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Relevance of low testosterone to non-alcoholic fatty liver disease

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Abstract

Non-alcoholic fatty liver disease (NAFLD) is a condition where there is excess accumulation of triglycerides in the liver in the absence of excess alcohol consumption. It ranges from simple steatosis to non-alcoholic steatohepatitis (NASH), which can progress to fibrosis, cirrhosis, and hepatocellular carcinoma (HCC). NAFLD, one of the most common causes of chronic liver disease in Western populations, is the hepatic component of the metabolic syndrome (MetS) and is associated with increased visceral adipose tissue (VAT), insulin resistance, and dyslipidemia. Studies have also shown that testosterone deficiency is associated with increased VAT and insulin resistance in males while hyperandrogenemia has been associated with increased risk of insulin resistance and VAT in females. Thus, the aims of this review are to discuss the available experimental and epidemiological data evaluating the association between testosterone and NAFLD, to discuss the potential clinical relevance of these data, and to identify gaps in the literature.

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Keywords

Liver diseases; sex hormone-binding globulin; hepatology; endocrinology; obesity

Introduction

Non-alcoholic fatty liver disease (NAFLD)

Non-alcoholic fatty liver disease (NAFLD) refers to a condition where there is an excess buildup of triglycerides in the liver parenchyma in the absence of excess alcohol consumption.[1] It ranges from simple steatosis to non-alcoholic steatohepatitis (NASH), which can progress to fibrosis, cirrhosis, and hepatocellular carcinoma (HCC). Simple steatosis is a relatively benign condition and is defined as the presence of fat comprising 5% of the liver weight in the absence of inflammation.[2] In contrast, NASH is diagnosed by the additional presence of inflammation and hepatocyte injury, with the most defining features being hepatocyte ballooning and the formation of Mallory bodies:[3] eosinophilic intermediate filament proteins found in the cytoplasm of damaged hepatocytes. NAFLD is considered to be the hepatic component of the metabolic syndrome (MetS) and is associated with increased visceral adipose tissue (VAT), insulin resistance, and dyslipidemia.[4] NAFLD has been found to be present in 20-30% of the population of Western countries [5] and is the leading cause of chronic liver disease in the U.S.[6] The global prevalence of NAFLD continues to rise in conjunction with rapid increases in rates of obesity and diabetes in recent years.[7]

In males, testosterone deficiency is associated with increased VAT and insulin resistance,[8] and testosterone replacement in this setting has been found to reduce fat deposition;[9] while hyperandrogenemia has been associated with increased risk of insulin resistance and VAT in females.[10; 11] Also, some reports suggest an association between testosterone and other sex hormones and severity of liver disease.[12] Thus, the primary aims of this review are to discuss the current available data evaluating the association between testosterone levels and NAFLD, to discuss the potential clinical relevance of these data, and to identify gaps in the literature.

Pathogenesis of NAFLD

The pathogenesis of NAFLD, although still not completely understood, appears to be multifactorial. The traditional model describing the pathogenesis of NASH is the two-hit hypothesis.[13] The first hit involves the accumulation of triglycerides and free fatty acids in the hepatic parenchyma due to an imbalance between the influx and synthesis of lipids and the export and β-oxidation of lipids. This makes the liver susceptible to injury caused by second hits such as inflammation, adipokines, gut-derived endotoxins, mitochondrial dysfunction, and oxidative stress. However, recently, there has been speculation that free fatty acids themselves can directly cause injury to the liver as they undergo esterification with glycerol to form triglycerides in hepatocytes and activate an inflammatory pathway.[14] Furthermore, a third hit has been recently proposed in the pathogenesis of NAFLD.[14] In a healthy liver, progenitor cells differentiate and replace dead hepatocytes. The third hit

portrays the inability to regenerate dead hepatocytes in the presence of oxidative stress in NAFLD, thus worsening liver damage.

