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High estradiol/testosterone ratio increased the risk of metabolic dysfunction-associated steatotic liver disease in men with type 2 diabetes mellitus

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Keywords
E2/T ratio, Metabolic dysfunction-associated steatotic liver disease, Type 2 diabetes mellitus

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ABSTRACT

Background: The association between estradiol/testosterone (E2/T) ratio and metabolic dysfunction-associated steatotic liver disease (MASLD) remains controversial. Moreover, few studies have explored their relationship in men with type 2 diabetes mellitus. We aimed to investigate the association of the E2/T ratio with MASLD in type 2 diabetes mellitus male patients.

Methods: This real-world observational study was performed in 1441 male type 2 diabetes mellitus patients. MASLD was determined by abdominal ultrasonography. The clinical characteristics and prevalence of MASLD were compared across the E2/T ratio quartiles. The association of the E2/T ratio and quartiles with MASLD was also evaluated using binary logistic regression.

Results: After adjusting for age and diabetes duration (DD), MASLD prevalence significantly increased across the E2/T ratio quartiles (37.7%, 42.6%, 53.1%, and 69.3%, respectively, $P < 0.001$ for trend). Fully adjusted logistic regression showed that both the E2/T ratio (OR: 2.201, 95% CI: 1.380–3.511, $P = 0.001$) and quartiles ($P = 0.001$) were positively associated with MASLD in males with type 2 diabetes mellitus. Furthermore, C-reactive protein (CRP) levels were significantly higher in patients with MASLD compared with those without ($P < 0.001$), and obviously increased across the E2/T ratio quartiles after controlling for age and DD ($P = 0.016$ for trend).

Conclusions: The E2/T ratio was independently and positively associated with the increased risk of MASLD in male type 2 diabetes mellitus patients, which may be attributed to the close association between the E2/T ratio and inflammation. The E2/T ratio may serve as a simple and practical indicator to assess the risk of MASLD in male type 2 diabetes mellitus patients.

INTRODUCTION

Both estradiol (E2) and testosterone (T) are key steroid hormones commonly associated with reproductive function. However, they also play important roles in regulating overall physiological processes, such as bone growth, cognitive function, cardiovascular health, and metabolic health[1,2]. Therefore, abnormal sex hormone levels may contribute to various diseases including metabolic disorders. For example, a nationally representative study conducted in young and middle-aged adult men in the United States found that low levels of total E2 were strongly associated with an elevated risk of cardiovascular disease (CVD) mortality after more than 27 years of follow-up[3]. Furthermore, a case–control study reported that male patients with metabolic syndrome (MetS) had significantly lower serum

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T levels and notably higher serum E2 levels compared to healthy participants[4]. These findings highlight the critical roles of E2 and T in overall health, especially metabolic health.

Considering the interaction between E2 and T, the E2/T ratio was first described by Chopra and colleagues in the context of hepatic cirrhosis in 1973[5]. They found that the most consistent abnormality in males with hepatic cirrhosis was a super-normal E2 to T ratio, which suggested a potential role of the altered balance between circulating estrogen and androgen in the pathogenesis of gynecomastia in these patients[5]. Compared to individual measurements of E2 and T, the E2/T ratio provides a more comprehensive approach to exploring potential synergistic effects of sex hormones, and has been widely used to investigate the relationship between sex hormones and various clinical conditions[6–8]. More importantly, the E2/T ratio has been found to be linked to metabolism-related diseases such as metabolic syndrome and osteoporosis since 2015[9–11]. For example, a longitudinal study performed in European men observed that a lower E2/T ratio might be associated with a decreased risk of developing metabolic syndrome[10].

As one of the metabolic disorders, metabolic dysfunction-associated steatotic liver disease (MASLD), formerly known as non-alcoholic fatty liver disease (NAFLD), is the leading cause of chronic liver disease and affects up to one-third of the global population[12]. Interestingly, premenopausal women exhibit a significantly lower incidence of MASLD compared with postmenopausal women and men of the same age[13,14], suggesting that serum E2 may be a protective factor against the development of liver steatosis. Additionally, some studies also reported the close association between serum T levels and the risk of MASLD[15–17]. For example, a meta-analysis including 13,721 men and 5,840 women revealed that high serum T levels were associated with a decreased risk of MASLD in men, but an increased risk in women[17]. The role of androgens in MASLD differs between men and women, but the close relationship between sex hormones and MASLD is undeniable.

However, few studies have examined the association between the E2/T ratio and MASLD, and with inconsistent findings[18–20]. For example, a cross-sectional study demonstrated that a high E2/T ratio was independently associated with an increased risk of hepatic steatosis in male patients with chronic hepatitis B[18]. Similarly, another study reported significantly higher E2/T ratios in males with compensated or decompensated non-alcoholic liver cirrhosis compared with healthy males[19]. In contrast, a prospective study of 79 boys, including 15 overweight and 64 obese, revealed no significant difference in the E2/T ratio between those with and without MASLD[20]. Therefore, the association between the E2/T ratio and MASLD in males remains unclear and controversial.

It is well-established that type 2 diabetes mellitus patients have a higher risk of developing MASLD than the general population. Several studies have explored the association between sex hormones and MASLD in type 2 diabetes mellitus[21,22]. For example, a cross-sectional study by Zhang *et al.*[21] found that low serum total T was significantly associated with MASLD prevalence in men but not in women with type 2 diabetes mellitus. Similarly, another study reported that male type 2 diabetes mellitus patients with NAFLD had significantly lower serum T levels and higher E2 levels compared to those without NAFLD[22]. However, to our knowledge, no studies have investigated the correlation between the E2/T ratio and MASLD in male patients with type 2 diabetes mellitus. Therefore, the aim of this real-world study is to investigate the relationship between the E2/T ratio and MASLD in Chinese men with type 2 diabetes mellitus.

MATERIALS AND METHODS

Study design and population

The data of this cross-sectional, real-world study were obtained from our registry study (MVCDP study: A real-world study of macrovascular complications in diabetic populations; clinical trial registration number: ChiCTR1800015893). This study was in accordance with the Declaration of Helsinki and was approved by the ethics committee of Shanghai Sixth People's Hospital Affiliated to Shanghai Jiao Tong University School of Medicine (Approval number: 2018-KY-018(K)). The male patients with type 2 diabetes mellitus were consecutively recruited from our department between January 2003 and December 2012. The inclusion criteria for this study were as follows[23]: diagnosis of type 2 diabetes mellitus according to the WHO criteria; age ≥17 years old; availability of complete clinical data; and documented abdominal ultrasound measurements. In addition, the exclusion criteria were as follows: incomplete clinical data; without data of serum E2 and T; without abdominal ultrasonography results; liver injury caused by drugs, viral hepatitis, and other reasons except for drinking. Finally, 1,441 males with type 2 diabetes mellitus were included in this investigation. Based on the serum E2/T ratio, the subjects were divided into quartiles in the present study.

All patients were asked about their diabetes duration (DD), history of hypertension, smoking habits, alcohol consumption, and medication use including lipid-lowering drugs (LLDs), insulin or insulin analogues (IIAs), insulin sensitizers, and metformin. In addition, the definitions of hypertension, smoking, drinking, and obesity have been fully described in our recent studies[24,25]. For the diagnosis of MASLD, we applied the criteria proposed by the multisociety Delphi consensus in 2023[26]. Briefly, hepatic steatosis was identified by ultrasonography in the presence of at least one cardiometabolic risk factor including: (a) BMI ≥25 kg/m^2 or WC >94 cm in men and >80 cm in women, (b) HbA1c ≥5.7%, (c) SBP ≥130 mmHg or DBP ≥85 mmHg, (d) plasma TG ≥150 mg/dL, or (e) plasma HDL-c <40 mg/dL in men and <50 mg/dL in women. Given that our study population consisted of diabetic patients, all individuals with hepatic steatosis detected by abdominal ultrasound were classified as MASLD. Written informed consent was obtained from all subjects.

Medical examinations and laboratory tests

Each patient received a comprehensive physical examination and laboratory tests. Physical examinations included height, weight, hip circumference, waist circumference (WC), and blood pressure. Body mass index (BMI) and waist-to-hip ratio (WHR) were calculated in accordance with our previous studies[27,28].

