Associations between sex hormones and metabolic-associated fatty liver disease in a middle-aged and elderly community

Weijie Cao *, Yiting Xu *, Yun Shen, Yufei Wang, Xiaojing Ma and Yuqian Bao

Abstract. Metabolic-associated fatty liver disease (MAFLD) was proposed by an international expert consensus to replace non-alcoholic fatty liver disease (NAFLD) in 2020. Previous studies have shown that sex hormones are strongly linked to NAFLD development. This study aims to explore whether sex hormones are associated with MAFLD and liver fat content (LFC) in a middle-aged and elderly community. The study included 732 subjects aged 50–80 years enrolled from communities. MAFLD was diagnosed using the 2020 International Expert Consensus. LFC was calculated using parameters from abdominal ultrasound images. Serum estradiol (E2), total testosterone (TT), sex hormone-binding globulin (SHBG), FSH, and LH were measured by chemiluminescent microparticle immunoassay. MAFLD was diagnosed in 107/304 (35.2%) men and 154/428 (35.2%) women. After adjustments for confounding factors, logistic regression analysis showed that SHBG was negatively correlated with MAFLD in men (OR, 0.95 [0.93–0.97], p < 0.001). In women, SHBG and FSH were negatively correlated with MAFLD (OR, 0.95 [0.94–0.97], p < 0.001; OR, 0.97 [0.96–0.98], p < 0.001). Multivariate linear regression analysis showed that SHBG was a negative factor for LFC in both men (standardized β = –0.188, p < 0.001) and women (standardized β = –0.184, p < 0.001). FSH was a negative factor for LFC in women (standardized β = –0.082, p = 0.046). SHBG was negatively correlated with MAFLD in middle-aged and elderly men and women. Moreover, FSH was negatively correlated, and bioactive testosterone was positively correlated with MAFLD in women. These findings suggest a relationship between sex hormones and MAFLD.

Key words: Metabolic associated fatty liver disease, Sex hormone-binding globulin, Liver fat content

ECTOPIC FAT DISTRIBUTION is a stronger determinant of metabolic health than increased fat mass [1]. Increased liver fat content (LFC) is closely related to insulin resistance, obesity, diabetes, and other metabolic dysfunctions [2, 3]. Non-alcoholic fatty liver disease (NAFLD) is an important manifestation of ectopic fat deposition, and metabolic dysfunction usually occurs before diagnosis [4]. To better understand fatty liver disease, a panel of experts from 22 countries took the initiative to propose the replacement of NAFLD with metabolic-associated fatty liver disease (MAFLD) in 2020 [5]. This definition is based on the presence of metabolic dysfunction, including, but not limited to, hypertension, type 2 diabetes, and dyslipidemia, rather than the absence of other factors such as alcohol abuse and other chronic liver diseases.

Sex hormones play a vital role in metabolic diseases [6, 7]. Sex hormone-binding globulin (SHBG), a plasma...
protein that specifically binds sex hormones, has been traditionally considered the primary transporter of active sex hormones. Previous studies showed that a lower serum SHBG level indicates a higher risk of metabolic syndrome, type 2 diabetes, and cardiovascular disease [8]. Sex hormones are also recognized to have crucial influences on body fat metabolism and distribution. Studies have shown that androgen in men could suppress lipoprotein lipase activity, reduce fat accumulation, and promote steatolysis [9]. SHBG has also been proven to correlate negatively with body fat, especially abdominal fat accumulation [10].

Previous studies revealed that sex hormones are strongly linked to the occurrence and development of NAFLD [11, 12] and are also closely related to metabolic diseases and body fat distribution. However, as MAFLD definitions have only been recently developed, the association between sex hormones and MAFLD remains unclear. Therefore, this study aimed to explore whether serum sex hormone levels are associated with MAFLD and LFC in community populations.