Insulin resistance, a component of the metabolic syndrome, has been shown to promote NAFLD in epidemiological, experimental, and human studies.[15] Since insulin suppresses lipolysis in adipose tissue, in a state of insulin resistance, there is an increased influx of fatty acids to the liver.[14] Hyperinsulinemia increases the expression of sterol regulatory element binding protein-1c (SREBP-1c), a transcription factor which up-regulates *de novo* lipogenesis. It also inhibits β-oxidation of free fatty acids in the mitochondria and increases the degradation of very low-density lipoprotein (VLDL) in the liver, leading to an increase in hepatic fat accumulation.[16] In addition to insulin playing a potential role in the pathogenesis of NAFLD, many factors including lipid metabolites can interfere with the insulin-signaling cascade and exacerbate insulin resistance, leading to a vicious cycle between insulin resistance and NAFLD.[17]

Obesity and increased VAT may also play a role in the pathogenesis of NAFLD. In obesity, excess lipids can deposit ectopically into other organs such as the liver.[18] Thus, both insulin resistance and excess VAT cause an increased influx of free fatty acids to the liver, leading to NAFLD.[19] Increasing visceral obesity also increases pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin 6 (IL-6), and c-reactive protein (CRP) and decreased production of the anti-inflammatory adipokine, adiponectin, which can also play a role in the development of NAFLD.[20]

Diagnosis of NAFLD

Most patients with NAFLD are asymptomatic. If symptoms present, they usually are nonspecific and include fatigue and abdominal discomfort. On physical exam, an enlarged, palpable liver can be present in a patient with NAFLD, but this sign is not universal and not specific. Hence, there should be evidence of hepatic steatosis by imaging or histology, with secondary causes of fat accumulation within hepatocytes such as significant alcohol consumption and viral infection excluded in order to establish a diagnosis of NAFLD.[21] Liver biopsy remains the gold standard to diagnose NAFLD/NASH and associated fibrosis; however, it is rarely performed for this purpose as it is expensive and has a risk of morbidity and mortality due to its invasiveness. Further, there are also limits related to data obtained by liver biopsy given well-known sampling variability and pathologist interpretation; these are significant considerations for missed diagnosis of NASH NASH-cirrhosis, or NAFLD-related HCC. Although non-invasive imaging modalities such as ultrasound (US), computed tomography (CT), and magnetic resonance imaging (MRI) can show the presence of a fatty liver, do not always correlate with other histological features.[22] Additionally, patients with NAFLD may have elevated (GGT) levels. However, these lack sensitivity and specificity.[23] Algorithms that combine liver enzymes with other widely available biomarkers or clinical data including the FIB-4 and NAFLD Fibrosis Score are also available and have high negative predictive values for determining presence of advanced NAFLD-associated fibrosis with similar positive predictive values.[24] Acute phase proteins have also been evaluated for predicting NASH. Cytokeratin-18 fragments, markers of hepatocyte apoptosis, have shown to be useful in distinguishing steatosis from NASH.[25] Nevertheless, the use of

serum biomarkers has not been approved yet in diagnosing NAFLD in routine clinical practice.[21]

Testosterone

Testosterone circulates in the body either free (2%), loosely bound to albumin which is produced in the liver (38%), or tightly bound to its main carrier which is also synthesized in the liver: sex-hormone binding globulin (SHBG) (60%).[26] Bioavailable testosterone (BT), which consists of the sum of free testosterone (FT) and albumin-bound testosterone, can bind to and activate the androgen receptor (AR) in target tissues. It can also be converted to dihydrotestosterone (DHT), the strongest activator of the AR, or to estradiol (E2). On the contrary, testosterone bound to SHBG cannot bind to the AR and is unable to be taken up by target tissues. Plasma SHBG concentrations are increased by a variety of conditions including aging, hyperthyroidism, hypogonadism, and hepatic cirrhosis and decreased in obesity and diabetes mellitus.[27] Because SHBG can be a potential confounding variable when evaluating androgen and estrogen levels in conditions such as obesity, aging, and liver disease, only FT and BT levels should be measured in these settings.

Equilibrium dialysis is the gold standard for measuring FT.[28] However, it can be affected by sample dilution, pH, and changes in temperature.[29] Immunoassays for quantifying FT are commonly available, but lack sensitivity.[30] Liquid chromatography-tandem mass spectrometry (LC-MS/MS) has improved sensitivity and shows hope in becoming the reference technology to detect testosterone levels.[31] Additionally, FT and BT levels can be calculated by using the measurements of SHBG and serum total testosterone levels. The calculated FT and BT have shown to be in agreement with equilibrium dialysis.[28] BT can also be measured by precipitating SHBG with ammonium sulfate. However, since ammonium sulfate can precipitate a small amount of albumin-bound testosterone, BT can be falsely underestimated.[32]

