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Sex dimorphism and metabolic profiles in management of MAFLD

MALFD, sex differences and metabolomics

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Abstract

Metabolic-associated fatty liver disease (MAFLD) refers to the build-up of fat in the liver associated with metabolic dysfunction and has been estimated to affect a quarter of the population worldwide. Although metabolism is highly influenced by the effects of sex hormones, studies of sex differences in the incidence and progression of MAFLD are scarce. Metabolomics represents a powerful approach to studying these differences and identifying potential biomarkers and putative mechanisms. First, metabolomics makes it possible to obtain the molecular phenotype of the individual at a given time. Second, metabolomics may be a helpful tool for classifying patients according to the severity of the disease and obtaining diagnostic biomarkers. Some studies demonstrate associations between circulating metabolites and early and established MAFLD, but little is known about how metabolites relate to and encompass sex differences in disease progression and risk management. In this review, we will discuss the epidemiological metabolomic studies for sex differences in the development and progression of MAFLD, the role of metabolic profiles in understanding mechanisms and identifying sex-dependent biomarkers, and how this evidence may help in the future management of the disease.

Key Words: MAFLD; sex differences; metabolic profiles; metabolism

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Core Tip: Metabolic-associated fatty liver disease (MAFLD) refers to the build-up of fat in the liver associated with metabolic dysfunction and has been estimated to affect a quarter of the population worldwide. Metabolomics represents a powerful approach to studying metabolic disease, including MAFLD, and to identify potential biomarkers and putative mechanisms. Some studies demonstrate associations between circulating metabolites and early and established MAFLD, but little is known about how

metabolites relate to and encompass sex differences in disease progression and risk management. In this review, we will discuss the role of metabolic profiles in understanding mechanisms and identifying sex-dependent biomarkers, and how this evidence may help in the future management of the disease.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a spectrum of liver diseases that occurs with different stages ranging from steatosis to cirrhosis or hepatocellular carcinoma. In 2020, the classical conception of NAFLD was revised and consequently, a new entity called metabolic-associated fatty liver disease (MAFLD) was defined. Unlike NAFLD, MAFLD does not exclude patients who consume alcohol or those who have other liver diseases. MAFLD prioritizes and values the metabolic involvement of the liver and the consequences of liver metabolism impairment on the progression of the disease [1]. At the clinical level, MAFLD seems more useful for the study of the advanced stages of the disease, since, by focusing on the metabolic dysfunction, MAFLD allows to include a greater number of patients and individuals at risk than the classic definition of NAFLD [2]. However, as expected for recently proposed definitions, the criteria for the use of MAFLD as a clinical entity is not unanimous and NAFLD is still the most used term [3]. Currently, the prevalence of NAFLD and MAFLD is continuously increasing in both adult and child populations due to the epidemics of obesity and type 2 diabetes mellitus. Although MAFLD and NAFLD share a large part of their clinical profile and produce similar long-term outcomes, the actual trend for increase mortality for both of them reflect different situations. Increased liver-related mortality among NAFLD patients seems driven by NAFLD-related liver complications and extra-hepatic diseases such as cardiovascular disease, extra-hepatic cancers, or kidney diseases [4]. MAFLD patients show greater risk for all-cause mortality and an equal risk for cause-specific mortality comparing to NAFLD patients [5].

The prevalence of MAFLD is very high worldwide with numbers rapidly increasing in low- and mid—income countries. In adulthood, the average prevalence of MAFLD

worldwide in 2015 was 25%, being higher in South America (31%) and the Middle East (32%) [6]. More recent studies show even higher rates of MAFLD with an estimated prevalence in adults as high as 30%, being even higher in the Middle East and North Africa (MENA) (≈ 43%) followed by South America and Asia (≈ 33%) [7]. The numbers at a young age are especially worrisome. In childhood, one of the greatest risk factors that contribute to the initiation and development of MAFLD is obesity. In overweight and obese children, the prevalence of MAFLD can reach up to 36%. Nevertheless, the worldwide prevalence of MAFLD among the general paediatric population is 8% with the highest rate in Central America and the Middle East [8, 9].