Materials and Methods

Study population

Volunteer subject participants were recruited from Shanghai communities from October 2015 to July 2016. A total of 732 subjects with complete data, consisting of men and women aged 50–80 years, were included. The following were excluded: premenopausal women; those with a known history of cardiovascular or cerebrovascular disease, malignant tumors or intracranial space-occupying lesions, severe liver or kidney dysfunction, thyroid dysfunction, a history of hyperthyroidism, or hypothyroidism, severe anemia, or treatment with steroids or thyroxine. The Ethics Committee of the Sixth People’s Hospital Affiliated to Shanghai Jiao tong University approved this study, and all subjects provided written informed consent before participation. All subjects received standardized questionnaires, including past and present illness and treatment, physical examinations, and biochemical determinations. “Menopausal woman” was defined as a woman with 12 consecutive months of amenorrhea without other medical reasons [13].

Anthropometric and biochemical measurements

Height, weight, waist circumference, and blood pressure were measured using standardized methods [14]. Body mass index (BMI) = weight (kg)/height² (m²). Venous blood samples were collected after a 10-h overnight fast to detect fasting blood glucose (FPG), fasting insulin (FINS), total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL–C), low-density lipoprotein cholesterol (LDL–C), albumin (Alb), C–reactive protein (CRP), alanine aminotransferase (ALT), alanine aminotransferase (AST), alkaline phosphatase (ALP), glutamyl transferase (GGT), and creatinine (CR). In addition, blood samples were collected after 75-g oral glucose tolerance tests to determine the 2-hour plasma glucose (2hPG) and glycated hemoglobin (HbA1c). All laboratory indicators were measured using standard methods. The homeostasis model assessment of insulin resistance (HOMA–IR) was computed as follows: HOMA–IR = FINS (mU/L) × FPG (mmol/L)/22.5 [15].

Serum estradiol (E2), total testosterone (TT), SHBG, FSH, and LH were detected on Abbott Architect i2000SR analyzer by chemiluminescence microparticle immunoassay (kits from Abbott GmbH & Co. KG, Wiesbaden, Germany). Free testosterone (FT) was calculated based on Vermeulen’s formula [16]: FT = ([T] – N × [FT])/Kt ([SHBG] – [T] + N[FT]). Bioactive testosterone (BT) was also calculated = N × [FT]; where Kt = 1 × 10⁴ L/mol, N = Ka × Ca + 1, Ka = 3.6 × 10⁴ L/mol, and Ca is Alb level. The sensitivity of E2, TT, SHBG, FSH, and LH measurements were <2.5 pg/mL, <0.1 ng/mL, <0.3 mmol/L, <0.1 IU/L, and <0.1 IU/L, respectively.

Measurement of LFC and diagnostic criteria for MAFLD

All subjects underwent abdominal ultrasonographic examination by a trained sonographer using a Voluson 730 Expert B-mode ultrasonogram device (5.0-MHz transducer, GE Healthcare, Waukesha, WI, USA) and were blinded to the study design and the subject’s clinical details. Fatty liver was diagnosed through ultrasonography with the presence of at least two of the following four findings [17]: (1) diffusely increased echogenicity of the liver relative to the kidney or spleen, (2) ultrasound beam attenuation with decreased vessel signal, (3) poor visualization of intrahepatic structures, and (4) slightly enlarged liver with a blunt margin. As an index of fat accumulation in the liver, LFC was determined through an ultrasound-based liver fat quantification. This method of LFC measurement is based on the liver-kidney echo ratio and liver attenuation coefficient of the liver ultrasound image [18].

According to the International Expert Consensus statement on MAFLD in 2020, MAFLD in this study was diagnosed as follows [5]: liver fat accumulation based on liver ultrasound and the presence of any one of the following three conditions: overweight/obesity (BMI ≥23 kg/m²), presence of type 2 diabetes mellitus, or evidence of metabolic dysregulation. In addition, metabolic dysfunction was defined as the presence of at least two metabolic risk factors: (1) waist circumference ≥90 cm in men and ≥80 cm in women, (2) blood pressure ≥130/85
mmHg or antihypertensive therapy, (3) TG ≥1.7 mmol/L or lipid-lowering therapy, (4) HDL-C <1 mmol/L for men; 1.3 mmol/L for women, or drug therapy, (5) prediabetes: FPG 5.6–6.9 mmol/L, 2hPG 7.8–11.0 mmol/L, or HbA1c 5.7%–6.4%, (6) HOMA-IR ≥2.5, and (7) CRP >2 mg/L.