Low testosterone, NAFLD, and the metabolic syndrome in men

In men, testosterone deficiency has been associated with an increased accumulation of visceral adipose tissue and insulin resistance, which are factors contributing to the metabolic syndrome;[19] and higher levels of testosterone are associated with decreased central obesity.[33] Analysis from a cross-sectional study amongst elderly men showed that an increase of 1 standard deviation (SD) of total testosterone level (∼153 ng/dL or 5.31 nmol/L) correlated with a 57% reduced risk of the diagnosis of the metabolic syndrome.[34] In a population of middle-aged Finnish men, it was found that low total testosterone and SHBG can predict the development of metabolic syndrome and diabetes.[35] Moreover, testosterone replacement has been found to reduce insulin resistance in hypogonadal men with type 2 diabetes and/or MetS [36] and to reduce fat accumulation.[37] Further support of the role of androgens in NAFLD is suggested by a case report of a men who developed NAFLD after treatment of prostate cancer with a gonadotrophin agonist that induced profound hypogonadism.[38]. In spite of this strong association between testosterone and the metabolic syndrome, causality has not been established.[39]

Laboratory and Clinical Evidence Showing Association of Testosterone to NAFLD

Male animal data

To determine the relative contribution of gonadal status to diet-induced fat accumulation in the liver, a study was conducted in young male rats placed in four different groups: Intact eugonadal (I) rats fed a regular chow diet (I+RCD), I rats fed a high fat diet (I+HFD), castrated (C) rats on a high fat diet (C+HFD), and C rats with testosterone replacement on a high fat diet (C+HFD+T). Over a 15-week time period, the C+HFD had undetectable serum testosterone levels, while the C+HFD+T had serum testosterone levels that were significantly higher than those measured in the I+RCD and I+HFD groups. The hepatocytes of the I+HFD and C+HFD+T revealed only mild to moderate microvesicular steatosis. C +HFD had the largest macrovesicular accumulation of fat in hepatocytes, inflammation of the liver, and worst pathology score. These pathological features, which characterize NAFLD, decreased significantly with testosterone replacement in the C+HFD+T group in the absence of hyperglycemia, hyperinsulinemia, and insulin resistance. Consequently, this study suggests that testosterone deficiency enhances diet-induced accumulation of fat in the liver and the development of NAFLD, while testosterone replacement in frankly hypogonadal animals diminishes hepatic steatosis and inflammation, thereby possibly reducing the severity of NAFLD.[40] Previous studies have shown that food consumption decreases by castration and increases by testosterone replacement in male rats.[41] Although leptin levels were higher in HFD groups regardless of their testosterone status, other factors such as ghrelin could influence this association between testosterone and food consumption, thus maybe offering an alternate potential causal or modifying factor in the relationship between testosterone and diet-induced NAFLD. The timing of androgen exposure may also be important. For example, in sheep, maternal prenatal exposure to testosterone predisposes to fatty liver disease in the adult offspring of exposed ewes independently of central obesity. [42] One limitation of this study was the lack of measurement of DHT and inability to detect estradiol (E2) levels lower than 2 pg/mL or 7.34 pmol/L since testosterone is converted to these active metabolites in the body.

Clinical epidemiology

Studies in Men—Several epidemiological studies have investigated the association between testosterone and NAFLD in males such as a study in 1,944 Korean men.[43] This study showed that males with US-confirmed NAFLD had a statistically significant lower median baseline total testosterone level, measured by radioimmunoassay, than the control group (~418 ng/dL [14.5 nmol/L] vs. 496 ng/dL [17.21 nmol/L], $p<0.05$). After adjusting for potential confounders such as age, smoking, exercise, history of hypertension and diabetes, waist circumference, systolic blood pressure, glucose, high-density lipoprotein cholesterol, triglyceride, and erythrocyte sedimentation rate, a logistic regression model showed that as baseline total testosterone levels increased, the prevalence of NAFLD was lower at baseline and follow-up (odds ratio, 0.696 and 0.795 at baseline and follow-up, respectively). In a secondary analysis using Cox regression, higher baseline testosterone levels significantly hindered the development of NAFLD. However, this association was no

longer significant after adjusting for confounders, suggesting that other factors may also play a role.

