Liver Fibrosis is Independently Associated with Diabetic Peripheral Neuropathy in Type 2 Diabetes Mellitus

Jinya Huang¹, Rumei Li¹, Naijia Liu¹, Na Yi¹, Hangping Zheng¹, Qi Zhang¹, Lianying Zhou², Linuo Zhou¹, Renming Hu¹, Lu Bin¹

1. Department of Endocrinology and Metabolism, Huashan Hospital, Fudan University, 12 Wulumuqi Road, Shanghai 200040, China.
2. Wujing Community Health Service Center, Minhang District, Shanghai.

Correspondence:
Bin Lu, M.D., PhD., NO. 12 Wulumuqi Road, 4th Floor, Building 0, Shanghai 200040, China, E-mail: doctor_lubinfudan@126.com

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Abstract

Aims: Non-alcoholic fatty liver disease and type 2 diabetes mellitus are closely related and often occur simultaneously in patients. Type 2 diabetes increases the risk of diabetic peripheral neuropathy, resulting in intolerable pain and extremity amputation that reduces the quality of life. However, the role of non-alcoholic fatty liver disease in the pathogenesis of diabetic peripheral neuropathy remains unclear. Thus, we evaluated the correlation of liver fibrosis and steatosis, which are representative histological morphologies of non-alcoholic fatty liver disease, with diabetic peripheral neuropathy in type 2 diabetes patients.

Results: Among the 520 patients, the prevalence of liver steatosis and fibrosis and diabetic peripheral neuropathy were 63.0% (n=328), 18.1% (n=94), and 52.1% (n=271), respectively. The prevalence of diabetic peripheral neuropathy was significantly elevated in patients with liver steatosis (55.7% vs. 44.9%, p=0.03) and fibrosis (61.5% vs. 50%, p=0.04), and it increased as liver stiffness measurement increased. Additionally, both hepatic steatosis (OR=1.48 [1.04–2.11], p=0.03) and fibrosis (OR=1.60 [1.02–2.51], p=0.04) were correlated with diabetic peripheral neuropathy. After adjusting for age, sex, weight, height, BMI, waist hip ratio, duration of T2DM, blood glucose, HOMA-IR, blood pressure, serum lipid, liver enzyme, urea, uric acid, creatinine, and inflammatory factors, liver fibrosis remained associated with diabetic peripheral neuropathy (OR=2.24 [1.11–4.53], p=0.02).

Conclusion: The prevalence of diabetic peripheral neuropathy was elevated in patients with liver steatosis and fibrosis. Liver fibrosis was also independently associated with an increased risk of diabetic peripheral neuropathy.

Keywords: DPN, liver fibrosis, liver steatosis, NAFLD, nerve conduction study, type 2 diabetes

Introduction

Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease worldwide, with a global prevalence of 22–28% in the general population. It refers to the excessive
accumulation of triglyceride in the liver in the absence of competing liver disease etiologies, such as substantial alcohol consumption, chronic viral hepatitis, and use of medications that induce steatosis. With the progression of steatosis, lobular inflammation and pericellular fibrosis would occur, which is known as non-alcoholic steatohepatitis, leading to cirrhosis and hepatocellular cancer, and eventually to death\(^2\). Except for liver-related complications, increasing epidemiological researches have claimed a correlation between other metabolic disorders and NAFLD. For example, the incidence of cardiovascular disease (CVD) mortality in the NAFLD population is much higher than that of liver-related mortality\(^2\). Additionally, a meta-analysis with a sample size of 8,515,431 from 22 countries demonstrated CVD-specific and liver-specific mortalities of 4.79/1,000 person-years and 0.77/1,000 person-years, respectively, in NAFLD patients.

Given that CVD is one of the most common chronic complications of type 2 diabetes mellitus (T2DM), and the prevalence of NAFLD in T2DM patients is almost three times that in the general population\(^3,4\), tremendous epidemiological researches on the relationship between NAFLD and chronic vascular complications of diabetes have emerged\(^5\). Currently, NAFLD has been comprehensively reported to correlate with an increase in the incidence of CVD and chronic kidney disease in individuals with T2DM\(^5,6\). However, only a few studies have evaluated the relationship between NAFLD and diabetic peripheral neuropathy (DPN) in patients with T2DM and have produced conflicting results\(^7-12\).

