Normalization of γ-glutamyl Transferase Levels is Associated with Better Metabolic Control in Individuals with Nonalcoholic Fatty Liver Disease

Qianqian Ma  
Sun Yat-sen University First Affiliated Hospital

Xianhua Liao  
Sun Yat-sen University First Affiliated Hospital

Congxiang Shao  
Sun Yat-sen University First Affiliated Hospital

Yansong Lin  
Sun Yat-sen University First Affiliated Hospital

Tingfeng Wu  
Sun Yat-sen University First Affiliated Hospital

Yanhong Sun  
Sun Yat-sen University First Affiliated Hospital

Shi-Ting Feng  
Sun Yat-sen University First Affiliated Hospital

Junzhao Ye  
Sun Yat-Sen Institute for Social Sciences and Philosophy: Research Center for Humanities and Social Sciences

Bi Hui Zhong (zhongbh@mail.sysu.edu.cn)  
Sun Yat-sen University First Affiliated Hospital  
https://orcid.org/0000-0002-4638-7699

Research article

Keywords: Gamma glutamyl transferase, Nonalcoholic fatty liver disease, Liver function, Metabolic syndrome

DOI: https://doi.org/10.21203/rs.3.rs-112697/v1

License: © This work is licensed under a Creative Commons Attribution 4.0 International License.  Read Full License
Abstract

**Background:** The normalization of liver biochemical parameters usually reflects the histological response to treatment for nonalcoholic fatty liver disease (NAFLD). Researchers have not clearly determined whether different liver enzymes exhibited various metabolic changes during the follow-up of patients with NAFLD.

**Methods:** We conducted a retrospective analysis of patients with NAFLD who were receiving therapy from January 2011 to December 2019. Metabolism indexes, including glucose levels, lipid profiles, uric acid levels and liver biochemical parameters, were measured. Magnetic resonance imaging-based proton density fat fraction (MRI-PDFF) and liver ultrasound were used to evaluate steatosis.

**Results:** Overall, 1048 patients with NAFLD were included and received lifestyle and pharmaceutical interventions, including 637 (60.7%) patients with abnormal GGT levels and 467 (44.6%) patients with abnormal ALT levels. Patients with concurrent ALT and GGT abnormalities presented higher levels of metabolism indexes and higher liver fat contents than patients with single or no abnormalities. After 12 months of follow-up, the cumulative normalization rate of GGT was much lower than that of ALT (33% vs. 52%, P < 0.001). Greater weight loss resulted in higher cumulative normalization rates of GGT and ALT. Weight loss (OR = 1.27, 95%CI: 1.15–1.41, P < 0.001), ALT normalization (OR = 4.32, 95%CI: 1.87–9.96, P = 0.01) and HOMA-IR decreased to normal levels (OR = 3.48, 95%CI: 1.60–7.57, P = 0.01) were independent protective factors for GGT normalization. Elevated baseline GGT (OR = 0.99, 95%CI: 0.98–0.99, P = 0.01), CHOL (OR = 0.47, 95%CI: 0.23–0.96, P = 0.04) and fasting insulin levels (OR = 0.91,95%CI: 0.86–0.96, P = 0.01) were risk factors.

**Conclusions:** For NAFLD patients with concurrently increased ALT and GGT levels, a lower normalization rate of GGT was observed rather than ALT. Good control of weight and insulin resistance was reliable predictors of GGT normalization.

Background

Nonalcoholic fatty liver disease (NAFLD) currently known as metabolic associated fatty liver disease (MAFLD), is a clinical syndrome characterized by excess lipid storage in hepatocytes, which has been acknowledged as the most common chronic liver disease worldwide [1]. The incidence of NAFLD continues to increase rapidly, with an estimated 3.6 million patients diagnosed with NAFLD annually based on its global prevalence of up to 25% [2]. In addition to NAFLD progression from steatosis to steatohepatitis, fibrosis, cirrhosis, liver failure and carcinoma [3], NAFLD promotes extrahepatic metabolic disturbances, including hypertension, hyperuricemia, hyperlipemia, hyperglycemia, and eventually contributes to a poor prognosis, leading to cardiovascular disease, type 2 diabetes and other metabolic complications [4, 5]. Therefore, clinical parameters associated with the remission of metabolic abnormalities must be identified when monitoring the effectiveness of treatments for NAFLD.

The liver enzymes alanine aminotransferase (ALT) and γ-glutamyl transferase (GGT) are routine clinical biochemical markers of injured liver cells that are applied to screen for NAFLD or nonalcoholic hepatitis [6]. The primary physical location of ALT is the cytoplasm of liver cells, and its increased level in serum often indicates release from the liver due to cell death. GGT, which is stored in bile duct epithelial cells and hepatocyte microsomes, has long been regarded as a marker of hepatobiliary disease, drug-related liver injuries and excess alcohol consumption [7]. Based on the data from emerging studies, both elevated baseline serum ALT and GGT levels are significantly associated with insulin resistance, as well as other metabolic syndromes and an increased risk of long-term complications of myocardial infarction and stroke [8, 9]. A recent study from the TONIC trial reported that decreased serum ALT and GGT levels are associated with improvements in liver histology [10]. However, the relationship between the dynamic changes in GGT and ALT levels and their metabolic treatment responses during the treatment of patients with NAFLD remains to be elucidated [6]. This issue is of particular clinical importance, as these biochemical markers of hepatitis remission may exhibit inconsistent levels during therapy, namely, the levels of one of the markers decrease to the normal range while the levels of other markers remain abnormal, and relevant studies may be helpful to interpret the results of biochemical assessments conducted during the disease course.

In the present study, we aimed to explore the associations of decreased ALT and GGT levels and improvements in metabolic disturbances during the routine treatment of NAFLD over time. Furthermore, we compared the characteristics of patients with NAFLD who presented inconsistent decreases in the levels of ALT and GGT.

Methods

**Study design and patients**

This single-center retrospective cohort study was conducted in the NAFLD clinic of the First Affiliated Hospital of Sun Yat-sen University, China, from January 1, 2011 to December 31, 2019. The clinical research ethics committee of the First Affiliated Hospital of Sun Yat-sen University approved the research plan, and all subjects provided written informed consent. Subjects were recruited continuously, and the inclusion criteria were as follows: (1) patients aged greater than 18 years; (2) with complete anthropometric parameters, laboratory test results and abdominal liver ultrasonography; and (3) an established diagnosis of NAFLD. The diagnosis of NAFLD was defined as (1) liver histology or imaging (such as abdominal ultrasonography or Magnetic resonance imaging-based proton density fat fraction (MRI-PDFF)) manifesting liver steatosis; (2) no drinking history or previous history of alcohol consumption < 140 g/week in men or < 70 g/week in women; (3) no history of drug-induced liver disease, total parenteral nutrition,
hepatolenticular degeneration, autoimmune hepatitis and other specific diseases that may lead to fatty liver [11]. The exclusion criteria included (1) pregnant and breastfeeding women, (2) patients with the specific occupations of athletes or chemical workers, (3) patients with a concomitant malignant tumor or other severe diseases with organ dysfunction, and (4) patients with preexisting cardiovascular diseases or stroke.