Statistical analyses
Data were analyzed using SPSS version 20.0 (SPSS Inc, Chicago, IL, USA). All variables were tested for normality. Normally distributed variables are presented as mean ± standard deviation, and non–normally distributed variables are presented as median and interquartile range. Student’s t-test was used to compare two groups with normal distribution, whereas the Wilcoxon rank-sum test was used for skewed distribution. The χ² test was applied to categorical variables. Binary logistic regression was used to analyze the relationship between sex hormones and MAFLD. Factors affecting LFC in men and women were investigated by multivariate linear regression. All p values were two-tailed tests, and p < 0.05 was considered statistically significant.

Results
Clinical characteristics of study subjects
The average age of the study participants was 62.1 ± 5.1 years. MAFLD was diagnosed in 107/304 (35.2%) men and 154/428 (35.2%) women. Subjects with MAFLD presented with lower HDL-C levels and significantly higher values of BMI, waist circumference, systolic and diastolic blood pressures, FPG, 2hPG, FINS, HbA1c, HOMA-IR, ALT, GGT, TG, CRP, LDL-C, and LFC than those without MAFLD (all p < 0.05). Additionally, no significant difference was found in age, Alb, Cr, ALP, and TC between the MAFLD and non-MAFLD groups (all p > 0.05) (Table 1). The proportion of subjects on antihypertensive and hypoglycemic treatments in the MAFLD group was significantly higher than that in the non-MAFLD group (41.4% vs. 21.4%, p < 0.001; 13.4% vs. 8.7%, p < 0.05). The proportion on lipid-lowering treatment showed no difference between the MAFLD and non-MAFLD groups (5.4% vs. 7.6%, p > 0.05).

Associations between sex hormones and MAFLD
Subjects were stratified according to sex. In men, the levels of TT, FT, BT, and SHBG were significantly lower in the MAFLD group than those in the non-MAFLD group (all p < 0.05); however, there was no significant difference in E2, FSH, and LH levels between the two groups (all p > 0.05). Women with MAFLD had lower levels of SHBG, FSH, and LH, and higher levels of E2, BT, and FT than those without MAFLD (all p < 0.05), while the TT level did not differ significantly between the two groups (p > 0.05) (Table 2).

We performed correlation analysis to detect which factors are most strongly influenced by SHBG, and found that ALT was influenced by SHBG in both men and women (r = –0.124, p = 0.031; r = –0.141, p = 0.003, respectively), while AST had no significant association with SHBG in both men and women (both p > 0.05).
men, except for SBP, other metabolic indices were related to SHBG (all \( p < 0.05 \)). Among these, BMI, WC, and HDL-C were most influenced by SHBG (\( r = -0.361, p < 0.001; r = -0.380, p < 0.001; r = 0.376, p < 0.001 \), respectively). In women, all metabolic indices were related to SHBG (all \( p < 0.05 \)). Similarly, BMI, WC, and HDL-C were most influenced by SHBG (\( r = -0.361, p < 0.001; r = -0.380, p < 0.001; r = 0.376, p < 0.001 \), respectively) (Fig. 1).

Binary logistic regression models were constructed to analyze the associations between MAFLD and sex hormones. After adjusting for age, ALT, AST, ALP, GGT, and Cr, SHBG was negatively correlated with MAFLD in men (odds ratio [OR], 0.95 [0.93–0.97], \( p < 0.001 \)); however, other sex hormones were not correlated with MAFLD (all \( p > 0.05 \)). In women, after further adjustments for the duration of menopause, SHBG and FSH were negatively correlated with MAFLD (OR, 0.95 [0.94–0.97], \( p < 0.001 \); OR, 0.97 [0.96–0.98], \( p < 0.001 \), respectively), and BT was positively correlated with MAFLD (OR, 1.87 [1.03–3.39], \( p = 0.039 \)), while E2, LH, and FT were not correlated with MAFLD (all \( p > 0.05 \)).