Furthermore, DPN is a significant microvascular complication of T2DM that contributes to pain, numbness, ulceration, and amputation of the distal extremities, leading to compromised quality of life. The prevalence of DPN in the adult T2DM population varied significantly from 20% to 60% due to diverse detection methods and ethnicities. In China, the prevalence reached up to 61.8%\(^13\). Considering the high prevalence and high disability tendency, the etiology and potential treatment of DPN are worth exploring. Currently, the most effective treatments are glucose control and pain management. Nevertheless, the 7.8-year intensive therapy achieving glycemic control compared with conventional therapy demonstrated a decrease in the incidence of autonomic neuropathy but
not a decrease in the incidence of DPN in T2DM patients\textsuperscript{14}. Thus, an early diagnosis of DPN is also of necessity.

As mentioned previously, NAFLD directly or indirectly results in many liver-irrelated disorders. Given the high clustering of NAFLD and T2DM, the high prevalence of DPN in T2DM patients, and the ambiguous connection between NAFLD and DPN, we evaluated the relationship between NAFLD and DPN in individuals with T2DM for better management of DPN.

**Materials and Methods**

**Study population**

Patients aged over 18 years and diagnosed with T2DM according to the World Health Organization criteria from the Wujing community, Shanghai, China, were enrolled in the study using the cluster sampling method. All enrolled patients were admitted to one center for all clinical and laboratory assessments. Patients with excessive alcohol consumption (daily alcohol intake > 20 g in women and > 30 g in men), viral B and C hepatitis, autoimmune hepatitis, steatosis-inducing drug utilization, and hepatocellular carcinoma history were excluded from the study. In total, 520 adult T2DM patients were included. Written informed consent was obtained from all participants, and the study was approved by the Ethical Committee of Huashan Hospital.

**Demographic and laboratory evaluation**

All participants were gathered in the same community center. Age, sex, body weight, height, waist circumference, hip circumference, and blood pressure were recorded by one doctor. Additionally, fasting serum samples were collected for measurement of blood glucose, HbA1c, C peptide, insulin, lipid profile, liver and kidney function, and inflammatory factors.

**Evaluation of DPN**

Nerve conduction study (NCS) is the most reliable method, except for biopsy, of studying DPN in clinical trials. Hence, NCSs (NDI-097, Shanghai Haishen) were performed on the motor ulnar,
median, and peroneal nerves, and the sensory ulnar, median, and sural nerves in one community center with temperatures maintained at 22–26°C in our study. Distal motor latency (DML), motor nerve conduction velocity (MNCV), motor compound muscle action potential (CMAP), sensory nerve conduction velocity (SNCV), and sensory nerve action potential amplitude (SNAP) were evaluated. DML prolonging, MNCV slowing, or CMAP descending of the motor nerves was defined as motor nerve dysfunction. Meanwhile, SNCV slowing or SNAP descending of the sensory nerves was defined as sensory nerve dysfunction.

At least two nerve dysfunctions, with at least one belonging to the lower extremity nerves, were deemed to have DPN. The cut-off value of each parameter displayed in Table S1 was evaluated by our hospital because the variation in different laboratories and ethnicities was large.

The detailed protocol was performed as described in a previous study. Surface electrodes were used in this study. The recording electrodes were fixed to the skin of the patients using adhesive tape; the skin was prepared by disinfecting the surface. The stimulation and recording sites are listed in Table S2. The length of each nerve was measured using a flexible measuring tape. Finally, a ground electrode was placed between the stimulating and recording electrodes for safety.

