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RESEARCH ARTICLE

Alcohol intake and markers of liver health in patients with type 2 diabetes and metabolic dysfunction–associated steatotic liver disease

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Objective: The study evaluated the impact of low-level alcohol intake on liver health in patients with type 2 diabetes (T2DM) and metabolic dysfunction–associated steatotic liver disease (MASLD).

Methods: In this prospective study T2DM patients with MASLD (alcohol intake <20 g/day (women) and <30 g/day (men)) underwent a comprehensive clinical and laboratory evaluation at baseline (v1) and after 12 months (v2). Alcohol consumption was assessed using the AUDIT-C questionnaire and a detailed clinical interview. Markers of liver health were measured, and liver steatosis and fibrosis were evaluated with non-invasive indexes, including the Liver Risk Score (LRS), an indicator of the risk of liver fibrosis and liver-related events.

Results: The average alcohol intake was 0.47 [2.77] g/day. Patients with an average intake >10 g alcohol/day showed significantly higher levels of aspartate aminotransferase, gamma glutamyl transpeptidase (GGT), direct bilirubin, ferritin, and higher LRS (7.86±1.64 vs. 6.86 [1.46] vs. 6.49 [1.71]; p=0.0039) at v1 compared to those who consumed <10 g/day or were abstinent. At v2, the aminotransferases and LRS were higher in patients with an alcohol intake >10 g/day compared with the other groups. In the multivariable analyses, GGT (β=0.168;p=0.008) and male sex (β=0.417;p<0.001) were independently correlated with the average alcohol intake. Drinking more than one type of alcoholic beverage significantly increased the LRS (v1: 7.02 [1.38] vs. 6.69 [1.43], p=0.0387; v2: 6.88 [1.25] vs. 6.42 [1.24], p=0.0010).

Conclusions: In patients with T2DM and MASLD, even minimal alcohol consumption is associated with markers of liver injury and higher risk of liver-related outcomes.

Keywords: alcohol intake, metabolic dysfunction-associated steatotic liver disease, type 2 diabetes, liver injury

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Introduction

Metabolic dysfunction–associated steatotic liver disease (MASLD) has emerged as the main cause of chronic liver disease in adults worldwide, with an increasing prevalence mainly driven by obesity and type 2 diabetes mellitus (T2DM) [1, 2]. In the current classification of steatotic liver disease (SLD), MASLD represents a clinical condition characterized by hepatic steatosis in the context of cardiometabolic risk factors, excluding a significant alcohol intake (i.e. >20 g/day for women and >30 g/day for men) [3]. MASLD replaced the previous term, non-alcoholic fatty liver disease (NAFLD), thus reflecting the central role of metabolic dysfunction in the disease pathogenesis, and underscoring its multifactorial nature and clinical complexity [2, 3]. Traditionally, alcohol-related liver disease (ALD) and metabolic liver disease were viewed as distinct entities. However, the MetALD (metabolic dysfunction–associated alcohol-related liver disease) category was introduced in the new SLD classification, highlighting the co-existence of alcohol use above the minimal thresholds but below those typically associated with ALD (i.e. >50 g/day for women and >60 g/day for men), in the context of metabolic dysfunction [3-5]. This intermediate category raises critical questions regarding the cumulative and interactive effects of metabolic dysfunction and alcohol use on liver injury [6].

In this context, alcohol consumption requires renewed attention for MASLD patients as well, as even minimal alcohol intake, below the conventional "safe" limits, may have clinically relevant effects [7-9]. Emerging evidence indicates that patients with low but sustained alcohol intake are more susceptible to worsening of liver disease and fibrosis progression [10-14]. Therefore, these traditional limits for moderate drinking might not be adequate for all individuals. Patients with metabolic risk factors, especially those with T2DM, may be more vulnerable to the deleterious hepatic effects of alcohol use, even at doses previously considered non-harmful [2, 6]. T2DM itself is a strong, independent risk factor for the progression of liver fibrosis and the development of cirrhosis and hepatocellular carcinoma (HCC) [15, 16]. It is hypothesized that the synergistic effects of hyperglycemia, insulin resistance, and alcohol

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intake may amplify oxidative stress and hepatic inflammation, thereby accelerating fibrogenesis [2, 15]. However, studies specifically addressing the association between MASLD, T2DM, and the sub-threshold alcohol consumption are scarce, leaving significant gaps in knowledge.

Therefore, it is clinically important to clarify whether a truly "safe" level of alcohol intake can be defined in patients with T2DM and MASLD. While recent guidelines recommend minimizing alcohol intake in subjects with MASLD and complete abstinence if advanced fibrosis is present, current evidence does not adequately address those with early-stage liver disease and multiple metabolic risk factors [17]. Based on these considerations, the present study aims to investigate the impact of low-level alcohol consumption on liver health in patients with T2DM and MASLD.

Methods

Study population. The study was approved by the Ethics Committees of the Emergency County Clinical Hospital of Târgu Mureș (nr. 8120/05.04.2022), County Clinical Hospital of Târgu Mureș (nr. 4873/24.05.2022), and of George Emil Palade University of Medicine, Pharmacy, Science and Technology of Târgu Mureș (nr. 1806/22.06.2022) and was conducted in accordance with the guidelines of the Declaration of Helsinki. All patients signed an informed consent at study entry. Details regarding the material and methods used in the study were previously published [18, 19].