The prevalence of MAFLD seems to differ among men and women even at young ages. The prevalence of MAFLD in the juvenile population is higher in men than in women both before and after puberty [8, 10]. In the adult population, the prevalence of MAFLD is also higher in men than in pre-menopausal women. However, the incidence in post-menopausal women increases markedly suggesting a potential role for sex hormones in the mechanisms of the disease [7, 11].

The burden of the disease in the national health systems and the population is increasingly higher. Due to the increase in cases in children, the disease becomes chronic at a younger age. It is estimated that, in the coming decades, the incidence will continue increasing and so will the costs in health systems worldwide [7, 9]. We herein review the influence of sex hormones on hepatic and mitochondrial metabolism and how sex hormones contribute to the development of MAFLD. It seems critical to identify new risk and early disease biomarkers, which include relevant biological factors like sex in the risk estimation, for a future better management of the disease. ´Therefore, we specifically focus on if the sex variable is currently considered when stratifying patients in metabolomics studies and if it is used to search new biomarkers based on metabolomics in the development of MAFLD.

SEX AND LIVER METABOLISM

The liver is an organ with a key role in the homeostasis of human metabolism. Due to the architecture of the hepatic lobules and the arrangement of the portal triad with respect to the hepatic vein, there is a metabolic zonation in the liver, which leads to a liver metabolism not exactly uniform within the hepatic lobule [12]. The liver is an organ with a high metabolic rate and, among other functions, is responsible for regulating lipid and carbohydrate metabolism in the body. Regarding lipid metabolism, circulating lipids enter the hepatocyte and can (1) be oxidized for energy, (2) be esterified and form VLDL particles, and (3) stored and stay in the hepatocyte. In addition, the hepatocyte can synthesize new lipids by de novo lipogenesis (DNL). Regarding carbohydrate metabolism, the liver can store sugars in the form of glycogen or synthesize de novo carbohydrates depending on the needs of the body. Both lipid and carbohydrate metabolism are coupled and highly regulated. When the homeostasis of metabolism is altered, lipids can accumulate pathologically in the organ causing stress and cell damage and sugars can remain circulating in the blood aggravating insulin resistance [13].

Sex hormones are steroid hormones derived from the cholesterol molecule. All sex hormones can bind to receptors in the liver cells. Based on their chemical structure, they are classified into three large groups: estrogens, androgens, and progestogens [14]. The liver is actively involved in the metabolism and interconversion of these sex hormones [15]. Estrogens, the main female sex hormone, are divided into estrone (E1), 17β-estradiol (E2) and estriol (E3), being E2 the main one. Like any steroid hormone, 95% of estrogens travel through the bloodstream binding to steroid hormone-binding globulin (SHBG). The remaining 5% circulates freely. Estrogens bind to its receptors, estrogen receptors (ERs), present in the cell nucleus. There are two types, ERα and ERβ, which are differentially expressed depending on tissue [16, 17]. In addition, there is a third type of receptor called the G-protein-coupled estrogen receptor (GPER), which is located on the plasma membrane and is also important in estrogen signalling and cell function [18]. Androgens, the main male sex hormone, belong to a group of sex hormones that

includes among others, testosterone, and dihydrotestosterone (DHT). These hormones bind to androgen receptors (RA), located in the cell nucleus [14]. Finally, progesterone is a hormone released by the corpus luteum into the ovary. It is mainly responsible for the stimulation of the mammary glands, and the preparation and maintenance of pregnancy. Progesterone can bind to two nuclear progesterone receptors (PR), PR-A and PR-B, expressed primarily in areas of the brain. Other receptors for progesterone located on the plasma membrane have also been described [19]. In women, the ovaries are responsible for producing estrogens, progestogens and androgens, which can be aromatized and converted into estrogen. In men, Leydig cells present in the testicles produce testosterone, which can aromatize and become estrogen [17].