Analysis of multiple factors affecting LFC

Multivariate linear regression models were constructed to analyze the degree of influence of different factors on LFC. Age, waist circumference, BMI, Cr, ALT, AST, ALP, GGT, HOMA-IR, TC, TG, HDL-C, LDL-C, CRP, TT, SHBG, FT, and BT were independent variables, while LFC was the dependent variable. After adjusting for BMI, ALT, TG, and HOMA-IR, we found that SHBG was a negative factor for LFC in men (standardized \( \beta = -0.188, p < 0.001 \)). In women, upon the addition of the duration of menopause, FSH, and LH as independent variables, SHBG and FSH were negatively associated with LFC except for BMI, ALT, TG, and HOMA-IR. (standardized \( \beta = -0.082, p = 0.046 \), respectively) (Table 3).

Discussion

Our study showed that SHBG was negatively correlated with MAFLD in men, while in women, both SHBG and FSH were negatively correlated with MAFLD, but BT was positively correlated.

NAFLD is considered the hepatic manifestation of metabolic syndrome or insulin resistance [19], tightly linked to the present epidemic of obesity and diabetes [20-22]. In addition, NAFLD could coexist with other liver diseases, such as viral hepatitis, autoimmune disease, and alcoholic liver disease, with a synergistic effect on the progression of the disease. Therefore, in 2020, hepatologists from 22 countries proposed using the term MAFLD instead of NAFLD [5]. A recent study using the

### Table 2  Sex hormones of the study subjects

| Variables | Non-MAFLD | MAFLD |
|-----------|-----------|-------|
| Men       |           |       |
| \( E_2 \) (pmol/L) | 102.76 (84.41–121.11) | 102.76 (84.41–124.78) |
| TT (nmol/L)   | 18.84 (15.86–23.70)    | 14.82 (11.38–18.67)** |
| SHBG (nmol/L) | 45.0 (35.8–58.3)       | 34.1 (25.2–41.6)**    |
| FSH (IU/L)   | 8.65 (6.36–11.99)      | 8.10 (6.28–11.81)     |
| LH (IU/L)    | 6.38 (4.89–8.42)       | 6.35 (4.83–7.74)      |
| FT (nmol/L)  | 0.304 (0.266–0.363)    | 0.277 (0.234–0.338)*  |
| BT (nmol/L)  | 8.23 (7.17–9.77)       | 7.64 (6.38–9.26)*     |

Women

| Variables | Non-MAFLD | MAFLD |
|-----------|-----------|-------|
| \( E_2 \) (pmol/L) | 40.37 (18.35–47.71) | 44.04 (18.35–51.38)** |
| TT (nmol/L)   | 0.90 (0.73–1.08)    | 0.94 (0.76–1.18)     |
| SHBG (nmol/L) | 59.1 (44.1–75.9)    | 36.8 (29.4–46.7)**   |
| FSH (IU/L)   | 72.23 (55.78–86.33) | 54.14 (44.28–64.97)**|
| LH (IU/L)    | 29.83 (24.34–37.93) | 25.45 (19.16–30.84)**|
| FT (nmol/L)  | 0.011 (0.008–0.014) | 0.015 (0.012–0.019)**|
| BT (nmol/L)  | 0.28 (0.20–0.38)    | 0.40 (0.32–0.50)**   |
| Menopause duration (years) | 12 ± 6 | 11 ± 6 |

Non-MAFLD versus MAFLD, * \( p < 0.05 \), ** \( p < 0.01 \)