Between July 2022 and July 2023, subjects with T2DM and NAFLD (main inclusion criteria) over 30 years of age were invited to participate in the study. Main exclusion criteria were represented by other types of diabetes, other chronic liver diseases (including alcohol intake over 20 g/day for females and 30 g/day for males), severe autoimmune diseases, malignant diseases in the last 5 years, severe valvulopathies and pericardial collections. As the new term MASLD was endorsed in 2023, we have used it afterwards to characterize the study population, since all patients fulfilled the MASLD definition (liver steatosis associated with at least one cardio-metabolic risk factor, i.e. T2DM).

Clinical evaluation. At first visit (study entry, visit 1) a comprehensive assessment was performed, which included vital signs and anthropometric measurements, liver ultrasound (US) and laboratory evaluation, collection of demographic and medical data (medical history, therapy, lifestyle evaluation). After 12 months (± two weeks) a second evaluation was performed (visit 2), which included lifestyle evaluation (over the last year), vital signs, anthropometric measurements, US liver evaluation and measurement of several laboratory parameters.

Liver steatosis was confirmed by a gastroenterologist, independently of other study evaluations, through ultrasonographic assessment of several subjective parameters (brightness of the liver (liver-kidney contrast), the appearance of liver parenchyma, intrahepatic vessels, and diaphragm) [20]. This assessment was performed by using a Hitachi Arietta v70 system (Hitachi Ldt., Japan).

The information regarding patients' lifestyles was obtained through several questionnaires. Specifically, at visit 1 the amount of alcohol intake was assessed by filling out the AUDIT-C questionnaire, which is a brief screening tool consisting of three questions, each scored from 0 to 4 points. A significant alcohol intake was considered for a total AUDIT-C score ≥3 points in women and ≥4 points in men [21]. In addition, at both visits the alcohol intake was evaluated by filling out a more detailed form (with clinical interview) regarding the type, frequency and quantity of alcoholic beverages consumed over the last year. The average amount of daily alcohol intake was then calculated from the amount and frequency of intake data per each type of alcoholic beverage (spirits/distilled drinks, wine and beer), considering the pure alcohol content of 14 g per one glass of wine (of approximately 150 ml) and per one portion of spirits (of approximately 45 ml), and 12 g per one portion of beer (of 330 ml). The total average amount of daily alcohol intake was then calculated by adding up the amounts of alcohol intake per each beverage.

Blood pressure was measured in standard conditions. The anthropometric measurements were performed by standard methods and included weight, height, several circumferences and skinfolds' thickness. The body mass index (BMI) was calculated by dividing weight to height^2 (kg/m^2). In addition, several anthropometric parameters (such as % body fat, segmental fat, etc.) were obtained by using an InnerScan BC-545N segmental body composition monitor (Tanita, Japan).

Laboratory assessment. At first visit, blood samples were collected in fasting conditions for the analysis of the following parameters: complete blood count (CBC), glucose, glycated hemoglobin (HbA1c), C-peptide, lipid panel (total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides), uric acid, aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), gamma-glutamyl transpeptidase (GGT)), direct bilirubin, albumin, ferritin, haptoglobin, sex hormone binding globulin (SHBG), creatinine. After 12 months, a second blood sample was obtained in fasting conditions, for measurement of several parameters: CBC, metabolic panel (except HDL cholesterol), ASAT, ALAT, GGT, albumin, creatinine. The metabolic and liver panel, creatinine, and haptoglobin were analyzed by using a Cobas Integra 400plus system (Roche Diagnostic; Mannheim, Germany), the CBC by an automated hematology equipment (Mindray BC6200, India), while the C-peptide, ferritin and SHBG were measured by an immunometric assay (Immulite 2000 XPI system; Siemens Healthcare Diagnostics, Germany).

The insulin resistance and β cell function were estimated by using the Homeostatic Model Assessment (HOMA) calculator version 2.2.3 (HOMA-IR and HOMA-B, respectively), with C-peptide and blood glucose concentrations as input values [22]. The estimated glomerular

filtration rate (eGFR) was calculated with the CKD-EPI 2021 formula [23]. The Neutrophil-to-Lymphocyte Ratio (NLR) and the Systemic Immune-Inflammation Index (SIII) (platelet count x neutrophil count/lymphocyte count) were calculated as markers of inflammation [24].

Estimation of liver steatosis and fibrosis. In addition to the US data, the liver steatosis was estimated by two indices: Fatty Liver Index (FLI), and the Index of NASH (Non-alcoholic steatohepatitis) (ION). FLI was calculated with formula: FLI = (e0.953×loge (TG) + 0.139×BMI + 0.718×loge (GGT) + 0.053×waist – 15.745)/(1 + e0.953×loge (TG) + 0.139×BMI + 0.718×loge (GGT) + 0.053×waist – 15.745) × 100 (30 rules out liver steatosis and ≥ 60 rules in steatosis) [25]. ION was calculated by sex-specific formulas: ION = 0.02 x triglycerides (mg/dl) + 0.24 x ALAT (U/l) + 9.61 x HOMA-IR – 13.99 (for women) and ION = 1.33 x waist-to-hip ratio + 0.03 x triglycerides (mg/dl) + 0.18 x ALAT (U/l) + 8.53 x HOMA-IR – 13.93 (for men) (a score higher than 22 indicates steatosis, and higher than 50 is indicative of NASH) [26].

Liver fibrosis was estimated by using two indices: Fibrosis-4 (FIB-4) score and the NAFLD Fibrosis Score (NFS). FIB-4 was calculated as follows: FIB-4 = age (years) x ASAT (U/l) / [platelet (109/l) x ALAT1/2 (U/l)] (a score >2.67 rules in advanced fibrosis (F≥2), a score <1.3 rules out advanced fibrosis, while values between 1.3-2.67 are indeterminate) [27]. NFS was calculated with the following formula: NFS = –1.675 + 0.037 × age (years) + 0.094 × BMI (kg/m^2) + 1.13 × impaired fasting glucose (IFG)/DM (yes=1; no=0) + 0.99 × ASAT/ALAT – 0.013 × platelet (×10^9/L) – 0.66 × albumin (g/dL) (>0.676 indicates significant fibrosis (>F2), <–1.455 indicates no significant fibrosis, and values between –1.455 to 0.676 are undetermined) [28].