Hepatic metabolism is highly regulated and sex hormones have been shown to contribute significantly to this regulation. Up to 72% of the genes related to liver metabolism can be expressed differentially based on sex [20]. The effects of sex hormones on liver metabolism are summarized in Figure 1. Estrogens are directly related to a protective mechanism against liver fat accumulation by promoting lipolysis and inhibiting DNL. In addition, estrogens contribute to maintaining a hepatic cholesterol balance by promoting lipoprotein synthesis, the secretion of VLDL particles, increasing HDL production and eliminating oxidized LDL. They also improve mitochondrial function, increase free fatty acid (FFA) oxidation, improve glucose tolerance as well as insulin sensitivity and decrease inflammatory processes in the liver [14, 17, 20, 21]. Androgens also have some protective role against the development of hepatic steatosis. They can promote VLDL exocytosis, DNL inhibition, homeostasis of carbohydrate metabolism and mitochondrial beta-oxidation of FFA [14, 20]. The role of progesterone in fat liver metabolism and accumulation seems more diluted. Although this hormone is metabolized in the liver, the impact on woman liver health may be partially detrimental. High levels of progesterone in women are related to the development of insulin resistance and some liver damage [22]. Not only are the mechanisms explaining

these effects unclear, but also, more investigations are needed to confirm these observations.

There are certain situations in which the levels of sex hormones decrease both in men and women. This condition is known as hypogonadism and has been related to the development of MAFLD [21]. Menopause or estrogen hypogonadism, physiological conditions in which estrogen levels abruptly decrease, have a notable impact on women. Decreased estrogen levels in women can lead to increased hepatic steatosis by decreasing VLDL secretion and increasing DNL [14, 20, 23]. Decreased estrogen levels also encompass a decrease in the body's metabolic rate which contributes to weight gain and the development of obesity [16], factors that contribute to the incidence of insulin resistance and an increased risk of developing liver fibrosis [14, 20]. Healthy women have higher levels of estrogen than androgens. However, this situation is reversed in polycystic ovary syndrome (PCOS). PCOS is one of the most common endocrinopathies associated with young women that is characterized by high levels of androgens, lack of ovulation and the presence of polycystic ovaries. Hyperandrogenism associated with PCOS doubles the risk of developing MAFLD, and increases the incidence of obesity, insulin resistance and metabolic syndrome, all related to MAFLD, compared to female controls [24-26]. In men, hypogonadism is a syndrome that is defined by decreased testosterone levels. Hypogonadism due to testosterone deficiency has also been shown to increase the risk of developing hepatic steatosis, obesity, and insulin resistance [24, 27]. A recent meta-analysis revealed that total serum testosterone was decreased in men with MAFLD *vs* men without MAFLD [28]. Decreased androgen levels in men leads to an increase in circulating triglycerides [23], a decrease in VLDL secretion, an increase in DNL, insulin resistance, and increased body weight [14, 20], all of them related to MAFLD.

MITOCHONDRIAL METABOLISM, THE LIVER, AND SEX HORMONES

Mitochondria represent approximately 20% of the hepatocyte volume [29]. They carry out critical metabolic functions related to lipids, carbohydrates, and amino acids. The conversion of pyruvate to acetyl-CoA and its oxidation occurs through the tricarboxylic acid (TCA) cycle at the mitochondrial matrix. This cycle generates ATP, NADH, FADH2 and other important metabolites such as citrate, succinate, malate, and oxaloacetate. Citrate, a precursor molecule of lipogenesis, is synthesized in the mitochondrial matrix and exported to the cytosol for the initiation of DNL. Succinate is transformed into fumarate by the electron transport chain (ETC) for cellular respiration and ATP production. Malate and oxaloacetate may initiate the process of gluconeogenesis for glucose synthesis. In turn, acetyl-CoA can be used for the synthesis of ketone bodies in a process known as ketogenesis. The beta-oxidation of fatty acids, which generates a large amount of acetyl-CoA, also takes place in the mitochondrial matrix. The acetyl-CoA produced during beta-oxidation of fatty acids can also enter the TCA cycle or alternatively initiate ketogenesis [29, 30]. On the other hand, the ETC is not perfect and during respiration free radicals can be produced. As a consequence, the mitochondria are the place in the cell where more reactive oxygen species (ROS) are produced. Under physiological conditions, antioxidant systems cope with the accumulation of ROS and decrease the number of toxic molecules. However, under pathological conditions, the accumulation of ROS can affect the integrity of DNA, both mitochondrial and nuclear. In addition, mitochondria are related to the production of S-adenosylmethionine (SAM), a methyl group donor molecule. This molecule can modulate gene expression by producing epigenetic changes in DNA. Finally, the mitochondria act as a sensor of cell viability, being related to processes of apoptosis and necrosis [30]. All these events and processes taking place at the mitochondria combined with the predominant role of mitochondria in liver metabolism places this organelle at the centre of many mechanistic hypotheses about MAFLD pathogenesis [29].