Abbreviation: \( E_2 \), estradiol; TT, total testosterone; SHBG, sex hormone-binding globulin; FSH, follicle-stimulating hormone; LH, luteinizing hormone; FT, free testosterone; BT, bioavailable testosterone.
Fig. 1 Association between SHBG and ALT, AST, BMI, WC, SBP, DBP, TG, HDL-C, FPG, 2hPG, HbA1c, and HOMA-IR in men (A) and women (B)
data of the third National Health and Nutrition Examination Surveys 1988–1994, including more than 13,000 people, showed that MAFLD has a more practical and accurate definition than NAFLD, and it is more practical for identifying patients with fatty liver with a high risk of disease progression [23]. Furthermore, a study that enrolled 765 Japanese patients showed that the definition of MAFLD better identified liver stiffness evaluated by ultrasound. This provides the possibility of using ultrasound to assess liver fat in large-scale population-based studies and progressed understanding of association between MAFLD and sex hormones. In addition, an experimental study showed that the overexpression of SHBG could significantly reduce liver TG content and lipogenesis via reducing PPAR-γ by activating the ERK–1/2 MAPK pathway and reducing the key enzymes of lipogenesis [26]. Furthermore, a study using biopsied human liver tissue illustrated that lipid accumulation in the liver might significantly reduce SHBG gene expressions through the mediation of the nuclear transcription factor HNF4-α [27].

We demonstrated that FSH was negatively correlated with MAFLD and LFC in women. The mechanism underlying the association between FSH and LFC remains poorly understood. A previous study confirmed that FSH was negatively correlated with NAFLD in women over 55 years [28]. During the perimenopausal period, significant changes in women’s hormone levels are frequently observed. With a decrease in E2, FSH increases rapidly. However, more E2 is secreted in obese women due to E2 secretions shifting from an ovarian to a compensatory source in fat during the perimenopausal period [29]. Therefore, FSH decreases due to an E2 increase, explaining the relationship between FSH and LFC. In addition, several studies indicated that compared with TT, FT and BT were more accurate in predicting NAFLD prevalence among women [30, 31]. We also found that BT was positively correlated with MAFLD after adjusting for confounding factors and TT in women, consistent with the findings of previous studies. Compared with TT, BT can more accurately reflect the level of androgen in the body [16]. In women, elevated androgen levels are closely related to central obesity and insulin resistance [32-34]. However, in the multiple linear regression of LFC, BT and FT had no association with LFC, and its mechanism requires further exploration.

Table 3 Multivariate regression analysis on LFC in men and women

| LFC | Multivariate model | | |
|-----|-------------------|---|---|
|     | standardized β    | t  | p  |
| Men | SHBG -0.188       | -3.614 | <0.001 |
|     | BMI 0.245         | 4.439 | <0.001 |
|     | ALT 0.156         | 3.127 | 0.002 |
|     | TG 0.179          | 3.514 | 0.001 |
|     | HOMA-IR 0.115     | 2.225 | 0.027 |

For men, multivariate model included age, BMI, WC, HOMA-IR, TC, TG, HDL-C, LDL-C, CRP, ALT, AST, GGT, ALP, Cr, TT, SHBG, FT, BT. For women, multivariate model included age, BMI, WC, HOMA-IR, TC, TG, HDL-C, LDL-C, CRP, ALT, AST, GGT, ALP, Cr, SHBG, FSH, LH, FT, BT.

Abbreviation: LFC, liver fat content; TT, total testosterone; SHBG, sex hormone-binding globulin; FSH, follicle-stimulating hormone; LH, luteinizing hormone; FT, free testosterone; BT, bioavailable testosterone; BMI, body mass index; WC, waist circumference; HOMA-IR, homeostasis model assessment-insulin resistance index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ-glutamyl transpeptidase; ALP, alkaline phosphatase; Cr, creatinine; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; CRP, C-reactive protein;
There are some limitations to this study. First, the cross-sectional study design could not determine a causal relationship between changes in sex hormones and MAFLD. Therefore, prospective studies with a larger sample size are needed to validate these findings. Second, this study only explored the relationship between sex hormones and MAFLD in middle-aged and elderly people; hence, the results may not be generalizable to all age groups.

In conclusion, SHBG was negatively correlated with MAFLD in middle-aged and elderly men and women. Moreover, FSH was negatively correlated with MAFLD, and BT was positively correlated with MAFLD in women. These findings suggest a relationship between sex hormones and MAFLD.

Acknowledgments

We would like to thank all of the involved physicians and staff from the communities and clinical laboratory and ultrasound department of Shanghai Sixth People’s Hospital for dedicating their time and skills which have facilitated the completion of this study. Furthermore, we would like to thank all the participants for their dedication in data collection and laboratory measurements.