In addition, the Liver Risk Score (LRS), indicative of significant liver fibrosis and long-term liver-related outcomes (cancer, hospitalization and mortality), was calculated online (available online at https://www.liverriskscore.com), by inputting several parameters: age, sex, blood glucose, total cholesterol, ASAT, ALAT, GGT, and platelet count [29]. The risk of advanced liver fibrosis and liver-related outcome was indicated by a score ≥15 for high risk, 10–<15 for moderate risk, 6–<10 for low risk, and <6 for minimal risk [29].

Statistical analysis

Descriptive and inferential statistics were employed for data analysis. Normality of data was checked with the Kolmogorov-Smirnov test. Variables with normal distribution are presented as mean ± standard deviation (SD), while those with non-gaussian distribution as median (interquartile range (IQR)). The categorical data was analyzed by using the Chi square test. Continuous variables were compared by using the One-way ANOVA with Tukey post-test (for data with normal distribution) or Kruskal-Wallis test with Dunn post-test (for non-parametric data, or if the differences between the SDs were significant). The correlation between two sets of variables was analyzed by employing either the Pearson's test (if both sets of variables were normally distributed) or the Spearman's test (if one or both sets of variables had non-Gaussian distribution). To test the independent association between the alcohol intake and more than two sets of variables, the multiple regression analyses were applied. The statistical significance was set at $p<0.05$. GraphPad InStat3 software was mainly used for statistical analysis, and the IBM SPSS stat version 31.0.0.0 was additionally used.

Results

The average amount of alcohol intake in this group of 271 patients was 2.90±5.45 (0.47 [2.77]) g/day (2.63 [7.54] g/day for males, and 0.10 [0.49] g/day for females). Of all patients, 76.38% used alcohol (up to 20 g/day for women and up to 30 g/day for men): 8.49% had an intake of 10-30 (20) g/day, and 67.90% had a daily intake up to 10 g, while the rest reported that they did not use alcohol at all. The clinical and laboratory characteristics according to the three alcohol intake categories are presented in Table 1. For those who used alcohol, the average intake was 3.80±5.96 g/day (1.04 [4.86]) (3.79 [8.15] g/day for males, and 0.40 [0.83] g/day for females). A higher proportion of men had an average alcohol intake between 10-30 g/day, and a higher proportion of women were abstinent for alcohol. Subjects with an average alcohol intake >10 g/day had higher liver enzymes (ASAT, GGT), direct bilirubin, ferritin, higher LRS and red blood cell parameters compared to the other groups, but lower body adiposity.

There was a strong positive correlation between the amount of alcohol intake estimated by the first question of the AUDIT-C score and the average amount of alcohol intake calculated from data obtained with the specific questionnaire and clinical interview (r=0.87 [95%CI: 0.83; 0.89]; $p<0.0001$). Patients with a significant alcohol intake (median total AUDIT-C score: 4.0 [0.0]) had higher LRS values (7.35 [1.39] vs. 6.71 [1.42]; p=0.0007) compared with those with non-significant alcohol intake (median total AUDIT-C score: 1.0 [2.0]), as well as higher ASAT (26.27±9.43 U/l vs. 19.9 [10.21] U/l; p= -0.0129), GGT (34.88 [43.39] U/l vs. 28.75 [25.88] U/l; p=0.0193) and serum ferritin concentrations (118.0 [158.5] ng/ml vs. 88.65 [116.73] ng/ml; p=0.0192). There were no statistically significant differences between the two groups with regards to the two fibrosis markers, direct bilirubin, and ALAT.

After 12 months, alcohol consumption was reevaluated among 254 patients that returned for a second visit. The average alcohol intake was 2.16±4.13 (0.38 [2.0]) g/day (for males: 2.0 [5.93] g/day, and for females: 0.05 [0.47] g/day). Of all, 6.32% used between 10 and 30 (20) g of alcohol/day, 62.85% had an average daily alcohol intake less than 10 g/day, while 30.83% declared no alcohol intake at 12 months. The average alcohol intake among those who

Table 1. Study patients' characteristics (clinical, laboratory parameters, inflammatory and liver fibrosis and steatosis indices) based on average daily alcohol intake categories.