Mitochondria are also regulated by sex hormones. Estrogens stimulate the expression of mitochondrial proteins encoded in nuclear DNA as ATP synthase-related proteins or

ETC proteins. Estrogens offer protection against the degenerative effects of age by increasing antioxidant defences, increasing ATP levels, decreasing lipoperoxidation, and decreasing levels of ROS. In addition, estrogens co-regulate the processes of mitochondrial fusion and fission, enhancing mitochondrial biogenesis and inhibiting mitophagy and apoptosis. Estrogens also promote mitochondrial DNA transcription and stimulate oxygen consumption [31-33]. Androgens, on the other hand, stimulate mitochondrial biogenesis by increasing mitochondrial content (mitochondrial DNA and mitochondrial proteins), inhibiting mitophagy, maintaining the integrity of ETC and protecting these organelles from the degenerative effects of age [32, 34]. The impact of MAFLD in the interaction between sex hormones and mitochondria can be two-fold. First, MAFLD can alter sex hormone levels and consequently decrease the protective effect on the mitochondria (Figure 1). Second, the accumulation of FFA and sugars inside the hepatocyte can impact the metabolic functions in the mitochondrial matrix. The most dramatic consequences include alterations in the cell respiration pattern, with decreased ATP production and an overproduction of ROS. Beta-oxidation can also be hampered with FFA to be consumed by alternative pathways in peroxisomes (beta-oxidation) or microsomes (omega-oxidation), which in turn can increase ROS and toxic intermediates (dicarboxylic acids) production. All these alterations produce ultrastructural mitochondrial changes at different levels. Different studies report increased permeability of the outer and inner membranes, abnormal mitochondria shapes or deletion of mitochondrial DNA [35, 36]. All these facts suggest that the study of mitochondrial metabolism both in the cell and also by studying mitochondrial metabolism products in available biofluids (such as blood) may help in further investigating fatty liver disease mechanisms. The identification of early mitochondrial dysfunction, combined with other risk factors, like body mass index, sex, and age, may provide the basis for early detection and risk stratification in MAFLD management.

METABOLIC PROFILES TO CHARACTERIZE SEX DIMORPHISM IN MAFLD

Each molecule involved in the chemical reactions that take place in a living organism is called a metabolite. The set of metabolites involved in all the chemical reactions in a living organism is called the metabolome. Omics are a set of analytical sciences that are responsible for the study of a specific biological set. Currently, there are many types of omics, however, the big four omics are genomics, transcriptomics, proteomics, and metabolomics [37, 38]. There are mainly two analytical techniques that allow detecting (qualitative analysis) and quantifying (quantitative analysis) metabolites in biological samples. These are Nuclear Magnetic Resonance (NMR) and Mass Spectroscopy (MS) [39, 40]. With NMR and MS, the metabolites existing in a biological sample (serum, plasma, urine, faeces, cells, or tissue) at the time of measurement can be determined. Because the metabolome is at the end of the -omics cascade, metabolomics reflects changes that occur at the proteomic, transcriptomic, or genomic level. Consequently, interpretation of metabolite levels and metabolomic profile is highly complex. Subsequently metabolomics is more used for identifying relevant biomarkers and signatures and less used for providing mechanistic hints. Nevertheless, metabolomics can be a helpful tool in the study of diseases in which metabolic dysfunction is at the centre of the pathogenesis, such as MAFLD [41, 42].

The metabolome is sex-dependent since very early stages of life. In general, biological sex differences can be included into one of 3 groups: (1) sex dimorphisms, in which some biological trait is only expressed in men or women; (2) sex differences, in which a biological trait has a range of possibilities but is predominant in one sex with respect the other, and (3) conditions in which there is no obvious difference between sexes for some biological trait but differences can show up under some conditions like stress, disease or some pharmacological treatments [43, 44]. The extension of these three groups to metabolites as biological traits is straightforward. Metabolites, which are different between men and women only in MAFLD patients would fall within the third group and would represent ideal candidates for a stratified MAFLD risk model.