Disclosures

Funding/Support

This work was funded by the Shanghai Municipal Science and Technology Commission Medical Guide Project (19411964300).

Conflict of interest

None of the authors have any potential conflicts of interest associated with this research.

Authors’ Contributions

XJM and YQB conceived the work. WJC and YQB performed the statistical analyses. YTX, YS, JLT, YFX, and XJM contributed to data collection. WJC and YTX contributed to drafting the article. XJM and YQB revised the manuscript. All authors gave final approval for the published version.

References

1. Stefan N, Schick F, Häring HU (2017) Causes, characteristics, and consequences of metabolically unhealthy normal weight in humans. Cell Metab 26: 292–300.
2. Blüher M (2013) Adipose tissue dysfunction contributes to obesity related metabolic diseases. Best Pract Res Clin Endocrinol Metab 27: 163–177.
3. Klöting N, Fasshauer M, Dietrich A, Kovacs P, Schön MR, et al. (2010) Insulin-sensitive obesity. Am J Physiol Endocrinol Metab 299: 506–515.
4. Lallukka S, Yki-Järvinen H (2016) Non-alcoholic fatty liver disease and risk of type 2 diabetes. Best Pract Res Clin Endocrinol Metab 30: 385–395.
5. Eslam M, Newsome PN, Sarin SK, Anstee QM, Targher G, et al. (2020) A new definition for metabolic dysfunction-associated fatty liver disease: an international expert consensus statement. J Hepatol 73: 202–209.
6. Mayes JS, Watson GH (2004) Direct effects of sex steroid hormones on adipose tissues and obesity. Obes Rev 5: 197–216.
7. Hammond GL, Bocchinfuso WP (1996) Sex hormone-binding globulin: gene organization and structure/function analyses. Horm Res 45: 197–201.
8. Pugeat M, Nader H, Hogeveen K, Raverot G, Déchaud H, et al. (2010) Sex hormone-binding globulin gene expression in the liver: drugs and the metabolic syndrome. Mol Cell Endocrinol 316: 53–59.
9. Janssen I, Powell LH, Kazlauskaitė R, Dugan SA (2010) Testosterone and visceral fat in middle-aged women: the Study of Women’s Health Across the Nation (SWAN) fat patterning study. Obesity (Silver Spring) 18: 604–610.
10. Couillard C, Gagnon J, Bergeron J, Leon AS, Rao DC, et al. (2000) Contribution of body fatness and adipose tissue distribution to the age variation in plasma steroid hormone concentrations in men: the HERITAGE Family Study. J Clin Endocrinol Metab 85: 1026–1031.
11. Sarkar M, VanWagner LB, Terry JG, Carr JJ, Rinella M, et al. (2019) Sex hormone-binding globulin levels in young men are associated with nonalcoholic fatty liver disease in midlife. Am J Gastroenterol 114: 758–763.
12. Yim JY, Kim J, Kim D, Ahmed A (2018) Serum testosterone and non-alcoholic fatty liver disease in men and women in the US. Liver Int 38: 2051–2059.
13. Burger HG (1994) The menopause: when it is all over or is it? Aust N Z J Obstet Gynaecol 34: 293–295.
14. Jian C, Xu Y, Ma X, Shen Y, Wang Y, et al. (2020) Neck circumference is an effective supplement for nonalcoholic fatty liver disease screening in a community-based population. Int J Endocrinol 16: 7982107.
15. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, et al. (1985) Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28: 412–419.
16. Vermeulen A, Verdonck L, Kaufman JM (1999) A critical evaluation of simple methods for the estimation of free testosterone in serum. J Clin Endocrinol Metab 84: 3666–
17. Agbim U, Asrani SK (2019) Non-invasive assessment of liver fibrosis and prognosis: an update on serum and elastography markers. *Expert Rev Gastroenterol Hepatol* 13: 361–374.

18. Xia MF, Yan HM, He WY, Li XM, Li CL, *et al.* (2012) Standardized ultrasound hepatic/renal ratio and hepatic attenuation rate to quantify liver fat content: an improvement method. *Obesity (Silver Spring)* 20: 444–452.