	Average alcohol intake 10-30# g/day (n=23)	Average alcohol intake 0-10 g/day (n=184)	No alcohol intake 0 g/day (n=64)	p
Average alcohol intake (g/day)	**18.08±5.85*****,***	**0.85 [2.53]** ***,***	**0.0*****,***	**<0.0001**
Clinical parameters				
Age (years)	62.78±8.08	66.00 [11.0]	65.55±7.44	0.3832
Diabetes duration (years)	11.00 [7.0]	9.00 [6.0]	10.00 [5.75]	0.4678
Sex (F/M) (no/%)	1 (4.3)/22(95.7)	97 (52.7)/87(47.3)	51(79.7)/13(20.3)	<0.0001
BMI (kg/m^2)	35.57 [8.37]	33.305 [7.04]	34.355 [6.22]	0.0789
Waist circumference (cm)	116.10±11.50	111.13±11.56	111.67±11.88	0.1566
Hip circumference (cm)	110.73±9.83	107.00 [14.28]	109.59±10.69	0.7885
% body fat	31.58±5.32**,***	36.43±7.52**,*	39.25±6.40***,*	<0.0001
WHtR	0.67 [0.10]	0.68 [0.10]	0.70±0.08	0.0237
Systolic BP (mmHg)	137.97±12.64	133.50 [20.38]	136.90±17.65	0.1350
Diastolic BP (mmHg)	82.76±6.93	80.00 [12.25]	82.25 [11.88]	0.6279
Coffee intake (cups/day)	1.0 [1.0]	1.0 [0.88]	1.0 [0.50]	0.3345
Smoking (yes/no)	3/20	20/164	6/58	0.8802
Laboratory parameters				
Blood glucose (mg/dl)	147.77±23.97	138.32 [31.23]	132.42 [33.66]	0.3510
HbA1c (%)	7.03±0.78	6.80 [0.9]	6.80 [0.7]	0.6313
C-peptide (ng/ml)	3.32±1.73	3.20 [1.85]	3.56±1.85	0.8323
HOMA-IR	2.83±1.53	2.715 [1.68]	2.665 [2.16]	0.9308
HOMA-B	76.01±34.89	79.05 [45.85]	85.78±35.29	0.6482
Total cholesterol (mg/dl)	168.99±54.05	154.06 [45.82]	155.83 [43.25]	0.2889
HDL cholesterol (mg/dl)	47.56±9.09	42.745 [13.23]	45.82±9.085	0.0682
LDL cholesterol (mg/dl)	95.31±45.16	78.96 [37.58]	86.445 [39.46]	0.2022
Triglycerides (mg/dl)	147.89 [72.50]	156.77 [86.73]	145.16 [87.79]	0.7185
Uric acid (mg/dl)	6.00 [1.70]	5.625 [1.95]	5.96±1.47	0.1189
Albumin (g/dl)	4.64±0.24	4.62 [0.28]	4.65±0.25	0.9220
ALAT (U/l)	20.76 [21.73]	18.31 [15.16]	16.72 [15.29]	0.1303
ASAT (U/l)	26.25 [12.80]*	19.64 [10.25]*	20.83 [11.10]	0.0142
GGT (U/l)	40.57 [50.95]*,*	28.635 [24.86]*	29.005 [31.45]*	0.0257
Direct bilirubin (mg/dl)	0.26±0.07**,***	0.20 [0.09]**	0.175 [0.11]***	0.0006
Haptoglobin (g/l)	1.60±0.43	1.69±0.58	1.71±0.69	0.7485
Ferritin (ng/ml)	192.99±132.58*,**	96.60 [123.03]*	65.35 [109.65]**	0.0015
SHBG (nmol/l)	30.13±6.92	33.85 [18.00]	40.05±19.55	0.0738
Creatinine (mg/dl)	0.92±0.19	0.820 [0.26]	0.815 [0.30]	0.2095
eGFR (mL/min/1.73 m^2)	90.20±14.88	91.375 [20.93]	86.905 [25.39]	0.2424
Leucocyte count (10^3/µL)	7.23±1.59	7.78±1.83	7.49±2.06	0.2950
Red blood cell count (10^6/µL)	4.82±0.46	4.85±0.49	4.67±0.57	0.0642
Hemoglobin (g/dl)	15.17±1.33*,***	14.32±1.51*,**	13.25 [1.95] ***,**	0.0001
Hematocrit (%)	45.13±3.93**	43.45±4.32*	40.80 [5.97] **,*	0.0012
MCV (fL)	93.80±5.49**,**	90.30 [4.75]**	89.68±4.94**	0.0054
MCH (pg)	31.52±1.81***,***	29.85 [2.20]***	29.38±1.75***	<0.0001
MCHC (g/dL)	33.40 [0.60]***,***	33.00 [0.80]***	32.77±0.83***	<0.0001
Platelet count (10^3/µL)^	226.09±35.75	234.64±64.65	244.09±71.04	0.4395
Neutrophil count (10^3/µL)	3.82 [1.70]	4.415 [1.98]	4.46±1.42	0.1483
Lymphocyte count (10^3/µL)	2.26±0.55	2.215 [0.76]	2.32±0.78	0.9890
Monocyte count (10^3/µL)	0.52±0.13	0.47 [0.19]	0.46±0.12	0.2631
Eosinophil count (10^3/µL)	0.19±0.10	0.18 [0.17]	0.175 [0.12]	0.5344
Basophil count (10^3/µL)	0.05±0.03	0.04 [0.02]	0.04 [0.01]	0.4567
Inflammatory markers and liver fibrosis and steatosis indices				
NLR	1.789 [0.68]	2.063 [1.05]	1.846 [1.21]	0.2180
SIII	371.17 [170.77]	456.79 [312.63]	502.12±229.09	0.3614
Liver risk score	7.86±1.64*,**	6.86 [1.46]*	6.49 [1.71]**	0.0039
FIB-4^	1.68±0.72	1.34 [0.76]	1.33 [0.80]	0.5982
NFS	0.167±0.946	0.155 [1.44]	0.266±1.363	0.9659
FLI	95.00 [26.70]	88.05 [23.68]	89.55 [19.58]	0.2675
ION	21.36±15.15	20.25 [18.13]	23.37±18.06	0.8915