Venous blood samples were collected after an overnight fast and 2 h after breakfast to determine the levels of fasting C-peptide (FCP), 2-h postprandial C-peptide (2h PCP), fasting plasma glucose (FPG), 2-h postprandial plasma glucose (2h PPG), glycosylated hemoglobin A1C (HbA1C), fasting insulin (Fins), 2-h insulin (2h ins), total triglycerides (TG), alanine aminotransferase (ALT), r-glutamyltransferase (r-GT), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), serum uric acid (SUA), creatinine (Cr), and C-reactive protein (CRP). The calculation of estimated glomerular filtration rate (eGFR) and 24-h urinary albumin excretion (UAE) was well described in our previous studies[28,29]. Furthermore, serum E2 and T levels were detected using a chemiluminescence assay (Immulite 2000; Siemens, Erlangen, Germany)[30], and the E2/T ratio was also calculated. Consistent with our recent study, the type 2 diabetes mellitus patients with CRP >10 mg/L were excluded to eliminate the effect of acute inflammation[31].

Abdominal ultrasonography

Each subject underwent abdominal ultrasonography to diagnose hepatic steatosis, following the methodology described in our previous reports[24,32]. Briefly, experienced ultrasonographers, blinded to the participants' laboratory data, conducted hepatic ultrasound scans using a 3.5-MHz probe (SSc-370, manufactured by Aloka Co, Tokyo, Japan). Participants were positioned supine with their right arm overhead. Ultrasound images were obtained by placing the ultrasound probe in the intercostal space.

Statistical analyses

The data were statistically analyzed by SPSS version 15.0 software. Data with normal distribution were represented as mean ± standard deviations, whereas data with non-normal distribution were represented as median with interquartile range (25–75%). The differences among groups were determined by one-way analysis of variance with the least significant difference or the Kruskal–Wallis H test. The categorical variables were described as absolute numbers and percentages. Chi-square tests were applied to analyze categorical variables. Binary logistic regression analyses were used to assess the differences among the groups after controlling for confounding factors. Finally, receiver operating characteristics (ROC) analysis was performed to assess the ability of the E2/T ratio, E2, and T to predict MASLD.

In addition, six logistic regression models were constructed to evaluate the association of E2, T, and the E2/T ratio and quartiles with the presence of MASLD: Model 1 was unadjusted; Model 2 was adjusted for age and DD; Model 3 was further adjusted for smoking status, alcohol intake, obesity, and hypertension; Model 4 included additional adjustments for the use of LLDs, IIAs, metformin, and insulin sensitizers; Model 5 incorporated further adjustments for SBP, DBP, WC, WHR, and BMI; Model 6 included additional adjustments for TC, HDL-C, LDL-C, TG, eGFR, SUA, UAE, FPG, 2h PPG, CRP, HbA1C, FCP, 2-h CP, Fins, and 2h ins. The difference was statistically significant at $P < 0.05$.

RESULTS

Clinical characteristics of study subjects

Table 1 summarizes the clinical characteristics of the subjects. The current study included 1,441 male patients with type 2 diabetes mellitus, categorized into quartiles based on the E2/T ratio with cutoffs of <3.66, 3.66–6.84, 6.85–10.55, and >10.55. After adjusting for age, the prevalence of obesity, metformin use, WC, WHR, BMI, and serum E2 levels was significantly increased across the quartiles, while the levels of HDL-C and LDL-C were obviously decreased (all $P < 0.05$). In addition, there was a significant difference in DD, the use of LLDs and insulin sensitizers, and serum T levels among the four groups, even after adjusting for age (all $P < 0.05$). In contrast, there was no significant difference in hypertension, smoking, drinking, use of IIAs, SBP, DBP, TC, FPG, 2h PPG, HbA1C, FCP, 2h PCP, Fins, 2h ins, ALT, TG, r-GT, Cr, SUA, UAE, and eGFR among the four groups after adjusting for age.

Comparisons of MASLD prevalence in different groups

Figure 1 compares the prevalence of MASLD across serum E2/T ratio quartiles and stratified by age and DD classification. There was a significantly increased trend in the MASLD prevalence across the E2/T ratio quartiles after adjusting for age and DD (37.7%, 42.6%, 53.1%, and 69.3% for each quartile, $P < 0.001$ for trend) (Figure 1a). The prevalence of MASLD was significantly higher in the patients aged <65 years compared to those aged ≥65 years (56.4% vs 34.1%, $P < 0.001$) (Figure 1b). Additionally, the prevalence of MASLD was also obviously higher in the patients with DD <120 months than in those with DD ≥120 months (59.0% vs 36.7%, $P < 0.001$) (Figure 1d). Moreover, the prevalence of MASLD showed a significant decreasing trend with advancing age and prolonged DD (both $P < 0.001$ for trend) (Figure 1c,e).

Comparisons of serum E2/T ratio in different groups

Figure 2 compares the serum E2/T ratio in different groups. The E2/T ratio in the subjects with MASLD was significantly higher than in those without MASLD after controlling for age and DD ($P < 0.001$) (Figure 2a). The E2/T ratio was markedly higher in the patients aged ≤39 years compared with the other four groups, though no significant differences were observed among the latter ($P = 0.001$ for trend) (Figure 2d). However, the E2/T ratio showed no significant difference between the

Table 1 | Characteristics of the subjects according to E2/T ratio

Variables	Q1 (n = 361)	Q2 (n = 359)	Q3 (n = 360)	Q4 (n = 361)	P value	P value*
E2/T	<3.66	3.66–6.84	6.85–10.55	>10.56	–	–
Age (years)	56 ± 12	57 ± 12	56 ± 13	55 ± 15	0.213	–
DD (months)†	60 (8.5–120)	96 (24–144)	78 (24–144)	72 (12–132)	0.006	0.003
Hypertension (n, %)	178 (49.3%)	175 (48.7%)	177 (49.2%)	181 (50.1%)	0.986	0.785
Smoking (n, %)	196 (54.3%)	201 (56.0%)	210 (58.3%)	194 (53.7%)	0.600	0.596
Drinking (n, %)	116 (32.1%)	122 (34.0%)	101 (28.1%)	113 (31.3%)	0.380	0.354
Obesity (n, %)	125 (34.6%)	161 (44.8%)	195 (54.2%)	232 (64.3%)	<0.001	<0.001
LLDs (n, %)	118 (32.7%)	169 (47.1%)	169 (46.9%)	188 (52.1%)	<0.001	<0.001
IIAs (n, %)	253 (70.1%)	243 (67.7%)	235 (65.3%)	247 (68.4%)	0.577	0.569
Metformin (n, %)	199 (55.1%)	229 (63.8%)	232 (64.4%)	238 (65.9%)	0.012	0.014
Insulin sensitizers (n, %)	18 (5.0%)	60 (16.7%)	35 (9.7%)	38 (10.5%)	<0.001	<0.001
SBP (mmHg)	130 ± 18	131 ± 17	132 ± 16	131 ± 16	0.333	0.196
DBP (mmHg)	80 ± 10	80 ± 10	82 ± 10	81 ± 10	0.175	0.236
WC (cm)	88.3 ± 9.9	90.3 ± 10.2	92.5 ± 9.1	96.0 ± 10.4	<0.001	<0.001
WHR	0.92 ± 0.06	0.93 ± 0.06	0.94 ± 0.05	0.95 ± 0.06	<0.001	<0.001
BMI (kg/m^2)	24.10 ± 3.19	24.67 ± 3.33	25.37 ± 2.73	26.41 ± 3.39	<0.001	<0.001
TC (mmol/L)	4.67 ± 1.02	4.65 ± 1.19	4.66 ± 1.04	4.62 ± 1.30	0.953	0.874
FPG (mmol/L)†	7.93 (6.33–10.09)	7.59 (6.15–9.88)	7.76 (6.42–9.62)	7.86 (6.40–10.49)	0.259	0.67
2h PPG (mmol/L)†	13.93 (10.64–16.84)	12.68 (10.28–15.91)	13.02 (10.48–16.11)	13.09 (10.17–16.37)	0.076	0.481
HbA1C (%)	9.17 ± 2.30	8.97 ± 2.40	8.93 ± 2.15	9.18 ± 2.30	0.339	0.365
FCP (ng/mL)†	1.74 (1.12–2.70)	1.75 (1.08–2.46)	1.84 (1.19–2.59)	2.06 (1.40–2.91)	<0.001	0.643
2h C-P (ng/mL)	4.03 (2.15–6.74)	3.67 (2.25–6.33)	4.25 (2.53–6.05)	4.30 (2.64–7.15)	0.038	0.858
Fins (uU/mL)†	9.32 (5.92–16.58)	9.21 (5.60–15.69)	10.86 (7.04–16.84)	11.80 (7.77–19.25)	<0.001	0.212
2h ins (uU/mL)†	41.00 (25.29–59.83)	43.89 (27.31–69.53)	44.64 (30.25–71.17)	49.26 (32.16–75.25)	0.001	0.321
ALT (U/L)†	21 (15–30.5)	19 (14–29)	21 (16–31)	24 (16–35)	0.002	0.239
TG (mmol/L)†	1.40 (0.92–2.10)	1.20 (0.88–1.91)	1.46 (0.99–2.19)	1.64 (1.07–2.62)	<0.001	0.647
r-GT (U/L)†	26 (19–44)	25 (17.25–38.75)	27 (19–41)	28 (20–46)	0.004	0.469
HDL-C (mmol/L)	1.08 ± 0.28	1.08 ± 0.26	1.02 ± 0.24	0.94 ± 0.23	<0.001	<0.001
LDL-C (mmol/L)	2.94 ± 0.86	2.93 ± 0.87	2.87 ± 0.90	2.68 ± 0.82	<0.001	<0.001
Cr (μmol/L)†	73 (65–83)	71 (64–82)	73 (65–84)	73 (63–84)	0.202	0.13
SUA (μmol/L)†	318 (273–381)	317 (267–379)	330 (276–391)	347 (295–407)	<0.001	0.88
UAE (mg/24 h)†	11.16 (6.89–25.97)	10.88 (6.59–28.00)	11.83 (6.91–30.54)	13.24 (7.08–45.01)	0.102	0.953
eGFR (mL/min/1.73 m^2)†	107 (92–125)	111 (95–129)	107 (90–126)	111 (91–132)	0.311	0.131
T (μg/L)†	286.30 (11.08–415.11)	7.05 (4.58–17.06)	12.73 (7.55–16.00)	9.23 (6.85–11.74)	<0.001	0.003
E2 (ng/L)†	29.00 (17.00–43.44)	37.00 (24.00–93.29)	107.89 (63.72–137.58)	133.65 (106.27–166.50)	<0.001	<0.001