**Clinical estimations**

Subjects’ information was collected by administering structured questionnaires that included information about basic demographic characteristics (age and sex), previous diseases (hypertension and diabetes), medications, and the nicotine and alcohol consumption history. Height, weight, blood pressure, waist circumference and hip circumference were measured by experienced doctors. The blood pressure was measured in the right upper arm with an automatic electronic sphygmomanometer after the patient had rested for more than 15 min, and the average value from 3 successive measurements was recorded. Body mass index (BMI) was calculated as the weight (kg) divided by the square of height (m) [12]. The waist-to-hip ratio (WHR) was calculated as the waist circumference divided by the hip circumference (cm/cm) [12].

**Laboratory measurements**

Venous blood samples were collected after a fast for at least 8 hours for measurements. Liver biochemical and metabolic parameters, including ALT, aspartate aminotransferase cellulase (AST), GGT, lactate dehydrogenase, choline esterase, leucine arylamidase, glutamate dehydrogenase, direct bilirubin (DBil), total bilirubin (TBil), total bile acid (TBA), lipid profile, fasting blood glucose (FBG), fasting insulin (FINS) and uric acid (UA) levels, were measured. The levels of liver enzymes were measured with the enzymatic-colorimetric method using a conventional automated analyzer (Biochemical analyzer from beckman coulter, Au 5800 System), and the cut-off values for ALT and GGT levels were set to 40 U/ml and 50 U/ml, respectively, based on the normal reference value of the detection kits [13]. The homeostatic model assessment of insulin resistance (HOMA-IR) was calculated as follows: FINS (μU/mL) ×FBG (mmol/L)/22.5 [14]. A cut-off value of 2.69 was utilized to define insulin resistance (IR) [14].

**Radiology assessments**

All the subjects were examined using abdominal ultrasonography, and the ultrasound technicians did not know the aim of this study. Based on the signs of diffuse enhancement of the near echo, obvious attenuation of the far echo, contrast enhancement of the liver and kidney echo and unclear intrahepatic duct structure, fatty liver was preliminarily diagnosed. Magnetic resonance imaging-based proton density fat fraction (MRI-PDFF) with the IDEAL-IQ/Dixon sequence is considered a new acute and reproducible method to estimate the fat content of the whole liver and pancreas, as well as the thickness of the abdominal subcutaneous tissue [15]. A large number of studies have shown a similar accuracy and repeatability of the quantitative measurement of liver fat using MRI-PDFF to liver biopsy and biochemical examinations in vitro [16]. We used a 3.0 Tesla MRI with the following settings, as previously described: TE1 2.5 ms; TE2 3.7 ms; repetition time 5.47 ms; 5° flip angle; ± 504.0 kHz per pixel receiver bandwidth; and a slice thickness of 3.0 mm; the fat content was calculated in an irregularly shaped ROI covering the entire liver in 21 consecutive slices (max-area centered) of each patient that were manually placed by two trained radiologists [17]. Subjects with fatty liver were categorized as having a mild (5~10%), moderate (10~25%) and severe (more than 25%) disease according to the liver fat content. The boundary value of this classification has often been used in previous clinical trials to evaluate the effects of different treatment schemes on NAFLD [15, 16].

**Clinical follow-up and treatment**

All patients received recommendations for lifestyle interventions according to the Dietary Reference Intakes [18], the Dietary Guidelines [19] and World Health Organization Global Strategy on Diet, Physical Activity and Health [20]. The patients were guided to adjust their food consumption and to exercise three times/week for 30 min per session by an easy-to-carry brochure with personalized exercise and dietary prescriptions based on sex, age, BMI, occupation and medical history. For patients with indications for drug therapy to treat hyperlipidemia, hypertension, hyperglycemia or hyperuricemia, pharmacological therapy was added as recommended by the guidelines [19-23]. Briefly, these treatments included metformin or insulin for glucose control, benz bromarone for uric acid control, renin–angiotensin blockers or a calcium channel blocker for blood pressure control, a statin for low-density lipoprotein cholesterol (LDL-C) control and fibrates for triglyceride control [21-24]. The prescription of specific agents was determined by the supervising physicians. Clinical follow-up and additional pertinent patient data are also provided. Some patients with a BMI ≥ 25 kg/m² received orlistat (120 mg, three times daily) without additional treatment [25]. Orlistat intake was confirmed by the prescription and records of patient interviews during clinic visits.

The patients were subject to periodic reviews at 1, 3, 6, 9 and 12 months, and each visit should not be postponed for one month after the prescribed time. At each follow-up visit, the anthropometric parameters, metabolic indexes and liver biochemical parameters of the patients were measured again. MRI-PDFF was only performed in some subjects every 6 months.

**Statistical analysis**
All statistical calculations were conducted using SPSS statistics software (version 24.0, IBM, Chicago, IL, USA). The continuous variables are reported as means ± standard deviations (SD), and variables without a normal distribution are reported as medians with interquartile ranges (IQR). The Kruskal–Wallis rank sum test was used to compare non-normally distributed continuous variables between groups. Pearson's Chi-squared test was used to compare categorical data between groups. Multiple comparisons among groups were performed using ANOVA with the Bonferroni post hoc test. Logistic regression models with stepwise selection were used to estimate odds ratios (ORs) for the different stratifications of GGT levels in relation to metabolic parameters. A receiver operating characteristic (ROC) curve analysis was conducted to identify the factors predicting decreased GGT levels. P values for the trend (two-sided) were calculated and were considered statistically significant when they were less than 0.05.

Results

Baseline characteristics

A total of 1048 patients with NAFLD were included in this study and were divided into 4 groups based on the normalization of baseline ALT and/or GGT levels after follow-up: both ALT and GGT abnormal group (n = 415), ALT-only abnormal group (n = 52), GGT-only abnormal group (n = 222), and both ALT and GGT normalization group (n = 359) (Fig. 1). Significant differences in gender, age, fasting glucose level, prevalence of type 2 diabetes, hypertension, partial lipid metabolism and medications were not observed between groups (Table 1). The group with both abnormal ALT and GGT levels had a higher BMI (kg/m^2) (median 26.2 vs. 26.0 vs. 25.9 vs. 25.6, P < 0.001, Table 1), but the waist-hip ratio of this group was not increased. Among the 1048 patients with NAFLD, 637 patients presented with abnormal GGT levels, and the percentage of patients with abnormal GGT levels was 60.7%. Meanwhile, 467 patients presented with abnormal ALT, and the percentage of patients with abnormal ALT levels was 44.6%. Compared with the other three groups (ALT-only abnormal group, GGT-only abnormal group, and both ALT and GGT normalization group), the both ALT and GGT abnormal group had higher liver function indices, such as GGT, ALT, AST, alkaline phosphatase, total bilirubin, direct bilirubin, total bile acid, lactate dehydrogenase, leucine arylamidase and glutamate dehydrogenase levels (all, P < 0.05, Table 1). However, compared with the other three groups, a significant increase in blood lipid metabolism was not observed in the both ALT and GGT abnormal group (Table 1). Furthermore, compared with the other three groups, the group with both abnormal ALT and GGT levels had higher uric acid levels, fasting insulin levels, and HOMA-IR levels (all, P < 0.001, Table 1). Of the 630 patients with NAFLD who underwent MRI-PDFF, the group with abnormal levels of both ALT and GGT presented a significantly higher liver fat content (%) measured using MRI-PDFF than the other three groups (median 15.4 vs. 14.9 vs. 12.5 vs. 10.8, P < 0.001, Table 1), but no difference was identified in the pancreas fat content and abdominal subcutaneous fat thickness (all, P > 0.05, Table 1).
Table 1: Baseline characteristics of patients with NAFLD presenting different ALT and GGT statuses.