BMI=body mass index; WHtR=waist-to-height ratio; BP=blood pressure; HbA1c=glycated hemoglobin; HOMA-IR=Homeostatic Model Assessment of Insulin Resistance; HOMA-B=Homeostatic Model Assessment of Beta-cell function; HDL=high-density lipoprotein; LDL=low-density lipoprotein; ALAT=alanine aminotransferase; ASAT=aspartate aminotransferase; GGT=gamma glutamyl transpeptidase; SHBG=sex hormone-binding globulin; eGFR=estimated glomerular filtration rate; MCV=mean corpuscular volume; MCH=mean corpuscular hemoglobin; MCHC=mean corpuscular hemoglobin concentration; NLR=Neutrophil-to-Lymphocyte Ratio; SIII=Systemic Immune-Inflammation Index; FIB-4=Fibrosis-4 Index; NFS=NAFLD Fibrosis Score; FLI=Fatty Liver Index; ION=Index of NASH (Non-alcoholic steatohepatitis); #10-20 g/day for females; ^one outlier was excluded; *<0.05; **<0.01; ***<0.001

use alcohol was 3.12±4.66 (1.189 [3.48]) (for males: 2.87 [5.51] g/day and for females: 0.39 [0.86] g/day).

At visit 2, ASAT and ALAT values were higher in the group with average alcohol intake between 10-30 (20) g/day compared with the other two groups (for ALAT: 26.70±8.21 U/l vs. 14.81 [11.28] U/l (0-10 g alcohol intake/day, p<0.001) vs. 13.53 [8.97] U/L (no alcohol intake, p<0.001), p<0.0001; and for ASAT: 27.14±9.83 U/l vs. 19.59 [8.17] U/l (0-10 g/day group) vs. 19.04 [8.75] U/l (no alcohol intake, p<0.05), p=0.0341). No significant differences were noted for the other liver health markers, except for LRS. The LRS values were higher in patients with an average daily alcohol intake >10 g compared with those with an alcohol intake 0-10 g/day (p<0.01) and those with no alcohol intake (p<0.001) (7.67±1.12 vs. 6.68 [1.15] vs. 6.41 [1.52]; p=0.0003) (Figure 1).

Correlation between the average alcohol intake and liver parameters

In the bivariate analyses, the amount of daily alcohol intake correlated positively with several markers of liver health, LRS and CBC parameters, male sex, and negatively with SIII and body adiposity (Table 2). For the rest of clinical, laboratory parameters and indices there were no significant correlations noted with the average daily alcohol intake.

In the multivariable analysis with ASAT, ALAT, GGR, direct bilirubin, ferritin, creatinine, hemoglobin, SIII and WHtR as independent variables (model 1), GGT, hemoglobin and serum creatinine were correlated independently with the average daily alcohol intake (R^2=0.146, p<0.001) (Table 3). In the fully adjusted model (sex added as independent variable), GGT and (male) sex remained significantly associated with alcohol intake.

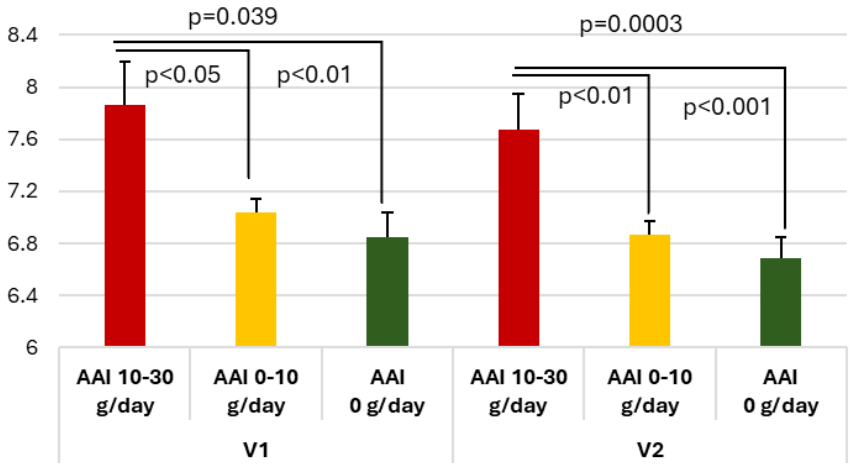

Fig. 1. The Liver Risk Score according to the three average daily alcohol intake categories at both visits (AAI=average alcohol intake; V1=visit 1; V2=visit 2).

Table 2. Clinical and laboratory parameters and indices significantly associated with the average daily alcohol intake in bivariate correlations analyses (at both study visits) in T2DM patients with MASLD.

	Visit 1		Visit 2	
	Correlation coefficient r [95%CI]	p	Correlation coefficient r [95%CI]	p
Sex (M)	0.54 [0.45; 0.63]	<0.0001		
BMI	-0.11 [-0.23; 0.02]	0.0789	-0.13 [-0.25; -0.004]	0.0374
% Body fat	-0.41 [-0.50; -0.30]	<0.0001	-0.40 [-0.50; -0.29]	<0.0001
WHtR	-0.19 [-0.31; -0.07]	0.0014	-0.18 [-0.30; -0.06]	0.0037
Direct bilirubin	0.23 [0.11; 0.35]	0.0001	NA	
GGT	0.18 [0.06; 0.30]	0.0025	0.12 [-0.01; 0.24]	0.0621
ALAT	0.10 [-0.02; 0.22]	0.0902	0.24 [0.12; 0.36]	0.0001
ASAT	0.08 [-0.04; 0.20]	0.1674	0.14 [0.01; 0.26]	0.0309
Creatinine	0.21 [0.09; 0.32]	0.0005	0.15 [0.02; 0.27]	0.0174
Ferritin	0.26 [0.14; 0.37]	<0.0001	NA	
Red blood cell count	0.15 [0.03; 0.27]	0.0140	0.06 [-0.07; 0.19]	0.3334
Hemoglobin	0.33 [0.22; 0.44]	<0.0001	0.24 [0.12; 0.36]	<0.0001
Hematocrit	0.29 [0.17; 0.40]	<0.0001	0.22 [0.09; 0.33]	0.0005
MCV	0.23 [0.12; 0.35]	<0.0001	0.23 [0.11; 0.35]	0.0002
MCH	0.34 [0.22; 0.44]	<0.0001	0.25 [0.12; 0.36]	<0.0001
MCHC	0.33 [0.22; 0.44]	<0.0001	0.11 [0.02; 0.23]	0.0953
Platelets	-0.17 [0.29; -0.05]	0.0043	-0.16 [-0.29; -0.04]	0.0090
Neutrophils	-0.06 [-0.18; 0.07]	0.3439	-0.15 [-0.27; -0.02]	0.0172
Basophils	0.13 [0.01; 0.25]	0.0285	0.09 [-0.04; 0.21]	0.1670
Eosinophils	0.11 [-0.02; 0.23]	0.0832	0.17 [0.04; 0.29]	0.0079
SIII	-0.13 [-0.25; -0.01]	0.0313	-0.13 [-0.25; -0.002]	0.0398
Liver Risk Score	0.23 [0.11; 0.34]	0.0001	0.27 [0.15; 0.39]	<0.0001