Another study was conducted in 55 neurologically stable males admitted to a rehabilitation program in Sulmona, Italy because of chronic spinal cord injury.[44] One blood sample was taken to measure total testosterone levels by chemiluminescence immunoassay, while NAFLD was diagnosed by abdominal US. Twenty-seven out of the 55 patients were diagnosed with NAFLD; all had a higher body mass index (BMI), insulin resistance (HOMA-IR), triglyceride levels, and GGT values, and lower total testosterone and FT levels. However, multiple logistic regression analysis showed that only total testosterone and FT had an independent association with NAFLD, with males with lower total testosterone levels (<300 ng/dL or 10.4 nmol/L) having a 12-fold increase risk of NAFLD than those with higher total testosterone levels.

In a different retrospective cross-sectional study conducted on 495 healthy Korean adult men,[45] total testosterone was measured in serum by radioimmunoassay, and abdominal US was performed to diagnose NAFLD. The 251 subjects that had NAFLD had lower testosterone levels (398 ng/dL [13.81 nmol/L] compared to 483 ng/dL [16.76 nmol/L]) and adverse metabolic profiles such as higher VAT, HOMA-IR, and low-grade inflammation. After adjusting for VAT, insulin resistance, low-grade inflammation, age, smoking, diabetes, exercise, BMI, and triglycerides, an inverse relationship was shown between testosterone and NAFLD.

Cross-sectional data from the Multi-Ethnic Study of Atherosclerosis evaluated the association between sex hormones and liver fat. Testosterone, dehydroepiandrosterone (DHEA), SHBG, and E2 were measured using various immunoassays and BT was calculated. CT scan measured fatty liver. 2,899 males were included in the analysis. Men with fatty liver had lower total testosterone levels and SHBG levels and higher DHEA and E2 levels. These associations remained significant even after adjusting for age, ethnicity, sex, waist to hip ratio, hypertension, current smoking, and total cholesterol [46].

Studies in Women—The association between testosterone and NAFLD was also evaluated in 2,835 post-menopausal women in the Multi-Ethnic Study of Atherosclerosis. [46] Women with fatty liver had lower SHBG levels and higher E2 levels. However, unlike in men, there was no association between fatty liver and total testosterone or DHEA levels, suggesting gender differences in the association of testosterone to NAFLD. After adjusting for confounding variables, it was found that women in the highest tertile of BT and E2 were significantly more likely to have fatty liver than in the lowest tertile of BT and E2, respectively. One limitation specific to this study is that estrone, the main post-menopausal estrogen, was not measured.

In Greece, a cross-sectional study was done in 40 post-menopausal women where 22 were diagnosed with NAFLD by liver biopsy. They underwent blood sampling to measure serum SHBG and total testosterone by immunochemiluminescence while FT and BT were calculated. The women with NAFLD had lower SHBG levels and higher free testosterone and BT when compared to those women without NAFLD. After adjusting for age, BMI, and

waist circumference, only SHBG and BT levels were independently associated with NAFLD, suggesting that the role of androgens may be the opposite in women compared to men. One limitation of this study is the small sample size.[47] However, this is one of the first studies on testosterone and NAFLD that diagnoses NAFLD by liver biopsy, the gold standard to diagnose this disease.

A retrospective cross-sectional study conducted in 95 females with polycystic ovary syndrome (PCOS) to determine if androgens play a role in the association between NAFLD and PCOS, a condition often accompanied by hyperandrogenism. NAFLD was diagnosed in twenty-one of these women by US, minimal alcohol consumption (no more than 1 drink per week), and the absence of positive hepatitis serologies. Total testosterone, dehydroepiandrosterone sulfate (DHEAS), and 17-hydroxyprogesterone (17-OHP) levels measured by a chemiluminescent immunoassays were similar in women with and without NAFLD, suggesting that androgens may not play a role in the association of PCOS and NAFLD. However, confounding factors such as age, weight, and insulin resistance were not accounted for; for example, HOMA-IR was higher in the PCOS and NAFLD group, possibly influencing the results.[48]

On the contrary, in the United Kingdom a case control study was conducted in 29 women with PCOS and 22 healthy controls. Total testosterone and SHBG were measured by a chemiluminescence method, and the free androgen index (FAI) was calculated while hepatic steatosis was identified by proton magnetic resonance spectrometry. Women with hyperandrogenic PCOS, based on the FAI, had evidence of increased liver fat compared to women with normoandrogenic PCOS and healthy controls after adjusting for BMI, adipose tissue, and HOMA-IR. This data suggests that hyperandrogenic PCOS can increase the risk of hepatic steatosis independently of obesity and insulin resistance.[49]