Values are presented as mean ± SD, or medians with interquartile range or percentages. P value: the P-values were not adjusted for age for the trend. *P value: the P-values were adjusted for age for the trend. †Non-normal distribution of continuous variables.

patients aged <65 and ≥65 years, and between those with DD <120 and ≥120 months (both $P > 0.05$) (Figure 2b,c). Additionally, no significant difference was observed in the E2/T ratio among the different DD groups after adjusting for age ($P > 0.05$) (Figure 2e).

Comparisons of serum ALT and r-GT levels

Figure 3 illustrates the comparisons of serum ALT and r-GT levels between the patients with and without MASLD, as well as across serum E2/T ratio quartile groups. After adjusting for age and DD, type 2 diabetes mellitus male patients with MASLD exhibited higher serum ALT and r-GT levels than those without MASLD (both $P < 0.001$) (Figure 3a,c). However, serum ALT and r-GT levels were not significantly different among the E2/T ratio quartiles (both $P > 0.05$ for trend) (Figure 3b,d).

Comparisons of HOMA-IR, HOMA2-IR, and CRP

Figure 4 shows the comparisons of HOMA-IR, HOMA2-IR, and CRP in different groups. After correcting for age and DD, HOMA-IR, HOMA2-IR, and CRP values were significantly higher in the patients with MASLD than in those without MASLD (all $P < 0.001$) (Figure 4a,c,e). Furthermore, serum CRP levels in the first quartile were not the lowest among the serum E2/T ratio quartile groups, but an overall upward trend in CRP levels was observed ($P = 0.016$ for trend) (Figure 4f).

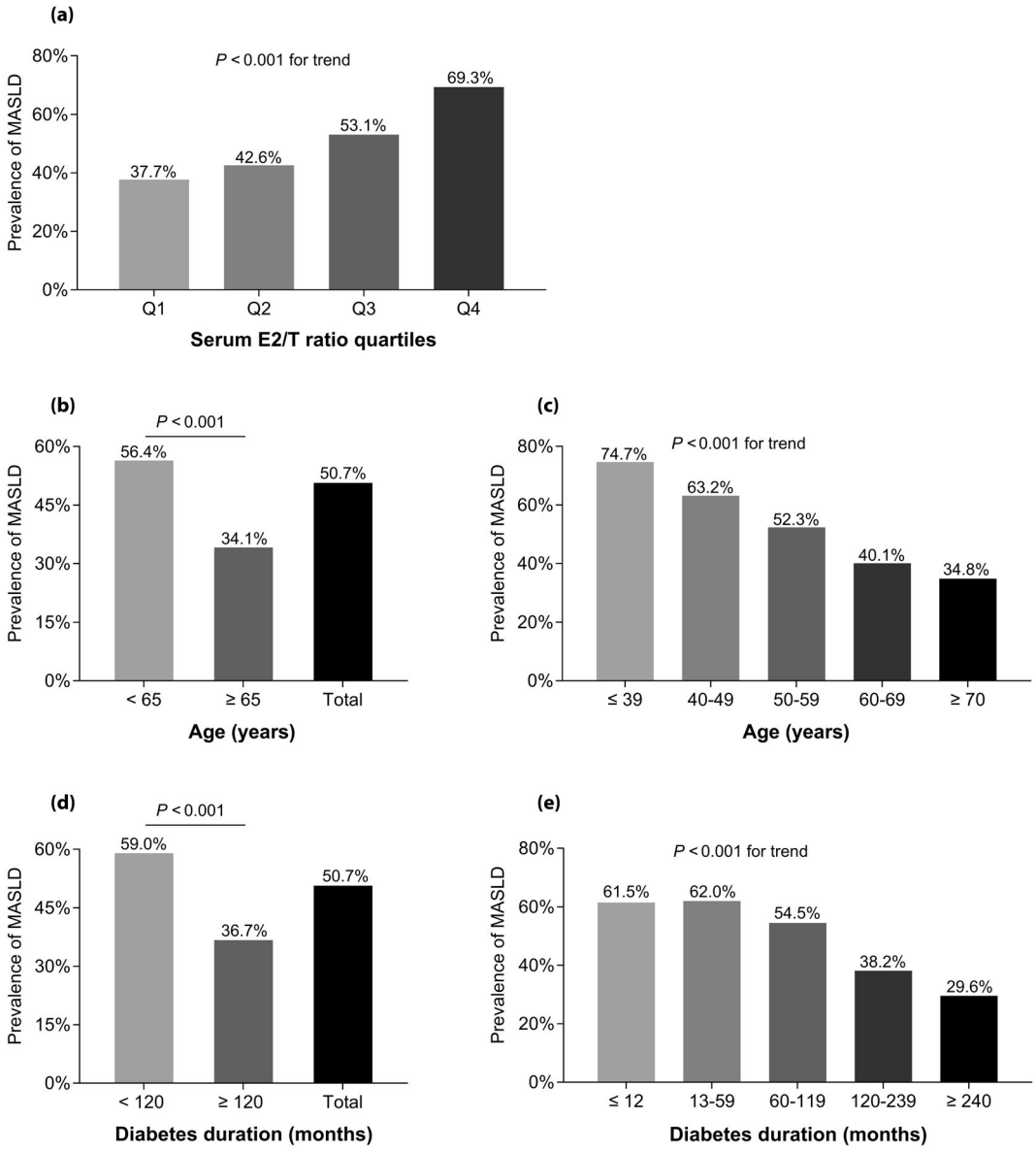

Figure 1 | Comparisons of MASLD prevalence in different groups. (a) Comparisons of MASLD prevalence across the serum E2/T ratio quartiles after controlling for age and diabetes duration (DD) (37.7%, 42.6%, 53.1%, and 69.3% in the first, second, third, and fourth quartiles, respectively, $P < 0.001$ for trend). (b) Comparison of MASLD prevalence between the subjects aged <65 and ≥65 years (56.4% vs. 34.1%, $P < 0.001$). (c) Comparison of MASLD prevalence stratified by age after adjusting for DD ($P < 0.001$ for trend). (d) Comparison of MASLD prevalence between the subjects with DD <120 and ≥120 months (59.0% vs 36.7%, $P < 0.001$). (e) Comparisons of MASLD prevalence stratified by DD after adjusting for age ($P < 0.001$ for trend).

However, there was no significant difference in the HOMA-IR and HOMA2-IR values across the E2/T ratio quartiles (both $P > 0.05$ for trend) (Figure 4b,d).