| Variables                  | Both GGT and ALT abnormal (N = 415) | ALT Abnormal only (N = 52) | GGT Abnormal only (N = 222) | GGT and ALT normalization (N = 359) | P | Post-hoc       |
|----------------------------|--------------------------------------|--------------------------|-----------------------------|-------------------------------------|---|----------------|
| Age, years                 | 39.6 ± 12.4                          | 40.9 ± 11.2              | 42.9 ± 11.5                 | 44.8 ± 12.5                        | 0.21 | 1# vs. 2#   |
| Male, n (%)                | 322 (77.6)                           | 38 (73.1)                | 167 (75.2)                  | 252 (70.2)                         | 0.13 | 1# vs. 3#   |
| BMI, kg/m²                  | 26.2 (24.9, 28.5)                    | 26.0 (23.2, 27.8)        | 25.9 (23.5, 27.4)           | 25.6 (23.5, 27.3)                  | < 0.001 | 2# vs. 3# |
| Waist-hip ratio            | 0.89 (0.86, 0.94)                    | 0.89 (0.85, 0.92)        | 0.90 (0.87, 0.94)           | 0.89 (0.84, 0.97)                  | < 0.001 | 2# vs. 4#   |
| Smoke, n (%)               | 87 (21.0)                            | 8 (15.4)                 | 44 (19.8)                   | 45 (12.5)                           | 0.01 | 3# vs. 4#   |
| Complication, n (%)        |                                     |                          |                             |                                     |      |               |
| Type 2 diabetes            | 30 (7.2)                             | 4 (7.6)                  | 15 (6.7)                    | 31 (8.6)                            | 0.84 |               |
| Hypertension               | 135 (32.5)                           | 16 (30.8)                | 65 (29.3)                   | 103 (28.7)                          | 0.68 |               |
| Liver biochemistry          |                                     |                          |                             |                                     |      |               |
| GGT, U/L                   | 84 (61,140)                          | 32 (27,37)               | 64 (50,103)                 | 25 (19,30)                          | < 0.001 | 1# vs. 2# |
| ALT, U/L                   | 96 (69,140)                          | 69 (57,94)               | 34 (26,41)                  | 24 (18,32)                          | < 0.001 | 1# vs. 3# |
| AST, U/L                   | 51 (38,76)                           | 40 (36,51)               | 27 (22,33)                  | 22 (19,26)                          | < 0.001 | 1# vs. 4# |
| Alkaline phosphatase, U/L  | 84 (70,98)                           | 76 (61,85)               | 78 (64,93)                  | 70 (58,84)                          | < 0.001 | 1# vs. 2# |
| Total bilirubin, umol/L    | 15.2 (11.2, 15.7)                    | 15.2 (12.2, 15.8)        | 15.2 (10.6, 15.5)           | 15.2 (11.5, 15.3)                  | 0.04 | 2# vs. 3#   |
| Direct bilirubin, umol/L   | 3.6 (2.6, 3.8)                       | 3.6 (2.6, 4.3)           | 3.4 (2.1, 3.6)              | 3.6 (2.4, 3.7)                      | 0.02 | 2# vs. 4#   |
| Total bile acid, umol/L    | 3.1 (2.2, 5.6)                       | 1.6 (1.2, 3.4)           | 2.6 (1.7, 4.2)              | 2.3 (1.6, 4.2)                      | 0.03 | 3# vs. 4#   |
| Lactate dehydrogenase, U/L | 201 (176,230)                        | 183 (164,199)            | 184 (168,205)               | 187 (171,210)                       | < 0.001 | 1# vs. 2# |
| Choline esterase, U/L      | 9064 (7921,10411)                    | 8647 (7687,9092)         | 8963 (8035,9800)            | 8997 (8074,10084)                   | 0.10 | 2# vs. 3#   |
| Leucine aminopeptidase, U/L | 73 (64,90)                           | 58 (52,65)               | 66 (59,78)                  | 55 (50,62)                          | < 0.001 | 3# vs. 4# |
| Glutamate dehydrogenase, U/L | 9.6 (5.5,14.0)                      | 5.3 (3.6,7.8)            | 5.2 (3.6,8.6)               | 3.3 (2.4,5.3)                       | < 0.001 | 1# vs. 2# |
| Metabolism                 |                                     |                          |                             |                                     |      |               |
| Uric acid, umol/L          | 424 (363,493)                        | 412 (355,506)            | 413 (353,484)               | 392 (331,447)                       | < 0.001 | 1# vs. 2# |
| Hyperuricemia, n%          | 170 (40.9)                           | 19 (36.5)                | 104 (46.8)                  | 97 (27.0)                           | < 0.001 | 1# vs. 3# |
| Cholesterol, mmol/L        | 5.5 (4.9,6.1)                        | 5.1 (4.6,5.5)            | 5.5 (4.9,6.3)               | 5.1 (4.5,6.2)                       | 0.11 | 3# vs. 4#   |

Data are median (first quartile, third quartile), n (%), or mean ± SD (standard deviation).

† mean N = 630; ‡ mean N = 628; ¶ mean N = 630.

# 1- both GGT and ALT abnormal group; 2- ALT abnormal only group; 3- GGT abnormal only group; 4- GGT and ALT normalization group.
### Comparison of metabolic control among the four groups stratified according to ALT and GGT levels