19. Jacobs M, van Greevenbroek MM, van der Kallen CJ, Ferreira I, Feskens EJ, *et al.* (2011) The association between the metabolic syndrome and alanine aminotransferase is mediated by insulin resistance via related metabolic intermediates (the Cohort on Diabetes and Atherosclerosis Maastricht [CODAM] study). *Metabolism* 60: 969–975.

20. Schindhelm RK, Heine RJ, Diamant M (2007) Prevalence of non-alcoholic fatty liver disease and its association with cardiovascular disease among type 2 diabetic patients. *Diabetes Care* 30: e94.

21. Marchesini G, Bugianesi E, Forlani G, Cerrelli F, Lenz M, *et al.* (2003) Non-alcoholic fatty liver, steatohepatitis, and the metabolic syndrome. *Hepatology* 37: 917–923.

22. Hua X, Sun Y, Zhong Y, Feng W, Huang H, *et al.* (2014) Low serum sex hormone-binding globulin is associated with non-alcoholic fatty liver disease in type 2 diabetic patients. *Clin Endocrinol (Oxf)* 80: 877–883.

23. Lin S, Huang J, Wang M, Kumar R, Liu Y, *et al.* (2020) Comparison of MAFLD and NAFLD diagnostic criteria in real world. *Liver Int* 40: 2082–2089.

24. Yamamura S, Eslam M, Kawaguchi T, Tsutsumi T, Nakano D, *et al.* (2020) MAFLD identifies patients with significant hepatic fibrosis better than NAFLD. *Liver Int* 40: 3018–3030.

25. Shin JY, Kim SK, Lee MY, Kim HS, Ye BI, *et al.* (2011) Serum sex hormone-binding globulin levels are independently associated with non-alcoholic fatty liver disease in people with type 2 diabetes. *Diabetes Res Clin Pract* 94: 156–162.

26. Wang X, Xie J, Pang J, Zhang H, Chen X, *et al.* (2020) Serum SHBG is associated with the development and regression of nonalcoholic fatty liver disease: a prospective study. *J Clin Endocrinol Metab* 105: dgz244.

27. Saez-Lopez C, Barbosa-Desongles A, Hernandez C, Dyer RA, Innis SM, *et al.* (2017) Sex hormone-binding globulin reduction in metabolic disorders may play a role in NAFLD development. *Endocrinology* 158: 545–559.

28. Luo J, Chen Q, Shen T, Wang X, Fang W, *et al.* (2018) Association of sex hormone-binding globulin with non-alcoholic fatty liver disease in Chinese adults. *Nutr Metab (Lond)* 15: 79.

29. Wang N, Li Q, Han B, Chen Y, Zhu C, *et al.* (2016) Follicle-stimulating hormone is associated with non-alcoholic fatty liver disease in Chinese women over 55 years old. *J Gastroenterol Hepatol* 31: 1196–1202.

30. Wang N, Kuang L, Han B, Li Q, Chen Y, *et al.* (2016) Follicle-stimulating hormone associates with prediabetes and diabetes in postmenopausal women. *Acta Diabetol* 53: 227–236.

31. Wang X, Li Q, Pang J, Lin J, Liu Y, *et al.* (2021) Associations between serum total, free and bioavailable testosterone and non-alcoholic fatty liver disease in community-dwelling middle-aged and elderly women. *Diabetes Metab* 47: 101199.

32. Klisic A, Kavaric N, Jovanovic M, Soldatovic I, Gligorovic-Barhanovic N, *et al.* (2017) Bioavailable testosterone is independently associated with Fatty Liver Index in postmenopausal women. *Arch Med Sci* 13: 1188–1196.

33. Mohler ER 3rd, Ellenberg SS, Lewis CE, Wenger NK, Budoff MI, *et al.* (2018) The effect of testosterone on cardiovascular biomarkers in the testosterone trials. *J Clin Endocrinol Metab* 103: 681–688.

34. Bianchi VE, Locatelli V (2018) Testosterone a key factor in gender related metabolic syndrome. *Obes Rev* 19: 557–575.