CI=confidence interval; BMI=body mass index; WHtR=waist-to-height ratio; ALAT=alanine aminotransferase; ASAT=aspartate aminotransferase; GGT=gamma glutamyl transpeptidase; MCV=mean corpuscular volume; MCH=mean corpuscular hemoglobin; MCHC=mean corpuscular hemoglobin concentration; SIII=Systemic Immune-Inflammation Index; NA= not applicable (not determined)

Table 3. Parameters independently associated with the average daily alcohol intake in the multivariable analyses in patients with T2DM and MASLD.

	Standardized β coefficient	95%CI	T ratio	p value
Model 1		R^2=0.146; p<0.001		
GGT	0.194	0.008; 0.041	2.894	0.004
Hemoglobin	0.132	0.007; 0.913	2.000	0.047
Creatinine	0.117	0.035; 5.637	1.994	0.047
Model 2		R^2=0.249; p<0.001		
GGT	0.168	0.006; 0.037	2.658	0.008
Sex (M)	0.417	3.051; 6.057	5.967	<0.001

SE=standard error; CI=confidence interval; GGT=gamma glutamyl transpeptidase; Model 1: independent variables were: ASAT, ALAT, GGT, direct bilirubin, ferritin, creatinine, hemoglobin, SIII and WHtR; Model 2: additional adjustment for sex

The analysis of the AUDIT-C questionnaire further substantiated these findings. The overall AUDIT-C score correlated with the GGT values (r=0.14 [95%CI: 0.02; 0.26]; p=0.0224), direct bilirubin (r=0.23 [95%CI: 0.11; 0.34]; p=0.0001), and serum ferritin concentrations (r=0.27; [95%CI: 0.15; 0.38]; p<0.0001), while the correlation with ASAT and ALAT were borderline (0.12 [95%CI: -0.01; 0.24]; p=-0.0564, and r=0.12 [95%CI: -0.004; 0.24]; p=-0.0500, respectively). The total AUDIT-C score did not correlate with the fibrosis scores (FIB-4 and NFS, p>0.05 for both), but the correlation with the LRS was significant (r=0.24 [95%CI: 0.12; 0.36]; p<0.0001).

Types of alcoholic beverages and liver health

Patients that used spirits (distilled beverages) had higher direct bilirubin values (at visit 1) compared to those that did not use this type of alcoholic beverage. Patients that used wine had higher ALAT values (at both visits), GGT and LRS (at visit 2) compared to those that did not drink it, while subjects that used beer had higher direct bilirubin and GGT (at visit 1), ALAT and ASAT (at visit 2) and LRS (at both visits) compared to those that did not drink beer (Supplemental table 1). The types of alcoholic beverage had no significant influence on liver fibrosis markers.

The analysis of liver markers and indexes according to the number of types of alcoholic beverages used by a subject showed no significant differences, except for direct bilirubin (at v1) and the LRS (at both visits) (Table 4). Patients that used two or three types of alcoholic beverages (spirits, wine and/or beer, in any combination) had higher LRS compared to subjects that used only one type or none.

Discussions

Excessive alcohol consumption is a well-known risk factor for hepatic disease, but it is still not clarified whether a light intake, below previously defined quantities, causes adverse liver effects in subjects with T2DM and MASLD. By exploring this interaction, we aimed to provide a clearer understanding of the clinical consequences of alcohol intake in this high-risk population and to provide more personalized monitoring and counseling strategies. Data in the literature is somewhat conflicting regarding the effects of modest alcohol intake on liver health, as some studies suggested a neutral or even a possible beneficial effect of low alcohol consumption on liver-related outcomes, while others showed detrimental effects of even a light intake [13]. A drawback is however the cross-sectional design of most studies and the variable definition of light/low/modest alcohol intake [13].

Our study highlighted significant associations between alcohol intake and various liver parameters in patients with T2DM and MASLD. Patients with an average alcohol intake of >10 g/day (up to 20 g/day for females and up to 30 g/day for males) had significantly higher liver enzymes (GGT, ASAT, ALAT), direct bilirubin, and serum ferritin values compared to those with lower average daily alcohol intake (<10 g/day) and abstainers. These findings suggest that even minimal alcohol consumption may induce subclinical alterations in liver function, highlighting its potential hepatotoxic impact in patients with T2DM and MASLD. These biochemical changes are significant because GGT is a sensitive biomarker not only of alcohol exposure, but also of oxidative stress and hepatocellular in-

Table 4. Liver enzymes, fibrosis markers and LRS according to the number of types of alcoholic beverages consumed by study patients (at both visits).