Association of E2/T ratio with MASLD

Table 2 displays the association of E2/T ratio with MASLD in male patients with type 2 diabetes mellitus. In Model 1, without adjusting for confounding factors, a higher E2/T ratio was associated with an increased risk of MASLD (OR: 1.871, 95% CI: 1.573–2.226, $P < 0.001$). After adjustment for age and DD in Model 2, the E2/T ratio remained independently and positively correlated with the presence of MASLD (OR: 2.023, 95% CI: 1.686–2.429, $P < 0.001$). Further controlling for smoking status, alcohol intake, obesity, and hypertension (Model 3, OR: 1.721, 95% CI: 1.407–2.105, $P < 0.001$); the use of LLDs, IIAs, metformin, and insulin sensitizers (Model 4, OR: 1.628, 95%

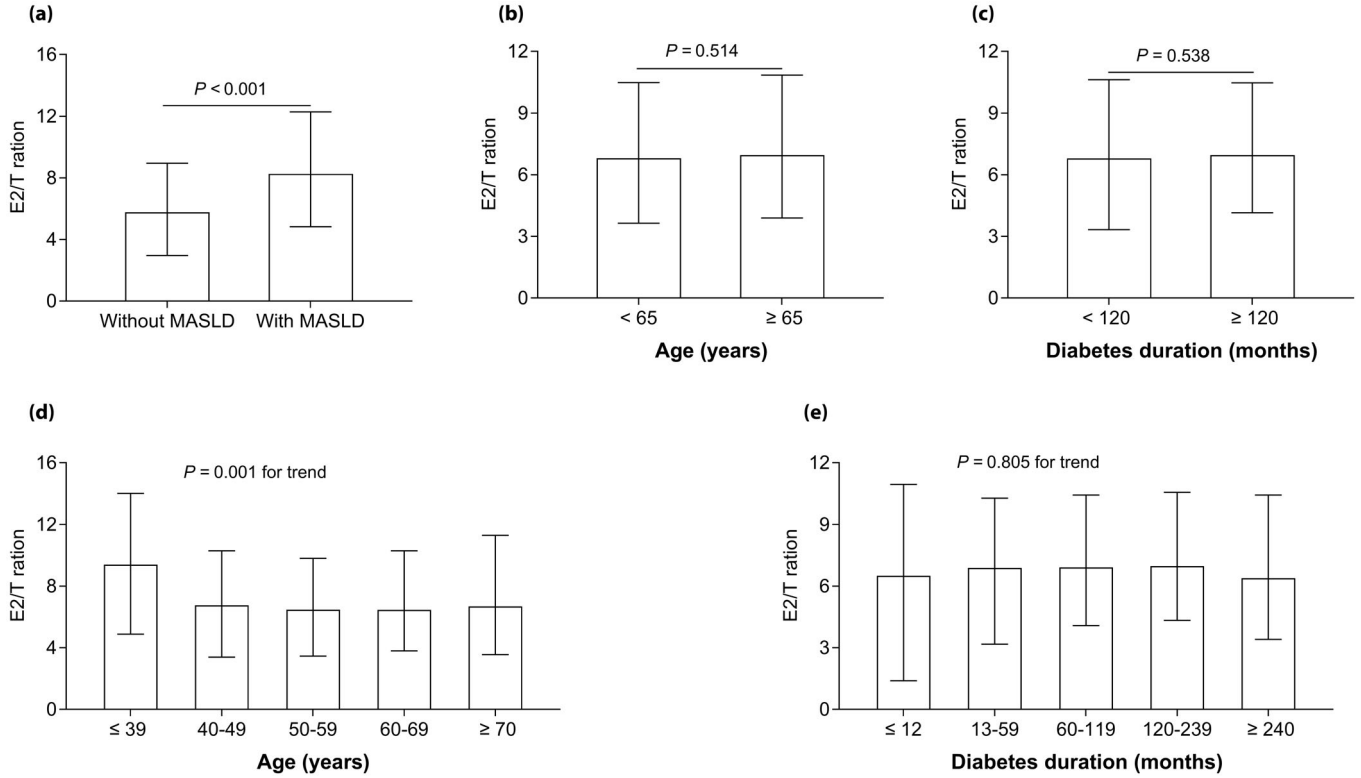

Figure 2 | Comparisons of serum E2/T ratio in different groups. (a) Comparison of serum E2/T ratio between the subjects with and without MASLD ($P < 0.001$). (b) Comparison of serum E2/T ratio between the subjects aged <65 years and ≥65 years ($P = 0.514$). (c) Comparison of serum E2/T ratio between the subjects with DD <120 months and ≥120 months ($P = 0.538$). (d) Comparisons of the E2/T ratio stratified by age after adjusting for DD ($P = 0.001$ for trend). (e) Comparisons of the E2/T ratio stratified by DD after adjusting for age ($P = 0.805$ for trend).

CI: 1.327–1.997, $P < 0.001$); SBP, DBP, WC, WHR, and BMI (Model 5, OR: 2.406, 95% CI: 1.607–3.604, $P < 0.001$); and TC, HDL-C, LDL-C, TG, eGFR, SUA, UAE, FPG, 2h PPG, CRP, HbA1C, FCP, 2-h CP, Fins, and 2h ins (Model 6), a significantly positive association between E2/T ratio and the presence of MASLD still existed (Model 6, OR: 2.201, 95% CI: 1.380–3.511, $P = 0.001$).

Association of E2/T ratio quartiles with MASLD

Table 3 presents the binary logistic regression analysis for the association between E2/T ratio quartiles and MASLD in male patients with type 2 diabetes mellitus. In the unadjusted analysis, individuals in higher E2/T ratio quartiles had a significantly increased risk of MASLD (Q1, 1.00 (reference); Q2, OR: 1.229, 95% CI: 0.912–1.656; Q3, OR: 1.870, 95% CI: 1.389–2.516; Q4, OR: 3.726, 95% CI: 2.727–5.074, $P < 0.001$ for trend) (Model 1). This positive association persisted after further adjustments for potential confounders, including age and DD (Q1, 1.00 (reference); Q2, OR: 1.391, 95% CI: 1.018–1.901; Q3, OR: 2.072, 95% CI: 1.516–2.820; Q4, OR: 4.211, 95% CI: 3.041–5.832, $P < 0.001$ for trend) (Model 2); smoking status, alcohol intake, obesity, and hypertension (Q1, 1.00 (reference); Q2, OR: 1.164, 95% CI: 0.822–1.648; Q3, OR: 1.728, 95% CI: 1.222–2.445; Q4, OR: 3.275, 95% CI: 2.269–4.726, $P < 0.001$ for trend) (Model 3); use of LLDs, IIAs, metformin, and insulin sensitizers (Q1, 1.00 (reference); Q2, OR: 1.081, 95% CI: 0.759–1.540; Q3, OR: 1.634, 95% CI: 1.150–2.323; Q4, OR: 3.023, 95% CI: 2.082–4.388, $P < 0.001$ for trend) (Model 4); SBP, DBP, WC, WHR, and BMI (Q1, 1.00 (reference); Q2, OR: 1.359, 95% CI: 0.807–2.289; Q3, OR: 1.858, 95% CI: 1.109–3.115; Q4, OR: 3.033, 95% CI: 1.776–5.180, $P < 0.001$ for trend) (Model 5); as well as TC, HDL-C, LDL-C, TG, eGFR, SUA, UAE, FPG, 2h PPG, CRP, HbA1C, FCP, 2-h CP, Fins, and 2h ins (Q1, 1.00 (reference); Q2, OR: 1.770, 95% CI: 0.952–3.292; Q3, OR: 2.036, 95% CI: 1.107–3.745; Q4, OR: 3.456, 95% CI: 1.838–6.499, $P < 0.001$ for trend) (Model 6). Across all adjusted models (Models 2–6), the association between E2/T ratio quartiles and the presence of MASLD remained significantly positive ($P \leq 0.001$).