Discussion

Summary of studies across genders and their limitations

The studies are summarized in Table 1. In the studies in men described above, there was an association between lower levels of total testosterone and the development of NAFLD. Free testosterone, BT, SHBG, DHEA, and other sex hormones like E2 were not measured in all the studies in men so definite conclusions cannot be made about their relationship to NAFLD. In the first two studies mentioned in women, NAFLD was associated with higher levels of BT. In the two studies showing the association between PCOS and NAFLD, one found that androgens may not play a role while the other found that increased androgens can predispose to hepatic steatosis. Other conclusions cannot be drawn at this time since different forms of testosterone and sex hormones were measured in these studies.

Immunoassays were used to measure the various sex hormones. This is a limitation since immunoassays lack sensitivity. Another limitation is that only one testosterone measurement was taken in some studies. Two samples are usually required to confirm the diagnosis of low testosterone. NAFLD and hepatic steatosis were diagnosed by abdominal US in many of the studies and US may not detect hepatic fatty infiltration <30%.[50] Subjects were excluded if they had consumed significant amounts of alcohol, which was defined differently across

these studies. Further, recall bias could have occurred when asking subjects if they had consumed significant alcohol or taken steatogenic drugs, excluding them from a clinical diagnosis of NAFLD. Although confounding variables were adjusted in most of the studies, all of them may not have been accounted for, causing limitations. Standardization of all these different factors would be important to take into consideration when future studies are conducted.

Possible mechanisms underlying the association of low testosterone to NAFLD

Several different mechanisms could explain the observed association between low testosterone and NAFLD in males. For instance, low testosterone leads to accumulation of VAT, which can cause insulin resistance and increased exposure of the liver to free fatty acids. Moreover, low serum testosterone is associated with increased inflammation.[51] Testosterone may also influence microRNAs in the liver or the activity of hepatic lipase as seen in male rats.[52] Since testosterone can be converted to DHT and E2 by the enzymes 5α-reductase and aromatase respectively, DHT and E2 may play a part in linking low testosterone and NAFLD in men. The deficiency or inhibition of 5α-reductase triggers the development of hepatic steatosis in male mice,[53; 54] suggesting low DHT levels may contribute to NAFLD. As DHT can initiate cell cycle arrest and apoptosis in androgen-sensitive liver cells by the PKR/eIF2a signaling cascade,[55] low levels of DHT may increase HCC risk and possibly have a role in the pathogenesis of NAFLD. Further, low testosterone is associated with elevated E2 in men and this could be due to greater conversion of testosterone to E2. E2 has been found to reduce lipogenesis in male rats by decreasing fatty acid synthase and the phosphorylation of acetyl coenzyme A [56], and thus, could affect testosterone's association with NAFLD.

Relevance of low testosterone and NAFLD association

A direct association between testosterone and NAFLD could help in the diagnosis of NAFLD. Testosterone levels could help predict the stage of NAFLD and prognosis of the disease or it may justify the screening of hypogonadal men for NAFLD. It is also possible that obesity causes both low testosterone and NAFLD since there is insufficient data to determine if the association between low testosterone and NAFLD is causal. However, if the relationship is causal, testosterone may also protect the liver from developing hepatic steatosis and slow down the progression to HCC.[53] Since estrogen/estrogen receptor and DHT/androgen receptor signaling decrease fatty acid and cholesterol synthesis and increase beta-oxidation,[56] and E2 has suggested as a protective factor for NAFLD in healthy men, [57] steroid hormones such as testosterone may have the ability to treat NAFLD in men. However, forms of oral testosterone that are 17-alpha-alkylated are hepatotoxic.[58], and so these forms would not be appropriate to use in this setting. Further, there has been an association between anabolic steroid abuse and HCC,[59] so caution must be taken when suggesting testosterone's possible therapeutic effect for NAFLD.