	Visit 1			Visit 2		
	2-3 types (n=104)	0-1 type (n=167)	p	2-3 types (n=95)	0-1 type (n=158)	p
ALAT	19.84 [17.66]	17.18 [13.78]	0.2066	16.65 [13.60]	14.27 [10.33]	0.0611
ASAT	21.08 [10.96]	19.97 [10.57]	0.4797	20.35 [9.12]	19.35 [8.52]	0.2147
GGT	32.33 30.96]	27.54 [21.83]	0.0170	29.45 [23.86]	25.76 [23.16]	0.1025
DBi	0.22 [0.11]	0.18 [0.09]	0.0008	NA		
LRS	7.02 [1.38]	6.69 [1.43]	0.0387	6.88 [1.25]	6.42 [1.24]	0.0010
FIB-4^	1.36 [0.83]	1.34 [0.75]	0.2206	1.48 [0.80]	1.37 [0.73]	0.2040
NFS	0.279 ± 1.326	0.100 [1.45]	0.6804	0.144 ±1.053	0.203 ± 1.205	0.6903

ASAT=aspartate aminotransferase; GGT=gamma glutamyl transpeptidase; DBi=direct bilirubin; LRS=Liver Risk Score; FIB-4=Fibrosis-4 Index; NFS=NAFLD Fibrosis Score; ^one outlier was excluded.

jury, and is recognized as an independent predictor of liver disease progression and of cardiovascular outcomes [6, 30, 31]. The deleterious effect of alcohol use on liver function was furthermore indirectly suggested by higher ferritin levels in subjects with an average alcohol intake >10 g/day. Ferritin is an iron-storage protein produced by hepatocytes and activated macrophages that is involved in the acute-phase response to injury and inflammation and is released by damaged hepatocytes upon injury [19, 32-34]. Previous research showed that chronic alcohol intake may increase ferritin levels, potentially causing iron overload, mainly in the presence of a liver condition [35, 36]. Excess iron is toxic to the liver and can generate harmful free radicals that damage liver cells, accelerating the progression of liver diseases [37-39].

Although other liver fibrosis indices (FIB-4 and NFS) were not significantly different between groups with different average daily alcohol intake, the LRS, which is an indicator of the risk of advanced liver fibrosis and liver-related outcomes, was significantly higher in individuals with an average alcohol intake of >10 g/day compared to the other two groups, both at the initial and the follow-up visit, suggesting that a relatively low but chronic alcohol intake can have a cumulative impact on the risk of liver fibrosis. Higher LRS and liver enzymes are in fact indicative of liver injury, and other research support our findings. In a large Finish cohort of 8,345 persons with hepatic steatosis, Åberg F. and colleagues showed that a daily alcohol intake of 10-19 g doubled the risk for advanced liver disease compared to lifetime abstainers [11]. Similarly, the study by Chang Y et al. demonstrated that a moderate alcohol intake (defined as 10-29.9 g/day for men and 10-19.9 g/day for women) was associated with worsening of liver fibrosis (assessed by FIB-4) compared to nondrinkers (adjusted Hazard Ratio=1.29 (1.18-1.40)), during 347,925.4 person-years of follow-up, in 58,927 Korean adults with NAFLD [10]. Additionally, a longitudinal biopsy study that included 285 participants with NAFLD, showed that patients with a modest alcohol intake (<2 drinks/day) had lower odds of ALAT reduction and histological improvement (of steatosis and NASH resolution) compared to those that did not use alcohol at follow-up (mean duration of 47 months) [40].

The study by Blomdahl et al. demonstrated a synergistic effect between moderate alcohol consumption (>66 g/week) and T2DM, leading to more advanced fibrosis in patients with MASLD [41]. Other studies appear to overall indicate a lower limit of daily alcohol intake for subjects with MASLD. Protopapas A. and colleagues suggested that an intake less than 10 g of alcohol/day might be allowed in patients without steatohepatitis or advanced fibrosis, provided a careful follow-up [13]. In line with this, our study also points towards a lower limit for alcohol consumption in patients with MASLD and T2DM, which is likely to be less than <10 g/day, supporting previous suggestions.

The mechanisms by which alcohol intake synergistically increase the risk of liver injury in MASLD are complex and involve generation of reactive oxygen species (ROS), leading to oxidative stress, which causes cellular damage, and inflammation, ultimately leading to fibrosis [42]. Alcohol metabolism significantly disrupts mitochondrial function and bioenergetics and makes hepatocytes more vulnerable to injury and cell death [43, 44]. Alcohol-induced mitochondrial dysfunction leads to disruption in hepatic lipid homeostasis, further exacerbated by excessive nutrient intake in the context of MASLD [43] The accumulation of toxic lipid intermediates promotes pro-inflammatory and pro-apoptotic pathways [45]. Chronic inflammation triggers fibrogenic signaling cascades, with activation of hepatic stellate cells, and excessive deposition of collagen [43, 45].