Associations of serum E2 and T levels with MASLD

Table 4 demonstrates the associations of serum E2 and T levels with MASLD in the subjects. In the unadjusted model (Model 1), higher serum E2 levels were significantly associated with the

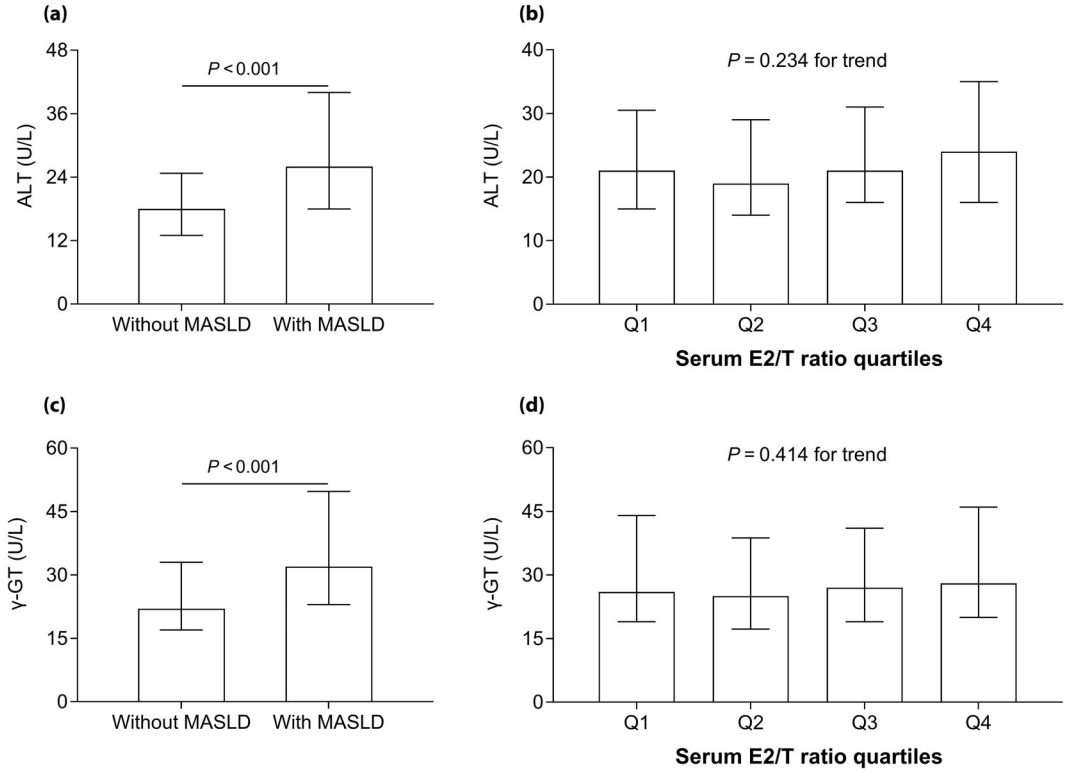

Figure 3 | Comparisons of serum ALT and r-GT levels. (a) Comparison of serum ALT levels between the subjects with and without MASLD after controlling for age and DD ($P < 0.001$). (b) Comparisons of serum ALT levels across the serum E2/T ratio quartiles after controlling for age and DD ($P = 0.234$ for trend). (c) Comparison of r-GT between the subjects with and without MASLD after controlling for age and DD ($P < 0.001$). (d) Comparison of r-GT across the serum E2/T ratio quartiles after controlling for age and DD ($P = 0.414$ for trend).

presence of MASLD (OR: 1.236, 95% CI: 1.112–1.373, $P < 0.001$). This association persisted after adjusting for age and DD (OR: 1.243, 95% CI: 1.114–1.388, $P < 0.001$) (Model 2), and remained robust after further adjustments for additional confounders in Model 3, 4, 5, and 6 (OR: 1.198, 95% CI: 1.057–1.358, $P = 0.005$; OR: 1.191, 95% CI: 1.049–1.352, $P = 0.007$; OR: 1.134, 95% CI: 0.979–1.314, $P = 0.093$; and OR: 1.212, 95% CI: 1.027–1.430, $P = 0.023$, respectively).

In Model 1, without adjusting for confounding factors, serum T levels were negatively correlated with MASLD (OR: 0.768, 95% CI: 0.691–0.854, $P < 0.001$). This inverse association remained significant after further adjustments for potential confounders, including age and DD (Model 2, OR: 0.734, 95% CI: 0.657–0.821, $P < 0.001$); smoking status, alcohol intake, obesity, and hypertension (Model 3, OR: 0.823, 95% CI: 0.727–0.932, $P = 0.002$); and the use of LLDs, IIAs, metformin, and insulin sensitizers (Model 4, OR: 0.851, 95% CI: 0.750–0.965, $P = 0.012$).

However, this association was no longer significant after additional adjustment for SBP, DBP, WC, WHR, and BMI (Model 5, OR: 0.898, 95% CI: 0.746–1.082, $P = 0.259$), as well as for metabolic and biochemical parameters including TC, HDL-C, LDL-C, TG, eGFR, SUA, UAE, FPG, 2h PPG, CRP, HbA1C, FCP, 2h CP, fasting insulin, and 2h insulin (Model 6, OR: 1.094, 95% CI: 0.869–1.378, $P = 0.023$).

The predictive performance of, E2, T, and the E2/T ratio for MASLD

Figure S1 presents the ROC curves using MASLD occurrence in type 2 diabetes mellitus patients as the state variable. The area under the curve (AUC) values of E2, T, and the E2/T ratio were 0.561, 0.423, and 0.636, respectively, indicating that the E2/T ratio exhibited better predictive performance for MASLD risk compared with E2 or T alone (Figure S1).

DISCUSSION

This large real-world study observed that the E2/T ratio was independently and positively correlated with the presence of MASLD in male patients with type 2 diabetes mellitus, even after adjusting for multiple confounding factors. Moreover, the close association between the E2/T ratio and MASLD may be attributed to chronic low-grade inflammation and obesity mediated by the E2/T ratio, which was indicated by the increase in CRP levels and WC across the E2/T ratio quartiles.