**Evaluation of liver steatosis and fibrosis**

FibroTouch (FT5000), which was as valid as FibroScan, was used to evaluate liver steatosis and fibrosis in our study. The liver stiffness measurement (LSM) value in FibroTouch showed high coincidence rate with hepatic fibrosis staging according to the liver biopsy which is the gold standard for diagnosis of cirrhosis and staging of fibrosis or steatosis. Transient elastography was performed by a certified physician who was blinded to the patients’ clinical data and manipulated according to the operations manual. Briefly, patients were placed in a standard supine position, with their right hands beneath their heads to broaden the intercostal space. Then, the probe was applied to the skin of the 7th–9th intercostal spaces in a vertical position where the coupling agent was smeared. The controlled attenuation parameter (CAP) was expressed as dB/m, and liver LSM was expressed in kPa.
The CAP and LSM were considered reliable only if 10 successful measurements were obtained, with an IQR/median of <30% and a success rate of ≥60%. The cut-off points of CAP and LSM were set at 240 dB/m and 9.5 kPa, respectively, according to Chinese thresholds\textsuperscript{20,21}. Therefore, individuals with CAP ≥240 dB/m and LSM ≥9.5 kPa were assigned to steatosis and fibrosis, respectively.

**Statistical analysis**

Continuous variables were presented as mean ± standard deviation when normally distributed and as median (interquartile range) when skewedly distributed. On the other hand, categorical variables were presented as percentages. Comparisons between two groups were performed using Student’s $t$-test for continuous variables and chi-square test for categorical variables. The risk of liver fibrosis and steatosis in the presence of DPN was estimated using binary logistic analysis. Missing data were eliminated. All statistical analyses were performed using SPSS for Windows (version 21.0; SPSS Inc., IBM, USA).

**Results**

**Clinical characteristics of the study population**

A total of 520 patients with T2DM were enrolled in this study. All basic clinical characteristics including age, sex, body mass index (BMI), duration of T2DM, glycemic control, β-cell function, blood lipid, blood pressure, inflammatory indicators, liver function, and kidney function indicators, are presented in column 2 of Table 1.

**Prevalence and clinical characteristics of patients with liver steatosis and fibrosis**

Among the 520 patients with T2DM, 63.0% (n=328) and 18.1% (n=94) had liver steatosis and fibrosis, respectively (Figure 1). Patients with liver steatosis (CAP ≥ 240 dB/m) were heavier than those with CAP less than 240 dB/m and were prone to abdominal obesity. Fasting insulin, C-peptide, Homeostatic Model Assessment of Insulin Resistance (HOMA-IR), and blood pressure levels were also much higher in patients with liver steatosis. The liver enzyme levels of patients...
with liver steatosis were elevated. In addition, serum uric acid levels were significantly increased. Neither creatinine nor urea levels were found to be altered. Furthermore, serum hsCRP levels were elevated in patients with liver steatosis (columns 2–4, Table 2).

Similarly, patients with liver fibrosis (LSM ≥9.5 kPa) were prone to abdominal obesity, and their fasting insulin, C-peptide, HOMA-IR, and blood pressure levels were also higher. Liver enzyme levels in patients with liver fibrosis were elevated. Additionally, their serum uric acid levels increased but without statistical significance. Neither creatinine nor urea levels were found to be altered. Serum hsCRP levels were also elevated in patients with liver fibrosis (columns 5–7, Table 2).

Prevalence and clinical characteristics of patients with DPN

Among the patients with T2DM, 52.1% (n=271) were diagnosed with DPN according to NCS (Figure 1). Patients with DPN were older, heavier, and taller. The number of diabetic courses was longer, and both fasting plasma glucose and HbA1c levels were higher. Additionally, both systolic and diastolic blood pressures were higher. Liver enzymes, kidney function, lipid patterns, and inflammatory indicators were not significantly different between patients with and without DPN. The LSM of DPN patients was also prone to be higher (5.80 [4.70–7.70] vs. 6.25 [4.80–8.58], p=0.09) (columns 8–10, Table 2).

Prevalence of DPN stratified by LSM and CAP

The prevalence of DPN was significantly elevated in patients with liver steatosis (55.7% vs. 44.9%, p=0.03, Figure 2c) and liver fibrosis (61.5% vs. 50%, p=0.04, Figure 2a). After grouping by quartile values, the prevalence of DPN was elevated as LSM increased (Figure 2b), but a positive relationship did not exist as the CAP increased (Figure 2d).