In addition to the amount of alcohol intake, other aspects, such as beverage type, drinking pattern, or other lifestyle patterns appear to be relevant [46]. In our study, the analysis concerning the type of alcoholic beverages showed no remarkable differences with regards to their impact on liver markers (although beer rather appeared more detrimental). This is somehow in contrast with previous results that suggested a less deleterious effect of wine (but not beer or non-wine drinks) [11, 47]. However, we did not evaluate the exclusive consumption of beverages, which might explain the differences in the results. It has been suggested that the non-alcoholic content of wine (e.g. polyphenols) might exert some beneficial effects (by reducing triglyceride levels, oxidative stress, inflammation, etc.) [48-50]. Our results indicated though that consuming two or three types of alcoholic beverages was associated with significantly higher LRS and GGT levels than consuming maximum one type of alcoholic beverages. This finding is valuable, suggesting that the cumulative effect of different ingredients in various drinks could be more damaging. Further studies are needed to elucidate the specific effects of different types of alcoholic beverages, their variety/combination and the pattern of alcohol intake on liver health/injury profile.

In conclusion, the results of our study challenge the traditional notion of a universal "safe" limit for alcohol intake. For patients with MASLD and T2DM, a history of even low-level alcohol consumption, below the MASLD definition thresholds, should be of concern. These findings are of clinical relevance, as they emphasize the need for attentive screening for alcohol intake in all patients with T2DM and MASLD and for close monitoring of their liver function. The synergistic effects of T2DM and alcohol consumption on liver health imply that a specific approach for alcohol intake recommendations should be implemented in this particularly vulnerable population, by setting lower limits of allowed intake or even advocating complete abstinence.

This study has several limitations. The liver biopsy (the gold-standard method) could not be used, but instead we have employed several well-accepted non-invasive indexes to define hepatic steatosis and fibrosis. The data regarding

alcohol consumption were based on self-reporting, which poses reasonable possibility of less accurate estimation of intake, and thus the interpretation of the results should be done with care. However, to minimize errors, we have used both the validated AUDIT-C questionnaire and the more detailed specific questionnaire with interview, and we found a good correlation between the two data. Nevertheless, future research should validate these findings in larger and more heterogenous populations. Furthermore, longer-term monitoring could offer a clearer understanding regarding the evolution of liver risk indices based on changes in alcohol consumption habits.

Conclusion

Even a minimal alcohol consumption, below the MASLD definition thresholds, is associated with indicators of liver injury and higher risk of long-term liver-related outcomes in patients with T2DM and MASLD. Drinking more than one type of alcoholic beverage significantly increased the Liver Risk Score.

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Authors' contributions

SC (Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Writing – original draft, review & editing)
DO (Investigation; Validation; Writing – original draft, review & editing)
ALR (Investigation; Validation; Writing – original draft, review & editing)

Conflict of interest

None to declare.

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Supplemental table 1. Types of alcohol beverages and liver health indicators (at both study visits)

V1	Spirits n=110	No spirits n=161	p	Wine n=136	No wine n=135	p	Beer n=111	No beer n=160	p
ALAT	18.00 [17.06]	18.17 [15.39]	0.6926	20.25 [16.17]	16.66 [14.34]	0.0480	19.47 [18.51]	17.39 [13.39]	0.2448
ASAT	19.90 [10.88]	20.80 [10.32]	0.4310	21.41 [9.68]	19.97 [11.62]	0.1846	21.09 [12.49]	19.96 [9.37]	0.3185
DBi	0.22 [0.12]	0.19 [0.10]	0.0171	0.20 [0.11]	0.180 [0.10]	0.0602	0.20 [0.10]	0.19 [0.12]	0.0269
GGT	28.83 [26.21]	29.01 [27.93]	0.4954	31.05 [23.95]	26.77 [28.37]	0.1903	32.37 [40.83]	28.19 [22.08]	0.0051
LRS	6.90 [1.58]	6.85 [1.48]	0.6580	7.04±1.07	6.69 [1.57]	0.1991	7.07 [1.42]	6.67 [1.5]	0.0049
FIB-4^	1.35 [0.71]	1.345 [0.84]	0.6673	1.36 [0.87]	1.34 [0.73]	0.7470	1.36 [0.87]	1.34 [0.74]	0.2769
NFS	0.155 [1.27]	0.223±1.38	0.9887	0.145 [1.62]	0.228±1.17	0.6419	0.190 [1.68]	0.110 [1.33]	0.6369
V2	**Spirits n=103**	**No spirits n=150**	**p**	**Wine n=125**	**No wine n=128**	**p**	**Beer n=89**	**No beer n=164**	**p**
ALAT	15.61 [11.38]	14.50 [12.57]	0.3076	16.74 [13.65]	14.00 [9.16]	0.0203	16.74 [13.86]	14.13 [10.64]	0.0402
ASAT	19.60 [8.18]	19.47 [9.53]	0.7837	19.97 [8.98]	19.50 [8.97]	0.3965	20.82 [9.68]	19.01 [7.81]	0.0120
GGT	27.58 [21.68]	26.87 [27.01]	0.8619	29.45 [25.50]	25.55 [22.83]	0.0358	27.99 [23.68]	26.87 [24.72]	0.2526
LRS	6.79 [1.28]	6.495 [1.29]	0.0674	6.87 [1.23]	6.40 [1.26]	0.0005	6.85 [1.25]	6.46 [1.27]	0.0081
FIB-4	1.46 [0.74]	1.38 [0.74]	0.4707	1.56±0.64	1.35 [0.71]	0.4979	1.45 [0.72]	1.38 [0.81]	0.2434
NFS	0.147±1.06	0.204±1.21	0.6963	0.119±1.14	0.241±1.16	0.4003	0.147 ±0.95	0.200±1.25	0.7078

ASAT=aspartate aminotransferase; DBi=direct bilirubin; GGT=gamma glutamyl transpeptidase; LRS=Liver Risk Score; FIB-4=Fibrosis-4 Index; NFS=NAFLD Fibrosis Score; V1=baseline visit; V2= second visit; ^one outlier was excluded