There are sex dimorphisms and sex differences in many metabolic processes of the organism, specifically of the liver, which are intrinsically related to many other differences detailed in the sections above, including sex hormones, and mitochondria function. Most metabolic pathologies, as MAFLD, affect differently men and women, with different risk factors, different disease progression and even difference prevalence under similar conditions [45]. The alteration of sex hormones, due to age or due to some pathological processes, and the development of MAFLD are strongly associated. Figure 2 shows a simplified summary of these links (Figure 2). Although most, if not all, epidemiological studies have information about the sex of the participants, only in the last decades -omics studies have analysed their data adjusting or stratifying by sex, and many suggested biomarkers are released with unisex models. Our knowledge about the influence of sex and sex hormones in metabolism strongly suggest that these analysis need to be stratified by sex (not just adjusted) for identifying sex-specific biomarkers and building sex-dependent risk models. Table 1 shows different studies which analysed the metabolome and the metabolic changes happening in established NAFLD accounting for the metabolic differences between men and women. A recent study suggests that cardiovascular risk in NAFLD patients could be stratified according to levels of Trimethylamine-N-oxide (TMAO), which is a gut microbiota-derived metabolite associated to cardiovascular risk [46]. In another study, women exhibited lower levels of TMAO than men. In addition, obese patients at higher risk of NAFLD also showed higher levels of TMAO. However, the authors did not stratify women by pre- or post-menopause and estrogen influence in these levels and associated risk [47]. In a different research, serum metabolomic profiles were measured in a young cohort at two time-points, at approximately 10 years old (T1) and 16 years old (T2). There were metabolites significantly different between MAFLD and controls at both time points and different between boys and girls. All the metabolites were related to lipid, amino acid, and carbohydrate metabolism [48]. Branched-chain amino acids (BCCA) are critical switches between health and disease [49]. A recent study analysed these metabolites in obese patients with different NAFLD severity. They concluded that there was a

correlation between BCCA levels, sex, and the degree of MAFLD severity. In women, the concentration of BCCAs in plasma was lower than in men. However, the levels of BCAA in women sharply increase with disease progression from control to NASH-fibrosis, whereas in men a parallel decrease was observed. Interestingly, there was a strong association between circulating BCAA and liver fibrosis only present in women [50]. Although liver disease can be diagnosed by non invasive measurements, the gold standard still remains the liver biopsy. Biopsy-confirmed MAFLD classified by degree of severity in simple steatosis, early NASH, and advanced NASH was used to obtain metabolomic profiles of liver disease progression in another metabolomic study. Although the sample size was low and the power of the study was limited, the authors identify some differences between different degrees of severity which did not correlate with differences between sexes [51]. Finally, a larger study also analysed serum metabolomics in 627 patients to classify them based on the degree of severity of the disease stratified by sex. The authors identified a set of common metabolic traits associated to liver disease severity regardless of sex and a different set of metabolites that changed specifically in men or in women. In the fibrosis stage, men presented 5 sex-specific metabolic differences whereas women show 17 sex-specific metabolic differences [52].

CONCLUSION

Metabolism is highly influenced by the effects of sex hormones. Specifically, hepatic metabolism and mitochondrial metabolism change depending on sex hormone levels. Recent studies show that individuals with altered estrogen or androgens levels have higher risk of developing fatty liver disease or progressing to more severe stages than those with normal levels. MAFLD is associated to metabolic alterations in liver and mitochondria. Among these, the identification of early mitochondrial dysfunction, combined with other risk factors, like body mass index, sex, and age, may provide the basis for early detection and risk stratification in MAFLD management. Because the metabolome is at the end of the -omics cascade, metabolomics reflects changes that

occur at the proteomic, transcriptomic, or genomic level. Metabolomics is a helpful tool in the study of diseases in which metabolic dysfunction is at the centre of the pathogenesis, such as MAFLD. In this review, we briefly explained the role of different metabolic compartments in the development of MAFLD and critically review current state-of-the-art evidence from metabolomic studies on sex dependency of fatty liver disease. Not only are more studies needed to clarify the role of metabolites and their use as biomarkers, but also, it is vital that future research includes the sex variable.

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