Associations of hepatic steatosis and fibrosis with the presence of DPN in patients with T2DM

The univariate logistic regression analysis demonstrated that both hepatic steatosis (OR=1.48,
p=0.03) and fibrosis (OR=1.60, p=0.04) were correlated with DPN. After adjusting for age, sex, weight, height, BMI, waist hip ratio, blood glucose, HOMA-IR, blood pressure, inflammatory indicators, and all other risk factors, only fibrosis remained associated with the presence of DPN (OR=2.24, p=0.02). (Table 3)

Discussion

In this large cohort of T2DM patients in the community sampled by cluster sampling, we found that liver fibrosis diagnosed by FibroTouch was associated with DPN diagnosed by NCS independently of BMI, plasma glucose, lipid profile, insulin resistance, blood pressure, serum liver enzymes, inflammatory factors, and other DPN risk factors. The risk of DPN in patients with LSM ≥9.5 kPa was more than twice of that in patients with LSM <9.5 kPa. Meanwhile, liver steatosis was also correlated with DPN but was not statistically significant after adjusting for BMI and other risk factors. In addition, we found a high prevalence of liver steatosis (63.0%) and fibrosis (18.1%) and DPN (52.1%) in the T2DM population, which is consistent with previous epidemiological studies.22,23

Although the relationship between NAFLD and diabetic microvascular complications, including diabetic kidney disease and retinopathy, has been researched comprehensively, only six currently available studies referred to DPN and drew contradictory conclusions. As shown in Table S3, five of the studies were unicentric cross-sectional studies from Italy, Australia, Korea, China, and India,8-12, while the other one was a multicenter cross-sectional study from Italy.7 Among them, Mantovani et al. demonstrated positive correlations between the prevalence of NAFLD diagnosed by ultrasonography and DPN assessed by the Michigan Neuropathy Screening Instrument method in adult outpatients with type 1 diabetes,8 but Vendhan et al. reported no difference in the risk of neuropathy (evaluated by vibratory perception threshold) stratified by NAFLD in young outpatients with type 1 diabetes.11 Distinct ethnicities, ages, and neuropathy diagnostic methods may have caused the inconsistency. Contradictions were also found in studies involving T2DM patients. Kim et al. and Lombardi et al. reported that both liver steatosis and fibrosis were not
correlated with DPN in outpatients with T2DM\textsuperscript{7,10}. Another study from China claimed negative correlations between the prevalence of NAFLD and the duration of diabetes and DPN in inpatients with T2DM, which was not comprehensively discussed in that article. From our perspectives, subjective bias of the diagnosis by ultrasound, the neuropathy symptoms and strict lifestyle interventions of patients with longer diabetic duration might be the explanations\textsuperscript{12}. In contrast, Williams et al. discovered a higher vibratory perception threshold associated with liver fibrosis due to NAFLD in inpatients with T2DM\textsuperscript{9}. In summary, currently, the effects of NAFLD on DPN have been elusive because of divergent diagnostic methods, ethnicity, and single-centered origin of the recruited population. Thus, further research is necessary.

Our results are valuable, for the correlation of DPN and liver steatosis and fibrosis secondary to NAFLD in a large cohort using FibroTouch and NCS simultaneously is first evaluated. FibroTouch is a valid and sensitive non-invasive approach to assess steatosis and fibrosis, similar to FibroScan\textsuperscript{17,24}. According to previous studies, the use of FibroScan in the prediction of DPN was applied in only two studies in which the diagnosis of DPN was mainly dependent on symptoms rather than NCS. In this study, thorough detection of nerves by NCS provided an objective evaluation of the extent of injury to large fiber nerves. Therefore, the correlation between liver fibrosis and DPN demonstrated by our results was credible and objective.