| Variables                  | Both GGT and ALT abnormal (N = 415) | ALT Abnormal only (N = 52) | GGT Abnormal only (N = 222) | GGT and ALT normalization (N = 359) | P      | Post-hoc |
|----------------------------|-------------------------------------|----------------------------|-----------------------------|------------------------------------|--------|----------|
| Hypercholesterol, n%       | 162 (39.0)                          | 16 (30.8)                  | 93 (41.9)                   | 132 (36.7)                         | 0.41   |          |
| Triglyceride, mmol/L       | 1.8 (1.3,2.4)                       | 1.7 (1.6,2.1)              | 1.9 (1.5,2.8)               | 1.6 (1.1,2.1)                      | < 0.001| 0.32     |
| Hypertriglyceride, n%      | 246 (59.3)                          | 30 (57.7)                  | 136 (61.3)                  | 166 (46.2)                         | < 0.001| 0.83     |
| HDL-cholesterol, mmol/L    | 1.1 (1.0,1.3)                       | 1.1 (1.0,1.2)              | 1.2 (1.0,1.4)               | 1.2 (1.0,1.4)                      | 0.74   |          |
| LDL-cholesterol, mmol/L    | 3.5 (3.0,4.0)                       | 3.4 (3.0,3.7)              | 3.5 (3.0,4.0)               | 3.4 (2.8,4.1)                      | 0.55   |          |
| Free fatty acid, mmol/L    | 537 (436,710)                       | 472 (380,542)              | 548 (393,646)               | 525 (426,693)                      | 0.47   |          |
| Apolipoprotein-A, mmol/L   | 1.3 (1.1,1.4)                       | 1.2 (1.2,1.3)              | 1.3 (1.2,1.5)               | 1.3 (1.1,1.4)                      | 0.37   |          |
| Apolipoprotein-B, mmol/L   | 1.0 (0.9,1.2)                       | 0.9 (0.8,1.2)              | 1.0 (0.9,1.2)               | 1.0 (0.8,1.2)                      | 0.15   |          |
| Apolipoprotein-E, mmol/L   | 49 (40,56)                          | 39 (33,43)                 | 47 (42,55)                  | 42 (34,49)                         | 0.13   |          |
| Lipoprotein-a, mmol/L      | 139 (52,203)                        | 142 (70,314)               | 132 (56,239)                | 137 (60,276)                       | 0.03   | 0.63     |
| Fasting glucose, mmol/L    | 5.1 (4.6,5.6)                       | 5.0 (4.5,5.4)              | 5.0 (4.6,5.4)               | 5.0 (4.6,5.4)                      | 0.47   |          |
| Fasting insulin, uU/mL     | 10.9 (7.7,15.0)                     | 9.5 (7.2,22.0)             | 8.4 (6.5,10.7)              | 8.2 (5.9,11.3)                     | < 0.001| 0.81     |
| HOMA-IR                    | 2.5 (1.5,3.4)                       | 2.4 (1.5,5.6)              | 2.0 (1.4,2.4)               | 1.8 (1.3,2.5)                      | < 0.001| 0.92     |
| LFC †, %                   | 15.4 (10.0,21.9)                    | 14.9 (9.3,20.8)            | 12.5 (7.6,19.1)             | 10.8 (7.1,14.8)                    | < 0.001| 0.61     |
| PFC ‡, %                   | 2.1 (1.6,2.6)                       | 2.1 (1.4,2.7)              | 2.1 (1.5,2.9)               | 1.9 (1.4,2.7)                      | < 0.001| 0.15     |
| ASFT ‖, mm                 | 23 (18,30)                          | 23 (19,25)                 | 23 (17,28)                  | 23 (18,27)                         | 0.62   |          |
| Medication                 |                                    |                            |                            |                                    |        |          |
| Anti-hyperlipidemic drug, n(%) | 129 (31.1)                     | 16 (30.8)                  | 66 (29.7)                   | 105 (29.2)                         | 0.95   |          |
| Anti-Diabetes drug, n(%)   | 29 (7.0)                            | 4 (7.7)                    | 8 (3.6)                     | 21 (5.8)                           | 0.35   |          |
| Uric acid lowering drug, n(%) | 33 (8.0)                      | 7 (13.5)                   | 18 (8.1)                    | 25 (7.0)                           | 0.45   |          |

Data are median (first quartile, third quartile), n (%), or mean ± SD (standard deviation).

† mean N = 630; ‡ mean N = 628; ‖ mean N = 630.

# 1- both GGT and ALT abnormal group; 2- ALT abnormal only group; 3- GGT abnormal only group; 4- GGT and ALT normalization group.
In the baseline group with abnormal levels of both ALT and GGT, 74 patients were excluded after a median follow-up of 10.6 months because they attended less than 2 follow-up visits within 12 months, and the remaining 341 patients were further analyzed. Both ALT and GGT normalization were observed in 74 (21.7%) patients, including 105 (30.8%) patients with ALT normalization alone, 39 (11.4%) patients with GGT normalization alone, and 123 (36.1%) patients with persistent abnormal levels of both enzymes (Fig. 1). Compared with the other three groups, the weight and BMI of both the ALT and GGT normalization groups exhibited the greatest decrease (weight %: 5.6 vs. 1.3 vs. 1.4 vs. 0.5, P < 0.001; BMI (kg/m²): 1.6 vs. 0.4 vs. 0.1, P < 0.001, Table 2). A similar trend was also observed in lipid metabolism-related parameters in the group with both ALT and GGT normalization, including CHOL, TG, LDL-C and APOB (all, P < 0.05, Table 2). Significant differences in fasting blood glucose levels, fasting insulin levels and HOMA-IR levels were observed among the four groups (all, P < 0.05, Table 2).
Table 2
Biochemical and metabolic changes from baseline to month 12 in 341 patients with NAFLD presenting abnormal levels of both GGT and ALT at baseline.

| Variables          | Both GGT and ALT normalization (N = 74) | ALT normalization Only (N = 105) | GGT normalization Only (N = 39) | Both GGT and ALT abnormal (N = 123) | P         | Post-hoc |
|--------------------|----------------------------------------|----------------------------------|--------------------------------|-------------------------------------|-----------|----------|
|                    |                                       |                                  |                                |                                     |           | 1# vs. 2# | 1# vs. 3# | 1# vs. 4# | 2# vs. 3# | 2# vs. 4# | 3# vs. 4# |
| Weight change, kg  | -4.7 ± 6.1                             | -1.1 ± 4.1                       | -1.1 ± 2.4                     | -0.4 ± 3.0                          | < 0.001   | < 0.001  | < 0.001  | 0.94      | 0.18      | 0.40      |
| Weight change, %   | -5.6 ± 6.9                             | -1.3 ± 5.5                       | -1.4 ± 3.0                     | -0.5 ± 3.9                          | < 0.001   | < 0.001  | < 0.001  | 0.94      | 0.26      | 0.39      |
| BMI, kg/m²         | -1.6 ± 2.0                             | -0.4 ± 1.2                       | -0.4 ± 0.8                     | -0.1 ± 1.1                          | < 0.001   | < 0.001  | < 0.001  | 0.94      | 0.13      | 0.33      |
| Waist-hip ratio    | -0.05 ± 0.2                            | -0.02 ± 0.1                      | 0.01 ± 0.1                     | -0.03 ± 0.2                         | 0.51      |          |          |          |           |           |
| ALT, U/L           | -70 (-88,55)                           | -43 (-73,29)                     | -27 (-37,-26)                  | -20 (-33,0)                         | 0.05      |          |          |          |           |           |
| AST, U/L           | -23 (44,17)                            | -14 (429,-7)                     | -5 (-13,-2)                    | 0 (-16,2)                           | 0.03      | 0.79     | 0.12     | 0.02      | 0.15      | 0.87      |
| GGT, U/L           | -46 (-57,28)                           | -26 (-82,-3)                     | -12 (-21,0)                    | 0 (-25,6)                           | < 0.001   | 0.48     | 0.01     | 0.18      | 0.01      | 0.97      |
| ALP, U/L           | -1 (-5,2)                              | -4 (-22,1)                       | 0 (-1,7)                       | -2 (-36,0)                          | 0.16      |          |          |          |           |           |
| TBil, umol/L       | -0.4 ± 6.5                             | -1.9 ± 9.5                       | -0.7 ± 5.0                     | -1.2 ± 8.2                          | 0.76      |          |          |          |           |           |
| DBil, umol/L       | -0.3 ± 2.1                             | -0.8 ± 4.2                       | -0.1 ± 1.5                     | -0.9 ± 4.7                          | 0.63      |          |          |          |           |           |
| TBA, umol/L        | 0.3 ± 3.4                              | 0.2 ± 4.2                        | -1.3 ± 3.4                     | 1.6 ± 8.1                           | 0.60      |          |          |          |           |           |
| LDH, U/L           | -9.6 ± 46.9                            | -4.9 ± 36.7                      | -6.6 ± 29.7                    | -9.3 ± 36.9                         | 0.96      |          |          |          |           |           |
| CHE, U/L           | -913 (-1668,539)                       | -1009 (-1339,576)                | -1046 (-1227,363)              | -338 (-511,-137)                    | 0.20      |          |          |          |           |           |
| LAP, U/L           | -4.6 ± 21.3                            | -9.4 ± 22.2                      | -1.6 ± 8.8                     | -6.6 ± 19.1                         | 0.77      |          |          |          |           |           |
| GLDH, U/L          | -3.4 ± 8.6                             | -1.3 ± 7.6                       | -2.7 ± 5.9                     | -2.1 ± 9.8                          | 0.86      |          |          |          |           |           |
| UA, umol/L         | -34 (-86,-4)                           | -25 (-154,49)                    | -26 (-58,2)                    | -19 (-55,16)                        | 0.28      |          |          |          |           |           |
| CHOL, mmol/L       | -1.2 ± 1.9                             | -0.3 ± 1.3                       | -0.2 ± 1.1                     | -0.6 ± 1.6                          | 0.01      | 0.01     | 0.01     | 0.02      | 0.79      | 0.25      | 0.29      |
| TG, mmol/L         | -0.7 ± 1.1                             | -0.2 ± 0.8                       | -0.1 ± 0.8                     | -0.3 ± 1.1                          | 0.01      | 0.02     | 0.01     | 0.01      | 0.73      | 0.38      | 0.34      |
| HDL-C, mmol/L      | -0.2 ± 0.5                             | -0.1 ± 0.3                       | 0.1 ± 0.2                      | -0.1 ± 0.6                          | 0.54      |          |          |          |           |           |           |
| LDL-C, mmol/L      | -0.8 ± 1.4                             | -0.3 ± 1.1                       | -0.2 ± 0.9                     | -0.3 ± 1.1                          | 0.02      | 0.01     | 0.02     | 0.01      | 0.72      | 0.84      | 0.81      |
| FFA, mmol/L        | -20 (-93,25)                           | -44 (-133,-6)                    | -95 (-150,55)                  | -17 (-159,20)                       | 0.37      |          |          |          |           |           |           |
| APOA, mmol/L       | -0.1 ± 0.4                             | 0.1 ± 0.4                        | 0.1 ± 0.3                      | -0.1 ± 0.5                          | 0.06      |          |          |          |           |           |           |
| APOB, mmol/L       | -0.1 ± 0.3                             | -0.1 ± 0.3                       | -0.1 ± 0.2                     | -0.2 ± 0.4                          | 0.03      | 0.82     | 0.79     | 0.02      | 0.93      | 0.02      | 0.12      |