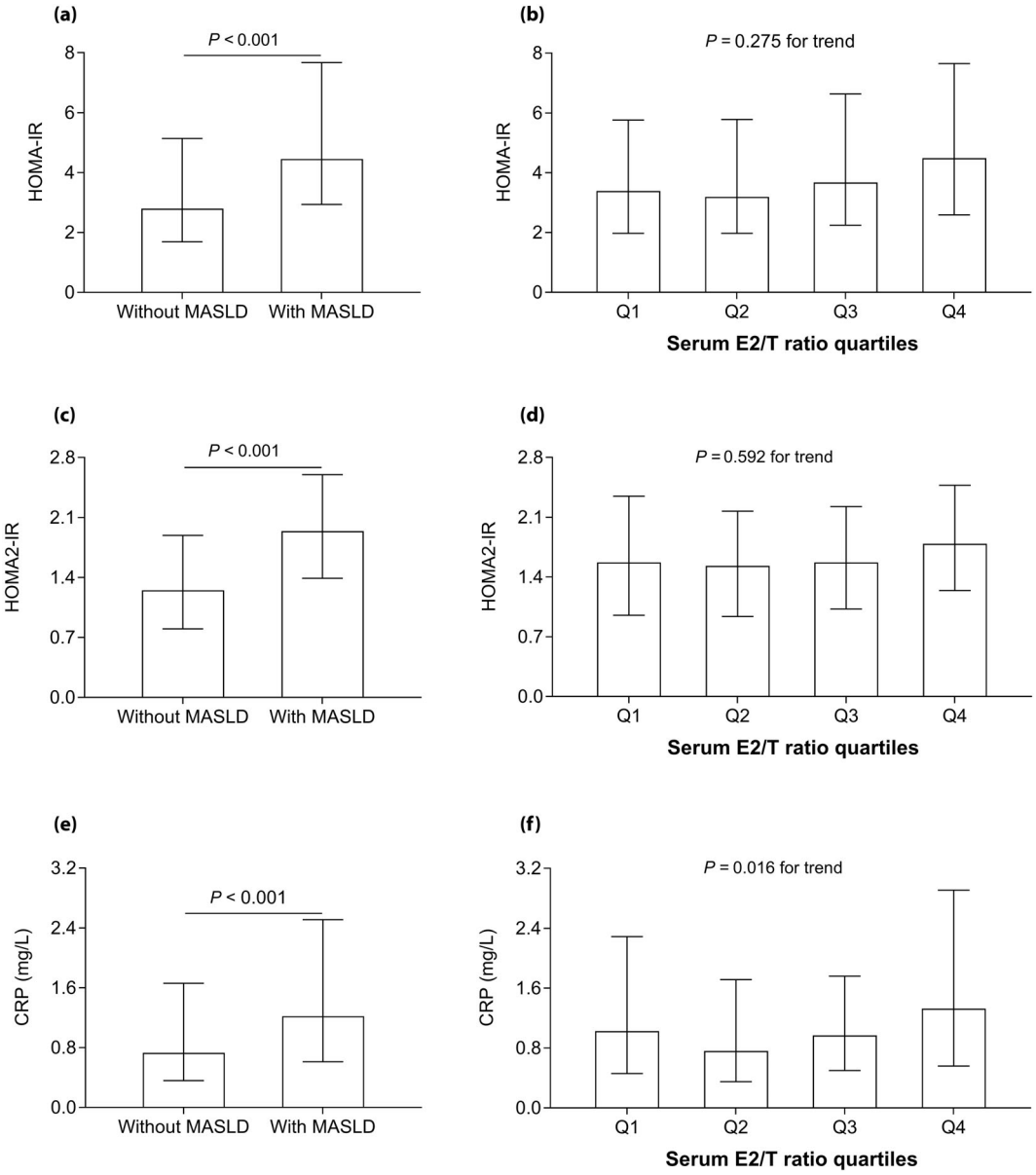

Figure 4 | Comparisons of HOMA-IR, HOMA2-IR, and CRP. (a) Comparison of HOMA-IR between the subjects with and without MASLD after controlling for age and DD ($P < 0.001$). (b) Comparisons of HOMA-IR across the serum E2/T ratio quartiles after controlling for age and DD ($P = 0.275$ for trend). (c) Comparison of HOMA2-IR between the subjects with and without MASLD after controlling for age and DD ($P < 0.001$). (d) Comparisons of HOMA2-IR across the serum E2/T ratio quartiles after controlling for age and DD ($P = 0.592$ for trend). (e) Comparison of CRP levels between the subjects with and without MASLD after controlling for age and DD ($P < 0.001$). (f) Comparisons of CRP levels across the serum E2/T ratio quartiles after controlling for age and DD ($P = 0.016$ for trend).

Furthermore, ROC analyses demonstrated that the E2/T ratio was a more effective predictor of MASLD in male patients with type 2 diabetes mellitus than either E2 or T alone. In contrast, no association between the E2/T ratio and MASLD was observed in female patients with type 2 diabetes mellitus, although these results are not presented here.

Interestingly, consistent with previous studies[33,34], we observed a negative correlation between MASLD prevalence and age in male type 2 diabetes mellitus patients. The higher prevalence of MASLD in younger patients may be explained by several interrelated factors. Middle-aged individuals are more prone to obesity, sedentary lifestyles, and socioeconomic

stressors, all of which increase MASLD risk, whereas retired elderly people often have more time to exercise and pay more attention to diet and health management[33]. In addition, older patients have higher overall mortality, partly attributable to fatty liver disease[35].

Table 2 | Associations of E2/T ratio with MASLD

	B statistic	OR	95% CI	*P* value
Model 1	0.627	1.871	1.573–2.226	<0.001
Model 2	0.705	2.023	1.686–2.429	<0.001
Model 3	0.543	1.721	1.407–2.105	<0.001
Model 4	0.488	1.628	1.327–1.997	<0.001
Model 5	0.878	2.406	1.607–3.604	<0.001
Model 6	0.789	2.201	1.380–3.511	0.001

Model 1: Unadjusted. Model 2: Age and DD. Model 3: Model 2 + smoking status, alcohol intake, obesity, and hypertension. Model 4: Model 3 + use of LLDs, IIAs, metformin, and insulin sensitizers. Model 5: Model 4 + SBP, DBP, WC, WHR, and BMI. Model 6: Model5 + TC, HDL-C, LDL-C, TG, eGFR, SUA, UAE, FPG, 2h PPG, CRP, HbA1C, FCP, 2-h CP, Fins, and 2h ins.

Our study also demonstrated an inverse association between MASLD prevalence and DD, in line with the findings of Popovic *et al.*[36] They reported that fasting insulinemia and insulin resistance assessed by the HOMA-IR index, both recognized risk factors for MASLD, were strongly negatively correlated with DD[36]. Furthermore, prolonged use of glucose-lowering medications in patients with longer DD, some of which may confer protective effects against MASLD, could also contribute to the reduced prevalence[37].

Androgens can be aromatized into estrogens, and their balance is crucial for maintaining normal physiological functions. Therefore, an imbalance between estrogen and androgen may contribute to the development of various diseases such as obesity and cardiovascular diseases, highlighting the potential utility of the E2/T ratio as an indicator of sex hormone interactions. Several studies have investigated the correlation between the E2/T ratio and clinical diseases including MASLD[18,20,38]. For example, Chen *et al.*[18] found that males with hepatic steatosis had a mean E2/T ratio of 17.75, which was significantly higher than the ratio of 12.49 in those without hepatic steatosis. Another study also observed that patients with liver cirrhosis had a significantly higher E2/T ratio than healthy controls[38]. However, a prospective study that included 79 boys found no

Table 3 | Association of E2/T ratio quartiles with MASLD

	ORs (95% CI)				*P* values for trend
	Q1	Q2	Q3	Q4	
Model 1	1	1.229 (0.912–1.656)	1.870 (1.389–2.516)	3.726 (2.727–5.074)	<0.001
Model 2	1	1.391 (1.018–1.901)	2.072 (1.516–2.830)	4.211 (3.041–5.832)	<0.001
Model 3	1	1.164 (0.822–1.648)	1.728 (1.222–2.445)	3.275 (2.269–4.726)	<0.001
Model 4	1	1.081 (0.759–1.540)	1.634 (1.150–2.323)	3.023 (2.082–4.388)	<0.001
Model 5	1	1.359 (0.807–2.289)	1.858 (1.109–3.115)	3.033 (1.776–5.180)	<0.001
Model 6	1	1.770 (0.952–3.292)	2.036 (1.107–3.745)	3.456 (1.838–6.499)	0.001

Model 1: Unadjusted. Model 2: Age and DD. Model 3: Model 2 + smoking status, alcohol intake, obesity, and hypertension. Model 4: Model 3 + use of LLDs, IIAs, metformin, and insulin sensitizers. Model 5: Model 4 + SBP, DBP, WC, WHR, and BMI. Model 6: Model5 + TC, HDL-C, LDL-C, TG, eGFR, SUA, UAE, FPG, 2h PPG, CRP, HbA1C, FCP, 2-h CP, Fins and 2h ins.

Table 4 | Associations of serum testosterone and estradiol levels with MASLD

	T levels				E2 levels			
	B statistic	OR	95% CI	*P* value	*B* statistic	OR	95% CI	*P* value
Model 1	−0.264	0.768	0.691–0.854	<0.001	0.212	1.236	1.112–1.373	<0.001
Model 2	−0.309	0.734	0.657–0.821	<0.001	0.218	1.243	1.114–1.388	<0.001
Model 3	−0.194	0.823	0.727–0.932	0.002	0.181	1.198	1.057–1.358	0.005
Model 4	−0.162	0.851	0.750–0.965	0.012	0.175	1.191	1.049–1.352	0.007
Model 5	−0.107	0.898	0.746–1.082	0.259	0.126	1.134	0.979–1.314	0.093
Model 6	0.090	1.094	0.869–1.378	0.445	0.192	1.212	1.027–1.430	0.023

Model 1: Unadjusted. Model 2: Age and DD. Model 3: Model 2 + smoking status, alcohol intake, obesity, and hypertension. Model 4: Model 3 + use of LLDs, IIAs, metformin, and insulin sensitizers. Model 5: Model 4 + SBP, DBP, WC, WHR, and BMI. Model 6: Model5 + TC, HDL-C, LDL-C, TG, eGFR, SUA, UAE, FPG, 2h PPG, CRP, HbA1C, FCP, 2-h CP, Fins and 2h ins.

significant association between the E2/T ratio and the presence of MASLD[20]. These discrepancies may be attributed to differences in study populations such as race and age, methods for measuring serum E2 and T levels, and diagnostic criteria for MASLD.

However, studies focusing on type 2 diabetes mellitus male subjects are scarce, and the association between the E2/T ratio and MASLD prevalence in this population remains unclear and needs to be further clarified. Only a few studies have investigated the relationship between either E2 or T alone and MASLD in male type 2 diabetes mellitus subjects[39,40]. For instance, a cross-sectional study of 1,005 male type 2 diabetes mellitus subjects demonstrated that higher serum T levels were significantly associated with a lower prevalence of NAFLD[40]. Contrarily, Shin *et al.*[39] found no significant difference in serum E2 and T levels among three subgroups based on the severity of fatty liver disease in men with type 2 diabetes mellitus. To address this gap, we therefore conducted the present study to investigate the association of the E2/T ratio and MASLD in type 2 diabetes mellitus male patients. Our study showed that a higher E2/T ratio was strongly associated with an increased risk of MASLD in type 2 diabetes mellitus male patients, even after adjusting for multiple confounding factors. Specifically, the MASLD prevalence rose progressively from the first to the fourth E2/T ratio quartile. Moreover, the risk of MASLD increased more than threefold when the E2/T ratio exceeded 10.55. Furthermore, ROC analysis using MASLD presence in male type 2 diabetes mellitus patients as the test variable confirmed that the E2/T ratio was a superior predictor of MASLD compared with E2 or T alone.

