very few men with severely reduced sex steroid concentrations) that adjust for multiple potential confounders report inverse associations of circulating testosterone and hepatic steatosis, these associations are in large part dependent on changes in circulating SHBG concentrations. This is consistent with studies of testosterone treatment enrolling men with modest reductions in circulating testosterone reporting modest or null effects on hepatic steatosis (see “Role of Sex Steroids in Hepatic Glucose and Lipid Metabolism: Clinical Studies” above).

Females

In females (see “Role of Sex Steroids in Hepatic Glucose and Lipid Metabolism: Preclinical Studies” and “Role of Sex Steroids in Hepatic Glucose and Lipid Metabolism: Clinical Studies”), there is consistent evidence from a variety of animal species and from women (both from the general population as well as

from women with PCOS) that androgen excess induces hepatic steatosis, findings that are clearly in the opposite direction to those in males. Similar to findings in males, however, estradiol exposure is protective against hepatic steatosis. In female rodents, ovariectomy, genetic deletion of ER α both in the whole animal as well as liver-targeted ER α inactivation (either by genetic deletion or knock down) promotes hepatic steatosis in the vast majority of studies. The protective effect of estradiol is supported by observational studies in community-dwelling women (demonstrating protection against hepatic steatosis by premenopausal status and with use of OCP or postmenopausal hormone therapy), as well as by observations demonstrating increased hepatic steatosis in women with gonadal dysgenesis owing to Turner syndrome. Small RCTs of estradiol treatment demonstrated improvements in hepatic transaminases and in hepatic insulin sensitivity.

Are the interactions of sex steroids with the liver direct or do they reflect systemic metabolic disturbances?

In rodent models (see “Role of Sex Steroids in Hepatic Glucose and Lipid Metabolism: Preclinical Studies”) there are several lines of evidence suggesting that manipulation of sex steroid signaling leads to hepatic steatosis independently of effects on systemic insulin resistance and/or adiposity. For example, male LARKO (75) and LERKO (86) mice have evidence of hepatic but not of skeletal muscle insulin resistance. Consistent with a dominant effect of hepatic AR signaling, insulin resistance did not occur after AR inactivation specifically in either adipose tissue (219, 220) or in skeletal muscle (221). However, insulin resistance was reported in neuronal ARKO mice (222), and hence hepatic AR action is not solely responsible for systemic insulin resistance. There is also evidence, both from male LARKO (75) as well as AromKO (79) mice, that hepatic insulin resistance precedes that of other tissues. Moreover, in female mice, low-dose androgen treatment caused hepatic insulin resistance without affecting total body adiposity (106). Interestingly, estrogen improves metabolic homeostasis, at least in part, by increasing the expression of hepatic fibroblast growth factor 21 (223, 224), a hepatokine with favorable effects on systemic glucose and lipid metabolism in rodents. Finally (see “Role of Sex Steroids in Hepatic Glucose and Lipid Metabolism: Preclinical Studies”), consistent with direct hepatic effects, sex steroids have been implicated in the transcriptional regulation of several key hepatic genes (e.g., AKT, ACC, FOXO1, SREBP-1) that play key roles in lipid and glucose metabolism (1).

In contrast to the preclinical studies, given the close association of NAFLD with other components of the metabolic syndrome, the human studies (see “Role of Sex Steroids in Hepatic Glucose and Lipid

“Similar to findings in males, estradiol exposure is protective against hepatic steatosis.”

Metabolism: Clinical Studies”) are less definitive. Although circulating sex steroids have been associated with NAFLD independent of multiple confounders, most of the human studies are observational. Moreover, fatty liver–associated comorbidities (such as obesity) may lead central suppression of the hypothalamic–pituitary–gonadal axis (225). Therefore, lowered steroids may be a consequence rather than a cause of hepatic steatosis. Given that hepatic steatosis may be associated with altered activity of the steroid hormone–metabolizing P450 system (226), alterations in hepatic clearance of sex steroids could further confound their association with fatty liver.

Development of hepatic fibrosis and hepatic carcinoma

Overall the data (see “Role of Sex Steroids in Hepatic Fibrogenesis and Carcinogenesis: Preclinical Studies” and “Role of Sex Steroids in Hepatic Fibrogenesis and Carcinogenesis: Clinical Studies”) are consistent that in both males and females, androgens promote and estrogens protect against hepatic fibrosis and HCC development. In rodents, males are more susceptible to HCC development (toxin or TGF- β induced) than are females, and interfering with AR signaling (orchidectomy in males, AR-degrading compounds or small interference RNAs, and liver-targeted deletion of the AR in both sexes) reduces HCC development. Estradiol has antifibrotic actions and protects against HCC development in rodents of either sex in a variety of experimental settings. Consistently in humans, male sex is associated with increased severity of hepatic fibrosis, increased risk of developing HCC, and increased HCC-associated mortality. Hepatic AR overexpression is an adverse prognostic indicator in most studies. In women, premenopausal status, compared with postmenopausal status, is associated with reduced and a longer duration of estradiol deficiency with increased severity of hepatic steatosis.