Data are median (first quartile, third quartile) or mean ± SD (standard deviation).

# 1- both GGT and ALT normalization group; 2- ALT normalization only group; 3- GGT normalization only group; 4- both GGT and ALT abnormal group.
| Variables       | Both GGT and ALT normalization (N = 74) | ALT normalization Only (N = 105) | GGT normalization Only (N = 39) | Both GGT and ALT abnormal (N = 123) | P      | Post-hoc |
|----------------|----------------------------------------|---------------------------------|---------------------------------|-------------------------------------|--------|----------|
| APOE, mmol/L   | -4.0 (-11.5,15.5)                      | 1.0 (-13.9,8.5)                 | 4.0 (-12.0,5.0)                  | -6.5 (-16.0,3.0)                    | 0.39   |          |
| LPA, mmol/L    | -70 (-140,40)                         | -7 (-71,74)                     | -34 (-116,19)                   | -30 (-102,63)                      | 0.61   |          |
| FBG, mmol/L    | -0.7 ± 1.6                            | -0.2 ± 1.3                      | -0.2 ± 1.2                      | -0.1 ± 1.4                         | 0.04   | 0.06     |
| FINS, uU/mL    | -2.9 ± 5.7                            | -1.2 ± 8.7                      | -1.1 ± 6.3                      | -0.7 ± 4.9                         | 0.02   | 0.04     |
| HOMA-IR        | -0.9 ± 1.3                            | -0.2 ± 2.1                      | -0.3 ± 1.2                      | -0.1 ± 1.4                         | 0.02   | 0.04     |

Data are median (first quartile, third quartile) or mean ± SD (standard deviation).

# 1- both GGT and ALT normalization group; 2- ALT normalization only group; 3- GGT normalization only group; 4- both GGT and ALT abnormal group.

Patients in the baseline group with abnormal levels of both ALT and GGT were monitored for 12 months to detect the normalization rate of GGT and ALT levels during treatment. After 6, 9, and 12 months of follow-up, the cumulative normalization rates of GGT levels were 17%, 25% and 33%, and the cumulative normalization rates of ALT levels were 27%, 41%, and 52% (all, P < 0.01, Fig. 2A), respectively. These patients were further divided into 5 groups according to the weight change after 12 months of treatment as follows: ≤ 3% (n = 232), 3–5% (n = 43), 5–7% (n = 30), 7–10% (n = 21) and > 10% (n = 15). In patients with a weight change ratio ≤ 3%, the cumulative normalization rates of GGT levels were significantly lower than in the other four groups (15% vs. 20%, P < 0.05, Fig. 2B).

We also described the changes in normalization rates in the ALT-only abnormal group and GGT-only abnormal group after 12 months of treatment. For the patients in the ALT-only abnormal group, the cumulative normalization rate of ALT levels was 62% after 12 months of follow-up (Fig. 2C), with the lowest cumulative normalization rates observed in patients with weight change ratios ≤ 3% (Fig. 2D). In the GGT-only abnormal group, the cumulative normalization rate of GGT levels after 12 months of follow-up was 37% (Fig. 2E), and a similar association with weight change was found (Fig. 2F).

**Predictors of GGT remission in patients with NAFLD after 12 months of treatment**

A univariate logistic regression analysis showed that weight loss, baseline levels of GGT, CHOL, TG and FINS and the normalization of ALT, UA, TG, FBG and HOMA-IR after treatment were independent factors influencing the recovery of GGT levels in 341 patients with both baseline ALT and GGT abnormalities (Table 3). After multivariate adjustment, weight loss (OR = 1.27, 95%CI:1.15–1.41, P < 0.001), normalization of ALT level (OR = 4.32, 95%CI:1.87–9.96, P = 0.01) and HOMA-IR level after treatment (OR = 3.48, 95%CI:1.60–7.57, P = 0.01) were independent protective factors for GGT normalization (Table 3). Baseline GGT level (OR = 0.99, 95%CI:0.98–0.99, P = 0.01), CHOL level (OR = 0.47, 95%CI:0.23–0.96, P = 0.04) and FINS level (OR = 0.91, 95%CI:0.86–0.96, P = 0.01) were independent risk factors related to GGT normalization (Table 3). In the subgroup of 179 patients with NAFLD whose ALT levels returned to normal after 12 months of treatment, the multivariate regression analysis showed that weight loss (OR = 1.56, 95%CI:1.27–1.92, P < 0.001) and decrease in HOMA-IR to normal levels after treatment (OR = 3.55, 95%CI:1.11–11.34, P = 0.03) remained independent factors related to GGT normalization (Table 3).
Table 3
Factors associated with GGT normalization in patients with NAFLD after 12 months of treatment predicted by the logistic regression model.