Interestingly, while serum ALT and r-GT levels were significantly higher in the subjects with MASLD than in those without, no significant differences were observed across the E2/T ratio quartiles in the present study. Contrary to our findings, a population-based study found significant differences in serum ALT levels among E2/T ratio quartiles in 4109 Chinese males older than 18 years of age[6]. Some factors, such as undiagnosed liver diseases and medications affecting liver function[41], may potentially influence liver enzyme levels, which may contribute to the discrepancies observed in the relationship between liver enzymes and the E2/T ratio across different studies. Our findings indicate that the E2/T ratio may be closely associated with the occurrence of MASLD, but not with the severity of the disease. Nevertheless, further longitudinal studies are needed to further determine the true association between the E2/T ratio and MASLD severity.

The close association between the E2/T ratio and MASLD may be attributed to chronic low-grade inflammation. CRP is a well-established marker of chronic low-grade inflammation, reflecting systemic inflammatory activity[42]. Both E2 and T have been implicated in modulating inflammatory and anti-inflammatory responses through various pathways[43,44]. A recent study demonstrated a significant inverse correlation between serum T levels and CRP levels in 280 male patients with type 2 diabetes mellitus[45]. Consistently, our study also showed a significant upward trend in serum CRP levels across the E2/T ratio quartiles. Moreover, serum CRP levels were also significantly higher in type 2 diabetes mellitus male patients with MASLD compared with those without. Therefore, these findings suggest that the positive correlation between the E2/T ratio and MASLD in males with type 2 diabetes mellitus may be mediated by chronic low-grade inflammation.

Notably, obesity may be another potential underlying factor in the association between the E2/T ratio and MASLD. Numerous studies have consistently demonstrated a positive correlation between the E2/T ratio and obesity, a well-established risk factor for MASLD[46–48]. For example, a previous study observed that obese individuals had the highest E2/T ratio, while overweight subjects exhibited moderate levels, both significantly higher than those in the control group[48]. Consistent with these findings, our study also found that obesity prevalence, WC, WHR, and BMI values markedly increased across the E2/T ratio quartiles. Thus, the type 2 diabetes mellitus male patients with elevated E2/T ratio were more likely to develop MASLD, which may be partially mediated by the strong association between obesity and the E2/T ratio.

Interestingly, the strong association between the E2/T ratio and the risk of MASLD was exclusively observed in male patients with type 2 diabetes mellitus, but not in female subjects. Regardless of menstrual phase, women generally have lower T levels and higher E2 levels than men. Several studies have reported that hepatic fat accumulation is related to low E2 levels[49,50]. For example, estrogen-deficient female rats exhibited lipid metabolism disorders such as elevated serum TC levels and increased hepatic lipid deposition compared with normal rats[49], suggesting a protective role of E2 against MASLD in females. Furthermore, females generally exhibit significantly lower T levels than males, which may also obscure the strong association between the E2/T ratio and MASLD in female patients with type 2 diabetes mellitus.

Our study has several limitations. Firstly, the cross-sectional design limited our ability to establish a causal relationship between the E2/T ratio and the development of MASLD in males with type 2 diabetes mellitus. Therefore, further longitudinal studies are needed to confirm the long-term effects of the E2/T ratio on MASLD in this population. Secondly, all participants in this study were Chinese males with type 2 diabetes mellitus, so the generalizability of our findings to other populations and races needs further validation. Thirdly, liver biopsy is the gold standard for diagnosing MASLD, but the diagnosis in the current study was based on abdominal ultrasonography. Despite this, ultrasonography remains the recommended first-line, non-invasive, and reliable tool for detecting liver steatosis in both clinical and population-based studies[51]. Fourthly, the present study lacked quantitative measures of MASLD severity such as fibrosis scores and steatosis grading assessed by liver transient elastography. Finally, only total serum T and E2 were measured in this study. Data on other hormone-related

parameters such as free T and sex hormone-binding globulin, which could provide further insight into the E2/T ratio, were unavailable. Future studies should include these measurements to provide a more comprehensive understanding.

CONCLUSION

In conclusion, the present study with a large sample provided further clinical evidence that the E2/T ratio is independently and positively correlated with the presence of MASLD in type 2 diabetes mellitus male subjects. This association might be attributed to the close relationship between the E2/T ratio and chronic inflammation, as well as obesity. The E2/T ratio might be used as a practical indicator for assessing the risk of MASLD in type 2 diabetes mellitus male patients, but further prospective studies are needed to obtain more convincing evidence.

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DISCLOSURE

The authors declare no conflict of interest.

Approval of the research protocol: The study protocol was approved by the ethics committee of Shanghai Sixth People's Hospital Affiliated to Shanghai Jiao Tong University School of Medicine (approval date, 15 May 2018; approval no. 2018-KY-018(K)). It conforms to the provisions of the Declaration of Helsinki.

Informed consent: All informed consent was obtained from the subjects or guardians.

Registry and the registration no. of the study/trial: April 27, 2018, ChiCTR1800015893.

Animal studies: N/A.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

1. Xiao X, Kennelly JP, Feng AC, *et al*. Aster-B-dependent estradiol synthesis protects female mice from diet-induced obesity. *J Clin Invest* 2024; 134: e173002.
2. Zhang Y, Chen M, Chen H, *et al*. Testosterone reduces hippocampal synaptic damage in an androgen receptor-independent manner. *J Endocrinol* 2024; 260: e230114.
3. Appiah D, Luitel S, Nwabuo CC, *et al*. Low endogenous estradiol levels are associated with elevated risk of cardiovascular disease mortality in young and middle-aged men in the United States. *Atherosclerosis* 2022; 361: 34–40.
4. Ali Hamza M, Abdulhameed A, Ali Mansour A. Total testosterone to estradiol ratio as a predictor marker of metabolic syndrome in males. *Arch Razi Inst* 2022; 77: 351–357.
5. Chopra IJ, Tulchinsky D, Greenway FL. Estrogen-androgen imbalance in hepatic cirrhosis. Studies in 13 male patients. *Ann Intern Med* 1973; 79: 198–203.
6. Chen Y, Chen Y, Xia F, *et al*. A higher ratio of estradiol to testosterone is associated with autoimmune thyroid disease in males. *Thyroid* 2017; 27: 960–966.
7. Dai W, Li Y, Zheng H. Estradiol/testosterone imbalance: impact on coronary heart disease risk factors in postmenopausal women. *Cardiology* 2012; 121: 249–254.
8. Ventetuolo CE, Baird GL, Barr RG, *et al*. Higher estradiol and lower dehydroepiandrosterone-sulfate levels are associated with pulmonary arterial hypertension in men. *Am J Respir Crit Care Med* 2016; 193: 1168–1175.
9. Dai W, Ming W, Li Y, *et al*. Synergistic effect of a physiological ratio of estradiol and testosterone in the treatment of early-stage atherosclerosis. *Arch Med Res* 2015; 46: 619–629.
10. Antonio L, Wu FCW, O'Neill TW, *et al*. Associations between sex steroids and the development of metabolic syndrome: a longitudinal study in European men. *J Clin Endocrinol Metab* 2015; 100: 1396–1404.
11. Xia JW, Tan SJ, Zhang XL, *et al*. Correlation of serum testosterone with insulin resistance in elderly male type 2 diabetes mellitus patients with osteoporosis. *J Diabetes Investig* 2015; 6: 548–552.
12. Fernandez CJ, Alkhalifah M, Afsar H, *et al*. Metabolic dysfunction-associated fatty liver disease and chronic viral hepatitis: the interlink. *Pathogens* 2024; 13: 68.
13. Zhang ZC, Liu Y, Xiao LL, *et al*. Upregulation of miR-125b by estrogen protects against non-alcoholic fatty liver in female mice. *J Hepatol* 2015; 63: 1466–1475.
14. Holcomb VB, Hong J, Núñez NP. Exogenous estrogen protects mice from the consequences of obesity and alcohol. *Menopause* 2012; 19: 680–690.
15. Zhang X, Mou Y, Aribas E, *et al*. Associations of sex steroids and sex hormone-binding globulin with non-alcoholic fatty liver disease: a population-based study and meta-analysis. *Genes (Basel)* 2022; 13: 966.
16. Park JM, Lee HS, Oh J, *et al*. Serum testosterone level within Normal range is positively associated with nonalcoholic fatty liver disease in premenopausal but not postmenopausal women. *J Womens Health (Larchmt)* 2019; 28: 1077–1082.
17. Jaruvongvanich V, Sanguankeo A, Riangwiwat T, *et al*. Testosterone, sex hormone-binding globulin and nonalcoholic fatty liver disease: a systematic review and meta-analysis. *Ann Hepatol* 2017; 16: 382–394.
18. Chen Q, Zhao L, Mei L, *et al*. Association of sex hormones with hepatic steatosis in men with chronic hepatitis B. *Dig Liver Dis* 2022; 54: 378–384.
19. Maruyama Y, Adachi Y, Aoki N, *et al*. Mechanism of feminization in male patients with non-alcoholic liver cirrhosis: role of sex hormone-binding globulin. *Gastroenterol Jpn* 1991; 26: 435–439.