Clinical implications

Although hepatic effects of severe sex steroid deprivation require further study, the current evidence suggests that modest reductions in circulating sex steroids observed in community-dwelling men are a marker rather than a rectifiable contributor to NAFLD. In such men, the focus of clinical care should be on early treatment of NAFLD-associated risk factors, particularly obesity, and care of liver disease–associated comorbidities to optimize metabolic and cardiovascular health and to prevent the progression of established liver disease to cirrhosis and its downstream complications. Overall, there is no evidence that testosterone replacement in men with established hypogonadism has hepatotoxic effects, although whether testosterone has carcinogenic effects in men with chronic liver disease has not been

excluded definitively. In women, the focus should likewise be treatment of NAFLD-associated risk factors and liver disease–associated comorbidities, and further trials should ascertain whether physiologic estradiol exposure or measures to reduce androgen actions have favorable hepatic effects.

Conclusions and Future Directions

Given the role of the liver as the body’s central metabolic hub and the evolutionary importance of successful reproduction, it is not surprising that there is a close crosstalk between the liver and the gonadal axis in both sexes (Fig. 1). Possibly because of the need to adapt to sex-specific reproductive needs, the liver displays a considerable degree of sexual dimorphism. This functional sex dimorphism has systemic consequences that extend beyond reproduction and involves sex differences in endobiotic and xenobiotic metabolism and in the susceptibility to NAFLD. Interestingly, a recent study reported that treatment of mice with 17- α estradiol resulted in a sex-specific lifespan extension in males, but not in females (227). This male-specific lifespan extension was associated with distinct changes suggestive of altered amino acid metabolism in the metabolomic profile of male but not female liver. In contrast, there was no evidence for no sex-dimorphism in the metabolic profile of skeletal muscle (227). The male-specific changes in the hepatic metabolome were abrogated by orchidectomy, inferring a role of testicular hormones in this sex dimorphic response to 17- α estradiol (227). Another recent study reported sex-dimorphic methylation profiles in the human liver that associate with changes in hepatic gene expression potentially playing a role in sex differences in circulating high-density lipoprotein (HDL) cholesterol concentrations (228). Although sex difference biomedical research is still in its infancy and not without methodological caveats (229), the preclinical and clinical data discussed in this review suggest that future studies examining sex-dimorphic disease expression should consider possible roles of the liver in contributing to such effects.

The current preclinical evidence suggests a role of androgens and estrogens in the pathogenesis of the full spectrum of NAFLD in both males and females, from simple steatosis to HCC. Although epidemiological studies consistently show sex-dependent differences in the prevalence of conditions encompassing the NAFLD spectrum that are consistent with the sex-dependent pathogenetic roles of sex steroids in preclinical studies, direct evidence for pathogenic roles of sex steroids in humans, although suggestive, is more limited. This is in part because the evidence is largely observational, and existing studies are largely limited by reliance of sex steroid measurements by suboptimal

methodology. Furthermore, there is a dearth of adequately designed and powered clinical trials of sex steroid treatment that inform on important health outcomes, such as cardiometabolic outcomes (effects on glucose and lipid metabolism, cardiovascular events) and progression to HCC, in men and women

with chronic liver disease. To better define the role of sex steroids in the pathogenesis of NAFLD, well-designed prospective studies with repeated LC-MS-based measurements focusing on clinical outcomes are required to determine the need for, and inform the design of, clinical trials of sex steroid treatment.

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Acknowledgments

Financial Support: M.G. is supported by National Health and Medical Research Council of Australia Project Grants APP1099173 and APP1062073.

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Disclosure Summary: M.G. and P.A. have received research support from Bayer. D.J.H. has received institutional grant support for investigator-initiated testosterone pharmacology studies and has provided expert testimony to antidoping tribunals and for testosterone litigation. M.E.W. has nothing to disclose.

Abbreviations

ACC, acetyl-coenzyme A carboxylase; ADT, androgen deprivation therapy; AR, androgen receptor; ARKO, AR knockout; AromKO, aromatase knockout; BMI, body mass index; DEN, diethylnitrosamine; DHT, dihydrotestosterone; ER, estrogen receptor; FoxO1, forkhead box protein O1; Gpr30, G protein-coupled receptor 30; GPER, G protein-coupled ER; HCC, hepatocellular carcinoma; HDL, high-density lipoprotein; HR, hazards ratio; LARKO, liver-targeted ARKO; LC-MS, liquid chromatography–mass spectrometry; LERKO, liver-targeted ER α knockout; MESA, Multi-Ethnic Study of Atherosclerosis; MTP, microsomal triglyceride transfer protein; NAFL, nonalcoholic fatty liver; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; NF- κ B, nuclear factor κ B; NHANES, National Health and Nutrition Examination Survey; OCP, oral contraceptive pill; PCOS, polycystic ovarian syndrome; PEPCK, phosphoenolpyruvate carboxykinase; PI3K, phosphoinositide 3-kinase; PPAR, peroxisome proliferator-activated receptor; RCT, randomized clinical trial; SEER, Surveillance, Epidemiology and End Results; SHBG, sex hormone-binding globulin; SMP30, senescence marker protein-30; SREBP-1c, sterol regulatory element-binding protein 1c; α ERKO, ER α knockout.