| Factors                              | Overall cohort (N = 341) | Achieve ALT normalization subgroup (N = 179) |
|--------------------------------------|--------------------------|---------------------------------------------|
|                                      | Univariate analysis      | Multivariable analysis                      | Univariate analysis | Multivariable analysis |
|                                      | OR (95%CI)               | p                                           | OR (95%CI)          | p                         |
|                                      |                          |                                             | OR (95%CI)          | p                         |
|                                      |                          |                                             | OR (95%CI)          | p                         |
| Baseline body weight, kg             | 1.01 (0.99–1.03)         | 0.15                                        | 1.01 (0.99–1.04)    | 0.29                      |
| Weight loss, %                       | 1.23 (1.14–1.33)         | < 0.001                                     | 1.27 (1.15–1.41)    | < 0.001                   |
|                                      |                          |                                             | 1.53 (1.26–1.84)    | < 0.001                   |
|                                      |                          |                                             | 1.56 (1.27–1.92)    | < 0.001                   |
| Hypertension a                       | 0.86 (0.54–1.38)         | 0.54                                        | 0.83 (0.35–1.95)    | 0.67                      |
| Smoke a                              | 0.16 (0.02–1.72)         | 0.13                                        | 0.17 (0.02–1.78)    | 0.21                      |
| Baseline ALT, U/L                    | 0.99 (0.99–1.01)         | 0.36                                        | 1.00 (0.99–1.01)    | 0.57                      |
| ALT decreased to normal a           | 17.91 (9.80–32.74)       | < 0.001                                     | 4.32 (1.87–9.96)    | 0.01                      |
| Baseline GGT, U/L                   | 0.67 (0.54–0.83)         | < 0.001                                     | 0.99 (0.98–0.99)    | 0.01                      |
|                                      |                          |                                             | 0.86 (0.75–0.99)    | 0.04                      |
|                                      |                          |                                             | 1.00 (0.99–1.01)    | 0.74                      |
| Change of TBil, umol/L              | 0.99 (0.97–1.02)         | 0.66                                        | 0.97 (0.93–1.02)    | 0.24                      |
| Change of DBil, umol/L              | 0.98 (0.92–1.05)         | 0.54                                        | 0.93 (0.81–1.06)    | 0.27                      |
| UA decreased to normal a            | 1.78 (1.10–2.89)         | 0.02                                        | 1.30 (0.66–2.57)    | 0.45                      |
|                                      |                          |                                             | 1.07 (0.49–2.33)    | 0.87                      |
| Baseline CHOL, mmol/L               | 0.60 (0.37–0.96)         | 0.03                                        | 0.47 (0.23–0.96)    | 0.04                      |
|                                      |                          |                                             | 0.73 (0.33–1.61)    | 0.43                      |
| CHOL decreased to normal a          | 0.58 (0.30–1.10)         | 0.09                                        | 4.58 (1.37–15.32)   | 0.01                      |
|                                      |                          |                                             | 2.73 (0.84–8.79)    | 0.09                      |
| Baseline TG, mmol/L                 | 0.79 (0.64–0.98)         | 0.03                                        | 0.82 (0.58–1.15)    | 0.26                      |
|                                      |                          |                                             | 0.87 (0.41–1.88)    | 0.73                      |
| TG decreased to normal a            | 2.37 (1.21–4.63)         | 0.01                                        | 1.84 (0.86–3.96)    | 0.12                      |
|                                      |                          |                                             | 2.29 (1.00–5.27)    | 0.05                      |
| Baseline LDL-C, mmol/L              | 0.98 (0.79–1.22)         | 0.91                                        | 0.63 (0.38–1.04)    | 0.07                      |
| LDL-C decreased to normal a         | 1.53 (0.96–2.44)         | 0.07                                        | 1.93 (0.87–4.26)    | 0.11                      |
| FBG decreased to normal a           | 2.37 (1.21–4.63)         | 0.01                                        | 2.22 (0.79–6.23)    | 0.13                      |
|                                      |                          |                                             | 1.04 (0.64–1.71)    | 0.86                      |
| Baseline FINS, uU/mL                | 0.93 (0.89–0.97)         | < 0.001                                     | 0.91 (0.86–0.96)    | 0.01                      |
|                                      |                          |                                             | 0.92 (0.85–0.99)    | 0.03                      |
|                                      |                          |                                             | 1.04 (0.94–1.14)    | 0.46                      |
| FINS decreased to normal a          | 2.26 (0.79–6.44)         | 0.13                                        | 3.33 (0.36–31.03)   | 0.29                      |
| HOMA-IR decreased to normal a       | 4.35 (2.36–8.01)         | < 0.001                                     | 3.48 (1.60–7.57)    | 0.01                      |
|                                      |                          |                                             | 3.85 (1.62–9.18)    | 0.01                      |
|                                      |                          |                                             | 3.55 (1.11–11.34)   | 0.03                      |

a Hypertension was defined as systolic blood pressure (SBP) ≥ 140 mmHg or diastolic blood pressure (DBP) ≥ 90 mmHg; Smoke was defined as smoking at least one cigarette in the last 1 month; ALT decreased to normal was defined as ALT level ≤ 40 U/L; UA decreased to normal was defined as uric acid level ≤ 420 umol/L for male and ≤ 360 umol/L for female; CHOL decreased to normal was defined as CHOL level ≤ 5.7 mmol/L; TG decreased to normal was defined as TG level ≤ 1.7 mmol/L; LDL-C decreased to normal was defined as LDL-C level ≤ 3.4 mmol/L; FBG decreased to normal was defined as FBG level ≤ 6 mmol/L; FINS decreased to normal was defined as FINS level ≤ 23 uU/mL; HOMA-IR decreased to normal was defined as HOMA-IR level ≤ 2.69.

We further explored the application of the factors identified by the logistic model as predictors of GGT normalization and found that the weight change, baseline GGT level, ALT normalization, baseline FINS level and normalization of HOMA-IR, but not CHOL level at baseline, exhibited significant values for areas under the ROC curves (P < 0.05, Fig. 3A). The combination of these factors obtained an area under curve (AUC) of 0.905 (P < 0.001, Fig. 3A). For patients with ALT remission, we identified that a weight change, normalization of HOMA-IR, and their combination were able to predict GGT remission with AUCs of 0.814, 0.729 and 0.905, respectively (all, P < 0.001, Fig. 3B).
Predictors of ALT remission in patients with NAFLD after 12 months of treatment

The univariate logistic regression analysis showed that changes in weight loss, baseline levels of ALT, TG and FINS and the normalization of GGT, UA, TG and LDL-C after treatment were independent factors influencing the recovery of ALT levels in subjects with both abnormal ALT and GGT levels at baseline (Table 4). After the multivariate analysis, weight loss (OR = 1.15, 95%CI:1.07–1.25, P = 0.01) and GGT level that decreased to normal (OR = 3.05, 95%CI:1.57–5.94, P < 0.001) remained independent protective factors for ALT normalization (Table 4). In 113 patients with NAFLD whose GGT levels returned to normal after 12 months of treatment, only weight loss was an independent protective factor influencing the recovery of ALT levels (OR = 1.14, 95%CI:1.01–1.29,P = 0.04, Table 4).
## Table 4
Factors associated with ALT normalization in patients with NAFLD after 12 months of treatment predicted by the logistic regression model.