Pathways implicated in DPN include the hexosamine pathway, advanced glycation end products accumulation, excess reactive oxygen species, inflammation, and insulin resistance\textsuperscript{25}. Patients with liver steatosis and fibrosis in this study were more likely to have abdominal obesity and to be in an insulin-resistant state. Moreover, blood pressure and plasma lipid patterns were worse. All the above-mentioned factors are widely accepted risk factors for NAFLD and DPN\textsuperscript{26}. In line with previous studies, elevated serum uric acid levels were observed in patients with liver steatosis. Uric acid is known to play an important role in metabolic diseases, including NAFLD, T2DM, and DPN, by triggering inflammation, mitochondrial oxidative stress, and insulin resistance\textsuperscript{27}. Thus, it is likely that their common etiologies contribute to DPN and NAFLD. In contrast, liver fibrosis per se, which increases the risk of DPN regardless of confounding factors, might also be involved.
in DPN pathogenesis. Bile acid is an amphipathic steroid molecule synthesized from cholesterol in the liver. Studies over the past two decades have suggested that bile acid may function as a signaling molecule through a variety of receptors to regulate their own synthesis and other metabolic processes, such as glucose, lipid, and energy homeostasis, and was implicated in the occurrence of liver fibrosis\textsuperscript{28,29}. TGR5, one of the receptors of bile acid, is widely distributed and expressed in neurons\textsuperscript{28}, which may mediate its role in the pathogenesis of DPN through inflammation. Interacting with TGR5, bile acid would inhibit NF-κB activation\textsuperscript{30,31}. Apart from DPN, liver fibrosis, but not steatosis, was independently associated with albuminuria\textsuperscript{32}, and regardless of the presence or severity of other histological features, fibrosis stage independently is the most relevant liver biopsy feature associated with overall and liver-related mortality or liver transplantation\textsuperscript{33}. Furthermore, inhibiting triglyceride synthesis improves hepatic steatosis but exacerbates liver damage and fibrosis. In other words, triglyceride synthesis actually helps to protect hepatocytes from lipotoxicity by buffering the accumulation of free fatty acids\textsuperscript{34}. However, the mechanism by which only fibrosis is independently associated with DPN remains obscure.

Currently, the most effective treatments for DPN are glucose control and pain management\textsuperscript{35}. However, the ACCORD randomized trial revealed that intensive glucose control did not reduce the risk of advanced measures of microvascular outcomes, and caused higher mortality, although delayed the onset of neuropathy\textsuperscript{36}. Thus, the relationship between liver fibrosis and DPN provides new insights into the etiology and early diagnosis of DPN and the development of a potential therapy.

However, this study has several limitations. Due to the cross-sectional design of the study, the causal relationship between NAFLD and DNP cannot be determined, but the preliminary correlation of NAFLD and the presence of DPN in T2DM individuals is still significant. Thus, future prospective studies and the underlying mechanism of the correlation between DPN and fibrosis needs further analysis. Furthermore, all recruited patients were from communities in Shanghai, China; therefore, generalization to patients with other ethnicities is inappropriate.

In conclusion, we found that the prevalence of DPN was higher in patients with liver steatosis...
and fibrosis secondary to NAFLD in the T2DM population from communities. Additionally, liver fibrosis is an independent risk factor for the presence of DPN after adjusting for all other confounding indicators.

Disclosure

The authors have no conflicts to disclose.

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**Figure 1: Prevalence of NAFLD and DPN in T2DM patients.**

Cohort number: 520 T2DM patients; Liver steatosis: CAP $\geq$ 240 dB/m; Liver fibrosis: LSM $\geq$ 9.5Kpa; DPN: diabetic peripheral neuropathy, At least dysfunction of 2 nerves and among whom at least 1 belonged to lower extremities nerves was deemed as DPN.

**Figure 2: Prevalence of DPN stratified by LSM and CAP.**

Cohort number: 520 T2DM patients; a, c: patients were grouped by cut-off values of LSM and CAP; b, d: patients were grouped by quartile values of LSM and CAP; DPN: diabetic peripheral neuropathy, at least dysfunction of two nerves and among whom at least one belonged to lower extremity nerves was deemed as DPN.