Androgens and NAFLD in women

The liver is one of the most sexually dimorphic organs per gene expression and profound differences in circulating hormones exist between genders. Also, there seems to be clear gender differences regarding the potential role of androgens in NAFLD. These differences

could be driven by several factors including that in women, higher androgen levels are in general associated with increased visceral adiposity and insulin resistance.[10] Though there may be a direct association between NAFLD and PCOS,[60] it is still uncertain if this association is due to the presence of high levels of testosterone or other metabolic factors such as obesity and insulin resistance.[61] DHT, rather than testosterone, may play a role in women with PCOS; long term DHT treatment in female mice displayed ovarian, endocrine, and metabolic characteristics of PCOS seen in humans.[62] The contrasting role of androgens in men and women regarding its contribution to NAFLD is far from understood. It is possible that the differences in testosterone levels per se, where women still have comparatively much lower testosterone levels than men with testosterone deficiency, or a potential protective effect of estrogens could explain these differences.

Future Studies

Due to the growing obesity epidemic, NAFLD is the most common cause of chronic liver disease in the U.S. This disease most often has an indolent course though a subset often develop more progressive liver diseases including NASH and NASH-cirrhosis, in whom there is an excess risk of HCC. There is experimental and clinical data suggesting testosterone may help prevent or ameliorate NAFLD in males. However, given limitations in studies, particularly the lack of randomized controlled trials, further investigations need to be conducted. The association between testosterone and NAFLD has been primarily evaluated in specific sub-groups of females (e.g., with PCOS); additional research is needed to establish testosterone's role in the development and progression of NAFLD in women across the age-spectrum within the general population. Gender specific research is crucial given that the burgeoning obesity epidemic also impacts women; even with women having a lower risk of NAFLD/NASH progression to HCC than men[63], there are a large number of women with NAFLD. Additionally, although experimental research suggests several mechanisms by which testosterone may influence NAFLD risk, additional epidemiological research in prospectively recruited and serially followed human populations is necessary to establish mechanisms in background of multiple common confounding factors in ‘real world’ populations including dietary intake, physical activity, and use of alcohol. Though physiological levels of testosterone were measured in the clinical studies, the impact of testosterone supplementation in the development of NAFLD should also be studied. These studies should also take into account long-term safety issues, particularly cardiovascular safety, since this has recently been questioned.[64]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Summary of data showing association between testosterone and NAFLD

Author (Year)	Location	Study design	Population	Total # of subjects	Main findings	Reference
Nikolaenko, et al. (2014)	USA	Experimental	Male rats	29	-T deficiency enhances diet accumulation of fat in liver -T replacement in hypogonadal animals diminishes hepatic steatosis	[40]
Seo, et al. (2015)	Seongnam, Korea	Cohort	Men	1944	-Total T was significantly lower in subjects with NAFLD than control group -As baseline total T increased, prevalence of NAFLD was lower at follow-up than baseline -Baseline total T did not independently predict the development or regression of NAFLD	[43]
Barbonetti, et al. (2015)	Italy	Cross-sectional	Men	55	-Low total T and FT were significantly and independently associated with NAFLD in neurologically stable men with chronic spinal cord injury	[44]
Kim, et al. (2012)	Seoul, Korea	Cross-sectional	Men	495	-Low total T is independently associated with NAFLD	[45]
Lazo, et al. (2015)	U.S.	Cross-sectional	Men	2899	-Men with fatty liver have low total T and SHBG and high DHEA and E2 -Fatty liver more likely in those with highest SHBG and E2	[46]
Lazo, et al. (2015)	U.S.	Cross-sectional	Women	2835	-Women with fatty liver have low SHBG and high E2 -Fatty liver more likely in those with the highest BT and E2 -No association found between fatty liver and low total T and high DHEA	[46]
Polyzos, et al. (2013)	Greece	Cross-sectional	Women	40	-Low SHBG levels and high BT levels associated with NAFLD	[47]
Kauffman, et al. (2010)	U.S.	Cross-sectional	Women (with and without PCOS)	95	-Total T, DHEAS, and 17-OHP levels similar between PCOS+NAFLD and PCOS-NAFLD groups	[48]
Jones, et al. (2012)	United Kingdom	Cross-sectional	Women (with and without PCOS)	51	-Hyperandrogenism PCOS determined by FAI increases risk of hepatic steatosis compared to non-hyperandrogenic PCOS and controls	[49]

Abbreviations: 17-OHP, 17-hydroxyprogesterone; BT, bioavailable testosterone; DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulfate; E2, estradiol; FAI, free androgen index; FT, free testosterone; NAFLD, nonalcoholic fatty liver disease; PCOS, polycystic ovarian syndrome; SHBG, sex-hormone binding globulin; T, testosterone