| Factors                      | Overall cohort (N = 341) | Achieve GGT normalization subgroup (N = 113) |
|------------------------------|--------------------------|---------------------------------------------|
|                              | Univariate analysis      | Multivariable analysis                      | Univariate analysis | Multivariable analysis |
|                              | OR (95%CI)               | p                                           | OR (95%CI)         | p                 |
|                              | Odds ratio               | p                                           | OR (95%CI)         | p                 |
| Baseline body weight, kg     | 0.99 (0.97–1.01)         | 0.47                                        | 0.99 (0.96–1.01)   | 0.28              |
| Weight loss, %               | 1.14 (1.06–1.22)         | < 0.001                                     | 1.15 (1.07–1.25)   | 0.01              |
| Hypertension<sup>b</sup>     | 0.85 (0.55–1.32)         | 0.47                                        | 0.58 (0.25–1.34)   | 0.21              |
| Smoke<sup>b</sup>            | 0.87 (0.08–9.79)         | 0.91                                        | 0.86 (0.05–8.51)   | 0.54              |
| Baseline ALT, U/L            | 0.90 (0.81–0.99)         | 0.04                                        | 0.99 (0.99–1.01)   | 0.47              |
| Baseline GGT, U/L            | 1.00 (0.99–1.00)         | 0.56                                        | 1.02 (0.99–1.03)   | 0.62              |
| GGT decreased to normal<sup>b</sup> | 15.03 (7.52–26.01)     | < 0.001                                     | 3.05 (1.57–5.94)   | < 0.001           |
| Change of TBil, umol/L       | 1.01 (0.98–1.03)         | 0.56                                        | 1.02 (0.95–1.09)   | 0.54              |
| Change of DBil, umol/L       | 1.01 (0.96–1.06)         | 0.85                                        | 1.01 (0.82–1.25)   | 0.92              |
| Change of CHE, U/L           | 1.00 (1.00–1.00)         | 0.47                                        | 1.00 (1.00–1.00)   | 0.71              |
| Change of LAP, U/L           | 0.99 (0.98–1.02)         | 0.87                                        | 0.99 (0.92–1.06)   | 0.76              |
| UA decreased to normal<sup>b</sup> | 2.46 (1.53–3.95)         | < 0.001                                     | 1.99 (0.92–4.35)   | 0.08              |
| Baseline CHOL, mmol/L        | 0.77 (0.50–1.18)         | 0.22                                        | 1.09 (0.47–2.49)   | 0.85              |
| CHOL decreased to normal<sup>b</sup> | 0.78 (0.44–1.37)         | 0.38                                        | 3.35 (0.96–11.67)  | 0.06              |
| Baseline TG, mmol/L          | 0.78 (0.64–0.95)         | 0.01                                        | 0.81 (0.61–1.06)   | 0.13              |
| TG decreased to normal<sup>b</sup> | 5.59 (3.47–9.03)         | < 0.001                                     | 1.53 (0.76–3.07)   | 0.23              |
| Baseline LDL-C, mmol/L       | 0.93 (0.76–1.14)         | 0.52                                        | 0.64 (0.38–1.09)   | 0.09              |
| LDL-C decreased to normal<sup>b</sup> | 1.89 (1.21–2.98)         | 0.01                                        | 1.78 (0.84–3.76)   | 0.13              |
| FBG decreased to normal<sup>b</sup> | 1.53 (0.76–3.08)         | 0.24                                        | 1.42 (0.40–3.26)   | 0.81              |
| Baseline FINS, uU/mL         | 0.96 (0.93–0.99)         | 0.04                                        | 0.95 (0.91–1.01)   | 0.06              |
| FINS decreased to normal<sup>b</sup> | 2.21 (0.78–6.32)         | 0.14                                        | 3.25 (0.35–30.28)  | 0.30              |
| HOMA-IR decreased to normal<sup>b</sup> | 1.51 (0.87–2.62)         | 0.14                                        | 1.18 (0.46–2.99)   | 0.73              |

<sup>b</sup> Hypertension was defined as systolic blood pressure (SBP) ≥ 140 mmHg or diastolic blood pressure (DBP) ≥ 90 mmHg; Smoke was defined as smoking at least one cigarette in the last 1 month; GGT decreased to normal was defined as GGT level ≤ 50 U/L; UA decreased to normal was defined as uric acid level ≤ 420 umol/L for male and ≤ 360 umol/L for female; CHOL decreased to normal was defined as CHOL level ≤ 5.7 mmol/L; TG decreased to normal was defined as TG level ≤ 1.7 mmol/L; LDL-C decreased to normal was defined as LDL-C level ≤ 3.4 mmol/L; FBG decreased to normal was defined as FBG level ≤ 6 mmol/L; FINS decreased to normal was defined as FINS level ≤ 23 uU/mL; HOMA-IR decreased to normal was defined as HOMA-IR level ≤ 2.69.
Dyslipidemia, insulin resistance and uric acid levels may be necessary to achieve GGT normalization, which may reduce inflammation and prevent treatment in patients with NAFLD, their associations with metabolic parameters were different. Our study emphasized that the rectification of Overall, although both serum ALT and GGT levels are useful markers for the noninvasive surveillance of the hepatic histological response to studies are warranted to clarify the relationships between the levels of GGT and other liver enzymes when monitoring NAFLD severity.

The current study had several limitations. First, the relationship between liver biochemical parameters and histological inflammation or the degree of fibrosis in patients with NAFLD was not estimated because the majority of these patients did not undergo liver biopsy in the follow-up period. Second, the findings of our study may not be generalizable to patients with NAFLD and other known causes of GGT abnormalities. Finally, additional studies are warranted to clarify the relationships between the levels of GGT and other liver enzymes when monitoring NAFLD severity.

Conclusions

Overall, although both serum ALT and GGT levels are useful markers for the noninvasive surveillance of the hepatic histological response to treatment in patients with NAFLD, their associations with metabolic parameters were different. Our study emphasized that the rectification of

In addition, 630 patients with NAFLD underwent MRI-PDFF to quantify liver fat. We identified a weak correlation between baseline GGT levels and the LFC (R = 0.08, P = 0.04, Fig. 4A). After 12 months of treatment, the correlation between GGT levels and LFC increased (R = 0.26, P = 0.01, Fig. 4B), as well as the changes in GGT levels and the changes in the LFC (R = 0.22, P = 0.02, Fig. 4C).

Discussion

In this prospective cohort study of 1048 consecutive patients with NAFLD with and without baseline increases in liver enzyme levels, we observed an association between increased GGT levels at baseline and higher triglyceride and cholesterol levels compared to patients with abnormal ALT levels alone. Moreover, for patients with concurrently increased ALT and GGT levels during follow-up, a lower normalization rate was observed for GGT than for ALT. Significant associations between the normalization of GGT levels and target uric acid, triglyceride and HOMA-IR levels, but not cholesterol levels, were observed in addition to weight loss. As shown in the present study, metabolic control was an independent protective factor for persistent abnormal GGT levels in NAFLD management.

GGT and ALT levels have been well accepted as important variables for screening NAFLD [6, 26, 27] because both indicators are tested using a simple routine method in clinical or epidemiologic settings. In the present study, the prevalence of simultaneous GGT and ALT abnormalities in Chinese patients with NAFLD was 39.6%, while the abnormal GGT rate was 21.2%, which is much higher than the prevalence of abnormal ALT levels (5.0%). These findings were similar to another large-scale cross-sectional study conducted in China, in which over 50% of patients with NAFLD presented abnormal serum liver enzyme levels [28]. Although both ALT, AST and GGT are considered markers of liver injury because they are released from disrupted hepatocytes in patients with NAFLD, the levels of GGT, a key enzyme involved in glutathione and cysteine metabolism, are increased not only in patients with NAFLD but also in patients with many other conditions, including oxidative stress, cholestatic liver disease and ethanol exposure [29]. In patients with NAFLD and oxidative stress, increased bile pressure secondary to steatosis (micro cholestasis) has been identified as an important pathogenic mechanism may help to explain its higher specificity for NAFLD than ALT.