**Table S1:** cut-off values for parameters of nerve conduction studies.

**Table S2:** sites for stimulation and recording of different nerves.

**Table S3:** Summaries of published researches about correlation between NAFLD and DPN.
Table 1: Basic clinical characteristics of whole population

| Characteristics                  | Overall (n=520) |
|----------------------------------|-----------------|
| Sex(M), n(%)                     | 227(43.6%)      |
| Age (years)                      | 64.82±6.51      |
| Weight (kg)                      | 64.66±10.09     |
| Height (m)                       | 1.62±0.08       |
| BMI (kg/m²)                      | 24.47±3.03      |
| WHR                              | 0.91±0.06       |
| Duration of T2DM (years)         | 8.21±5.59       |
| HbA1c (%)                        | 7.01±1.20       |
| FBG (mmol/L)                     | 7.39±2.16       |
| Fasting insulin (pmol/L)         | 16.60(8.89-26.57)|
| Fasting C peptide (pg/mL)        | 155.41(66.79-247.13)|
| HOMA-IR                          | 0.74(0.41-1.24) |
| Systolic pressure (mmHg)         | 130±9           |
| Diastolic pressure (mmHg)        | 80±6            |
| LDL (mmol/L)                     | 2.36±1.00       |
| HDL (mmol/L)                     | 1.01±0.40       |
| TC (mmol/L)                      | 4.30±1.29       |

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| Parameter          | Value                        |
|-------------------|------------------------------|
| TG (mmol/L)       | 1.60 (1.05-2.36)             |
| ALT (U/L)         | 9 (7-12)                     |
| AST (U/L)         | 16 (11.25-20)                |
| GGT (U/L)         | 19.5 (13-28)                 |
| Creatinine (umol/L) | 65.76±21.50                |
| Urea (mmol/L)     | 5.34±1.55                    |
| Uric acid (umol/L) | 284.30±90.62                |
| hsCRP (ng/ml)     | 3.25 (1.43-6.76)             |
| TNFα (pg/ml)      | 33.85 (18.93-98.01)          |
| IL6 (pg/ml)       | 10.63 (6.39-18.94)           |
| LSM               | 5.95 (4.70-8.20)             |
| CAP               | 259.71±41.63                 |

Note: Cohort size, n=520. Continuous variables are expressed either as mean ± SD for normal distributed variables and median (interquartile range) for skewed distributed data or as absolute and relative frequencies for categorical variables. Abbreviations: BMI: body mass index, WHR: waist hip ratio, FBG: fasting blood glucose, LDL: low density lipoprotein, HDL: high density lipoprotein, TC: total cholesterol, TG: triglyceride, LSM: liver stiffness measurement, CAP: fat attenuation parameter.
Table 2: Basic clinical characteristics of population stratified by CAP, LSM and DPN

| Characteristics         | CAP<240dB/m | CAP≥240dB/m | p     | LSM<9.5Kpa | LSM≥9.5Kpa | p     | none-DPN | p     | DPN     | p     |
|-------------------------|-------------|-------------|-------|------------|------------|-------|-----------|-------|---------|-------|
| Sex(M), n(%)            | 86(45%)     | 140(42.6%)  | 0.58  | 198(46.5%) | 28(29.8%)  | 0.004 | 86(34.1%) | 144(52.4%) | <0.001 |
| Age (years)             | 65.66±6.58  | 65.12±5.99  | 0.34  | 65.21±6.36 | 65.80±5.52 | 0.41  | 64.62±6.22 | 65.94±6.14 | 0.02   |
| Weight (kg)             | 59.28±8.77  | 67.69±9.39  | <0.001| 64.27±9.97 | 66.57±10.07| 0.03  | 62.82±9.80 | 65.72±10.16| 0.001  |
| Height (m)              | 1.62±0.08   | 1.63±0.08   | 0.20  | 1.63±0.08  | 1.60±0.07  | 0.002 | 1.61±0.08  | 1.63±0.07  | 0.008  |
| BMI (kg/m^2)            | 22.48±2.39  | 25.56±2.86  | <0.001| 24.02±2.81 | 26.24±3.58 | <0.001| 24.08±2.92 | 24.75±3.23 | 0.01   |
| WHR                     | 0.89±0.06   | 0.92±0.05   | <0.001| 0.90±0.05  | 0.93±0.06  | <0.001| 0.91±0.05  | 0.91±0.06  | 0.18   |
| Duration of T2DM (years)| 9.33±6.24   | 7.67±5.16   | <0.001| 8.27±5.65  | 8.18±5.44  | 0.89  | 7.38±5.29  | 9.10±5.53  | <0.001 |
| HbA1c (%)               | 6.87±1.32   | 7.06±1.06   | 0.09  | 6.93±1.16  | 7.27±1.15  | 0.01  | 6.79±0.95  | 7.20±1.37  | <0.001 |
| FBG (mmol/L)            | 7.20±1.93   | 7.41±2.14   | 0.28  | 7.25±2.00  | 7.71±2.32  | 0.06  | 7.10±1.65  | 7.62±2.53  | 0.007  |
| Fasting insulin (pmol/L)| 13.60(7.83-22.8)| 18.64(11.00-28.8) | <0.001| 16.17(8.61-24.8)| 21.29(12.26-34.8)| <0.001| 18.23(10.24-27.0) | 15.53(8.46-26.8) | 0.17   |
| HOMA-IR                 | 0.60(0.33-1.02) | 0.85(0.48-1.40) | <0.001| 0.71(0.39-1.16) | 1.04(0.66-1.70) | <0.001| 0.84(0.41-1.22) | 0.72(0.42-1.26) | 0.59   |
| Systolic pressure (mmHg)| 128±10      | 131±7       | <0.001| 130±9      | 133±8      | 0.001| 129±8      | 132±9      | <0.001 |