20. Kızılay DÖ, Tuhan H, İsmailoğlu E, *et al*. Relationship of sex hormones with obesity and nonalcoholic fatty liver disease in boys with gynecomastia. *J Tepecik Educ Res Hosp* 2023; 33: 190–196.
21. Zhang X, Xiao J, Liu Q, *et al*. Low serum Total testosterone is associated with non-alcoholic fatty liver disease in men but not in women with type 2 diabetes mellitus. *Int J Endocrinol* 2022; 2022: 8509204.
22. Cao L, Zheng R-d, Sun H-P, *et al*. Sex hormones are altered in male type 2 diabetes complicated with non-alcoholic fatty liver disease. *Int J Clin Exp Med* 2019; 12: 10857–10862.
23. Wang JW, Jin CH, Ke JF, *et al*. Serum iron is closely associated with metabolic dysfunction-associated fatty liver disease in type 2 diabetes: a real-world study. *Front Endocrinol (Lausanne)* 2022; 13: 942412.
24. Zhang ZH, Ke JF, Lu JX, *et al*. Serum retinol-binding protein levels are associated with nonalcoholic fatty liver disease in Chinese patients with type 2 diabetes mellitus: a real-world study. *Diabetes Metab J* 2022; 46: 129–139.
25. Ma YL, Ke JF, Wang JW, *et al*. Blood lactate levels are associated with an increased risk of metabolic dysfunction-associated fatty liver disease in type 2 diabetes: a real-world study. *Front Endocrinol (Lausanne)* 2023; 14: 1133991.
26. Rinella ME, Lazarus JV, Ratziu V, *et al*. A multisociety Delphi consensus statement on new fatty liver disease nomenclature. *J Hepatol* 2023; 79: 1542–1556.
27. Li LX, Zhao CC, Ren Y, *et al*. Prevalence and clinical characteristics of carotid atherosclerosis in newly diagnosed patients with ketosis-onset diabetes: a cross-sectional study. *Cardiovasc Diabetol* 2013; 12: 18.
28. Zhao CC, Wang JW, Chen MY, *et al*. High-normal serum bilirubin decreased the risk of lower limb atherosclerosis in type 2 diabetes: a real-world study. *Diabetol Metab Syndr* 2023; 15: 105.
29. Wang JW, Ke JF, Zhang ZH, *et al*. Albuminuria but not low eGFR is closely associated with atherosclerosis in patients with type 2 diabetes: an observational study. *Diabetol Metab Syndr* 2022; 14: 50.
30. Wang N, Shao H, Chen Y, *et al*. Follicle-stimulating hormone, its association with cardiometabolic risk factors, and 10-year risk of cardiovascular disease in postmenopausal women. *J Am Heart Assoc* 2017; 6: e005918.
31. Xu MR, Wang AP, Wang YJ, *et al*. Serum magnesium levels are negatively associated with obesity and abdominal obesity in type 2 diabetes mellitus: a real-world study. *Diabetes Metab J* 2024; 48: 1147–1159.
32. Wang YJ, Jin CH, Ke JF, *et al*. Decreased serum osteocalcin is an independent risk factor for metabolic dysfunction-associated fatty liver disease in type 2 diabetes. *Diabetes Metab Syndr Obes* 2022; 15: 3717–3728.
33. Lu H, Zeng L, Liang B, *et al*. High prevalence of coronary heart disease in type 2 diabetic patients with non-alcoholic fatty liver disease. *Arch Med Res* 2009; 40: 571–575.
34. Huang YP, Zhang S, Zhang M, *et al*. Gender-specific prevalence of metabolic-associated fatty liver disease among government employees in Tianjin, China: a cross-sectional study. *BMJ Open* 2021; 11: e056260.
35. Chen YL, Li H, Li S, *et al*. Prevalence of and risk factors for metabolic associated fatty liver disease in an urban population in China: a cross-sectional comparative study. *BMC Gastroenterol* 2021; 21: 212.
36. Popović L, Zamaklar M, Lalić K, *et al*. Analysis of the effect of diabetes type 2 duration on beta cell secretory function and insulin resistance. *Srp Arh Celok Lek* 2006; 134: 219–223.
37. Ferguson D, Finck BN. Emerging therapeutic approaches for the treatment of NAFLD and type 2 diabetes mellitus. *Nat Rev Endocrinol* 2021; 17: 484–495.
38. Li C, Lee F, Hwang S, *et al*. Spider angiomas in patients with liver cirrhosis: role of alcoholism and impaired liver function. *Scand J Gastroenterol* 1999; 34: 520–523.
39. Shin JY, Kim SK, Lee MY, *et al*. Serum sex hormone-binding globulin levels are independently associated with nonalcoholic fatty liver disease in people with type 2 diabetes. *Diabetes Res Clin Pract* 2011; 94: 156–162.
40. Yang LJ, Zhou JZ, Zheng YF, *et al*. Association of non-alcoholic fatty liver disease with total testosterone in non-overweight/obese men with type 2 diabetes mellitus. *J Endocrinol Investig* 2023; 46: 1565–1572.
41. Li J, Zhao Z, Jiang H, *et al*. Predictive value of elevated alanine aminotransferase for in-hospital mortality in patients with acute myocardial infarction. *BMC Cardiovasc Disord* 2021; 21: 82.
42. Supanji S, Romdhoniyyah DF, Sasongko MB, *et al*. Associations of ARMS2 and CFH gene polymorphisms with neovascular age-related macular degeneration. *Clin Ophthalmol* 2021; 15: 1101–1108.
43. Nicastri E, Marinangeli F, Pivetta E, *et al*. A phase 2 randomized, double-blinded, placebo-controlled, multicenter trial evaluating the efficacy and safety of raloxifene for patients with mild to moderate COVID-19. *EClinicalMedicine* 2022; 48: 101450.
44. Cutolo M, Montagna P, Brizzolara R, *et al*. Sex hormones modulate the effects of leflunomide on cytokine production by cultures of differentiated monocyte/macrophages and synovial macrophages from rheumatoid arthritis patients. *J Autoimmun* 2009; 32: 254–260.
45. Rovira-Llopis S, Bañuls C, de Marañon AM, *et al*. Low testosterone levels are related to oxidative stress, mitochondrial dysfunction and altered subclinical atherosclerotic markers in type 2 diabetic male patients. *Free Radic Biol Med* 2017; 108: 155–162.
46. Wang S-s, Li K, Liu Z, *et al*. Aerobic exercise ameliorates benign prostatic hyperplasia in obese mice through downregulating the AR/androgen/PI3K/AKT signaling pathway. *Exp Gerontol* 2021; 143: 111152.

47. Lenart-Lipińska M, Łuniewski M, Szydełko J, *et al*. Clinical and therapeutic implications of male obesity. *J Clin Med* 2023; 12: 5354.
48. Mendoza SG, Zerpa A, Velazquez E, *et al*. Sex hormones, lipids, lipoprotein cholesterols, and apolipoproteins in normal and obese subjects: atherogenic relationships. *Int J Obes* 1986; 10: 427–441.
49. Yu YM, Zhou BH, Yang YL, *et al*. Estrogen deficiency aggravates fluoride-induced liver damage and lipid metabolism disorder in rats. *Biol Trace Elem Res* 2022; 200: 2767–2776.
50. Hart-Unger S, Arao Y, Hamilton KJ, *et al*. Hormone signaling and fatty liver in females: analysis of estrogen receptor α mutant mice. *Int J Obes* 2017; 41: 945–954.
51. EASL-EASD-EASO clinical practice guidelines for the management of non-alcoholic fatty liver disease. *J Hepatol* 2016; 64: 1388–1402.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1 | The predictive performance of E2, T, and E2/T ratio for MASLD.