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|                         | 79±5 | 81±5 | <0.001 | 80±5 | 82±5 | 0.001 | 80±5 | 81±5 | <0.001 |
|-------------------------|------|------|--------|------|------|-------|------|------|--------|
| Diastolic pressure (mmHg) |      |      |        |      |      |       |      |      |        |
| LDL (mmol/L)            | 2.29±1.00 | 2.39±1.02 | 0.29  | 2.37±1.01 | 2.28±1.01 | 0.47  | 2.41±1.06 | 2.31±0.96 | 0.25 |
| HDL (mmol/L)            | 1.04±0.46 | 0.94±0.35 | 0.01  | 1.00±0.41 | 0.88±0.34 | 0.006 | 0.97±0.40 | 0.98±0.38 | 0.76 |
| TC (mmol/L)             | 4.09±1.29 | 4.35±1.36 | 0.04  | 4.25±1.35 | 4.25±1.33 | 0.99  | 4.32±1.37 | 4.20±1.30 | 0.36 |
| TG (mmol/L)             | 1.25(0.91-2.01) | 1.78(1.19-2.61) | <0.001 | 1.56(1.02-2.29) | 1.82(1.27-2.87) | <0.001 | 1.69(1.09-2.44) | 1.49(1.01-2.30) | 0.16 |
| ALT (U/L)               | 8.00(6.00-10.00) | 9.00(7.00-13.00) | <0.001 | 9.00(7.00-11.0) | 11.00(8.00-15.0) | <0.001 | 9.00(7.00-11.00) | 9.00(7.00-12.0) | 0.52 |
| AST (U/L)               | 15.00(11.00-18.00) | 17.00(12.00-22.00) | <0.001 | 15.00(11.00-19.0) | 17.50(11.25-24.0) | 0.03 | 16.00(11.00-20.0) | 15.50(12.00-20.0) | 0.96 |
| GGT (U/L)               | 17.00(11.75-22.00) | 22.00(15.00-32.00) | <0.001 | 19.00(12.00-27.0) | 23.00(15.00-38.0) | <0.001 | 19.00(13.00-26.5) | 20.00(13.00-29.0) | 0.49 |
| Creatinine (umol/L)     | 64.63±20.76 | 64.80±20.85 | 0.93  | 65.06±20.45 | 63.30±22.33 | 0.47  | 63.20±21.0 | 66.57±20.7 | 0.08 |
| Urea (mmol/L)           | 5.42±1.64 | 5.30±1.53 | 0.43  | 5.34±1.60 | 5.38±1.41 | 0.82  | 5.39±1.62 | 5.33±1.51 | 0.67 |
| Uric acid (umol/L)      | 258.93±84.21 | 296.80±94.23 | <0.001 | 279.19±91.23 | 297.76±96.28 | 0.09 | 283.8±88.5 | 282.6±95.5 | 0.89 |
| hsCRP (ng/ml)           | 1.95(0.82-4.41) | 4.14(1.97-7.52) | <0.001 | 2.97(1.29-6.03) | 4.56(2.14-8.26) | <0.001 | 3.29(1.59-7.08) | 3.20(0.96-6.04) | 0.12 |
| TNFα (pg/ml)            | 35.50(19.96-123.5) | 31.86(18.85-89.3) | 0.14 | 33.74(19.46-96) | 33.95(18.83-11) | <0.001 | 35.50(20.36-116.2) | 32.12(18.82-73.1) | 0.21 |
| IL6 (pg/ml)             | 10.77(6.34-20.4) | 10.63(6.46-18.3) | 0.72  | 10.72(6.22-18.1) | 10.51(6.88-18.5) | 0.39  | 9.42(5.75-22.31) | 10.47(6.40-17.3) | 0.92 |

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|      | 6) | 6) | 98) | 3) | 77) |
|------|---|----|-----|----|-----|
| LSM  | 5.30(4.33-6.90) | 6.60(5.00-9.40) | <0.001 | 5.50(4.50-6.90) | 12.30(10.90-14.40) | <0.001 | 5.80(4.70-6.90) | 6.25(4.80-8.58) | 0.09 |
| CAP  | 217.85±16.94 | 283.94±32.92 | <0.001 | 252.24±39.46 | 292.32±40.50 | <0.001 | 259.18±43.61 | 259.79±41.57 | 0.87 |

Note: Cohort size, n=520. Continuous variables are expressed either as mean ± SD for normal distributed variables and median (interquartile range) for skewed distributed data or as absolute and relative frequencies for categorical variables. Abbreviations: BMI: body mass index, WHR: waist hip ratio, FBG: fasting blood glucose, LDL: low density lipoprotein, HDL: high density lipoprotein, TC: total cholesterol, TG: triglyceride, LSM: liver stiffness measurement, CAP: fat attenuation parameter. P values represent results of comparisons between two groups with the chi‐squared test (for categorical variables), the unpaired Student’s t test and the Mann‐Whitney test (for normally and not normally distributed continuous variables). Statistical significant p values are in bold pattern.
| Mode  | Liver steatosis (OR, 95% CI) | p  | Liver fibrosis (OR, 95% CI) | p  |
|-------|------------------------------|----|-----------------------------|----|
| Mode 1| 1.48 (1.04-2.11)             | 0.03| 1.60 (1.02-2.51)            | 0.04|
| Mode 2| 1.63 (1.13-2.36)             | 0.01| 1.95 (1.21-3.12)            | 0.006|
| Mode 3| 1.40 (0.92-2.14)             | 0.12| 1.75 (1.07-2.88)            | 0.03|
| Mode 4| 1.47 (0.88-2.47)             | 0.15| 2.26 (1.22-4.18)            | 0.01|
| Mode 5| 1.59 (0.94-2.69)             | 0.09| 2.41 (1.29-4.50)            | 0.006|
| Mode 6| 1.59 (0.91-2.77)             | 0.10| 2.34 (1.23-4.46)            | 0.01|
| Mode 7| 1.56 (0.89-2.74)             | 0.12| 2.18 (1.13-4.22)            | 0.02|
| Mode 8| 1.78 (0.97-3.25)             | 0.06| 2.24 (1.11-4.53)            | 0.02|

Mode 1: liver steatosis or fibrosis per se;

Mode 2: on the basis of mode 1, adjusting age and sex;

Mode 3: on the basis of mode 2, adjusting weight, height, BMI and WHR;

Mode 4: on the basis of mode 3, adjusting duration of T2DM, HbA1c, FBG, fasting insulin, fasting c peptide and HOMA-IR;

Mode 5: on the basis of mode 4, adjusting LDL, HDL, TG and TC;

Mode 6: on the basis of mode 5, adjusting ALT, AST, GGT, urea, uric acid and creatinine;
Mode 7: on the basis of mode 6, adjusting systolic and diastolic blood pressure;

Mode 8: on the basis of mode 7, adjusting inflammatory factors including hsCRP, TNFα and IL6.
The diagram illustrates the percentage of patients with liver steatosis, liver fibrosis, and DPN. The percentages are as follows:

- Liver steatosis: 63.0%
- Liver fibrosis: 18.1%
- DPN: 52.1%