Both GGT and ALT normalization were proposed as predictors of a histological improvement in routine management of patients with NAFLD [10]. The dynamic association between liver biochemical markers and disease progression is complicated and multifactorial [10]. However, the differences in dynamic changes between serum GGT and ALT activity have seldom been reported during NAFLD treatment [30]. In this study, we described distinct decreasing trends in ALT and GGT levels after the intervention was initiated, with GGT levels presenting a much slower restoration than ALT levels. Interestingly, in addition to the common factor of weight loss, different factors have also been shown to be independently associated with ALT or GGT normalization. Persistent GGT abnormalities tended to be associated with poor metabolic control, including uric acid levels, triglyceride levels and HOMA-IR levels. Emerging evidence supports a close association between metabolic dysregulation and GGT levels in patients with NAFLD [31]. Another Brazilian study reported an increase in GGT levels as the degree of steatosis increased, as insulin resistance has been identified as the acknowledged mechanism driving hepatic de novo lipid synthesis, which would result in the increased release of fatty acids and the derived products, such as triglycerides and cholesterol [31]. Using a multivariate linear regression analysis, a significant positive correlation was observed between GGT and HOMA-IR (standard β = 0.252) in a population-based cross-sectional study conducted in a Chinese population [31]. In the Framingham offspring study with a 20-year follow-up period, GGT level quartiles at baseline exhibited dose-response effects on the occurrence of cardiac risk factors, including serum lipid profiles, blood glucose levels and the development of diabetes [32]. Our research expanded on a previous cross-sectional study investigating the correlation between changes in GGT levels with IR and related metabolic dysfunction for the first time, and our study provided novel findings that the degree of IR decreased to normal and other related indexes that were reduced to target levels by the treatment are potential predictors of restored GGT levels, suggesting that the clinical value of GGT differs from ALT as a noninvasive monitoring parameter to directly estimate the posttreatment severity of NAFLD [10].

The current study had several limitations. First, the relationship between liver biochemical parameters and histological inflammation or the degree of fibrosis in patients with NAFLD was not estimated because the majority of these patients did not undergo liver biopsy in the follow-up period. Second, the findings of our study may not be generalizable to patients with NAFLD and other known causes of GGT abnormalities. Finally, additional studies are warranted to clarify the relationships between the levels of GGT and other liver enzymes when monitoring NAFLD severity.
the deterioration of fibrosis in patients with NAFLD. In contrast, individuals with NAFLD presenting persistently elevated GGT levels may benefit from earlier intensive interventions with drugs for metabolic control, combined with an appropriate diet and exercise strategy.

Abbreviations

NAFLD: Nonalcoholic fatty liver disease; MRI-PDFF: Magnetic resonance imaging-based proton density fat fraction; BMI: Body mass index; GGT: Gamma-glutamyl transferase; ALT: Alanine aminotransferase; OR: Odds ratio; CI: Confidence interval; HOMA-IR: Homeostasis model assessment of insulin resistance; CHOL: Cholesterol; TG: Triglyceride; MAFLD: Metabolic associated fatty liver disease; T2DM: Type 2 diabetes mellitus; WHR: Waist-to-hip ratio; AST: Aspartate aminotransferase; ALP: Alkaline protease; TBil: Total bilirubin; DBil: Direct bilirubin; TBA: Total bile acid; LDH: Lactate dehydrogenase; CHE: Choline esterase; LAP: Leucine arylamidase; GLDH: Glutamate dehydrogenase; UA: Uric acid; HDL-C: High density lipoprotein-cholesterol; LDL-C: Low density lipoprotein-cholesterol; FFA: Free fatty acid; APOA: Apolipoprotein-A; APOB: Apolipoprotein-B; APOE: Apolipoprotein-E; LPA: Lipoprotein-a; FBG: Fasting blood glucose; FINS: Fasting insulin; IR: Insulin resistance; SD: Standard deviations; IQR: Interquartile ranges; ROC: Receiver operating characteristic; AUC: Area under curve; LFC: Liver fat content; PFC: Pancreas fat content; ASFT: Abdominal subcutaneous fat thickness.

Declarations

Acknowledgements

We are grateful to Professor Aihua Lin from Department of Medical Statistics, School of Public Health, Sun Yat-sen University provide assistance in statistical analysis to this study.

Authors’ contributions

BZ and JY participated in design and critical revision of the manuscript for important intellectual content. QM participated in baseline data collection, analysis and manuscript drafting. XL participated in data analysis and interpretation of results. CS, TW and YL completed baseline data collection, cases follow-up and data verification. YS and SF participated in critical revision of the manuscript for important intellectual content. All authors read and approved the final manuscript.

Funding

This study is funded by National Natural Science Foundation of China (81670518, 81870404, 81170392).

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The clinical research ethics committee of the First Affiliated Hospital of Sun Yat-sen University approved the research plan, and all subjects provided written informed consent.

Consent for publication

Not applicable.

Competing interests

All authors declare that they have no competing interests.

References

1. Younossi ZM, Koenig AB, Abdelatif D, et al. Global epidemiology of nonalcoholic fatty liver disease-meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology. 2016;64(1):73-84.
2. Estes C, Razavi H, Loomba R, et al. Modeling the epidemic of nonalcoholic fatty liver disease demonstrates an exponential increase in burden of disease. Hepatology. 2018;67(1):123-133.

3. Sheka AC, Adeyi O, Thompson J, et al. Nonalcoholic steatohepatitis: a review. JAMA. 2020;323(12):1175-1183.

4. Medrano M, Arenaza L, Migueles JH, et al. Associations of physical activity and fitness with hepatic steatosis, liver enzymes, and insulin resistance in children with overweight/obesity. Pediatr Diabetes. 2020;21(4):565-574.

5. Zhang Y, He H, Zeng YP, et al. Lipoprotein A, combined with alanine aminotransferase and aspartate aminotransferase, contributes to predicting the occurrence of NASH: a cross-sectional study. Lipids Health Dis. 2020;19(1):134.

6. Feng G, He N, Zhou YF, et al. A simpler diagnostic formula for screening nonalcoholic fatty liver disease. Clin Biochem. 2019;64:18-23.

7. Zhao W, Tong J, Liu J, et al. The dose-response relationship between gamma-glutamyl transferase and risk of diabetes mellitus using publicly available data: a longitudinal study in Japan. Int J Endocrinol. 2020;2020:5356498.

8. Rajput R, Ahlawat P. Prevalence and predictors of non-alcoholic fatty liver disease in prediabetes. Diabetes Metab Syndr. 2019;13(5):2957-2960.

9. Hernandez M, Klunder M, Nieto NG, et al. Pediatric visceral adiposity index adaptation correlates with HOMA-IR, Matsuda, and transaminase. Endocr Pract. 2018;24(3):294-301.

10. Newton KP, Lavine JE, Wilson L, et al. Alanine aminotransferase and gamma glutamyl transpeptidase predict histologic improvement in pediatric nonalcoholic steatohepatitis. Hepatology. 2020.

11. Eslam M, Sarin S K, Wong V W, et al. The asian pacific association for the study of the liver clinical practice guidelines for the diagnosis and management of metabolic associated fatty liver disease. Hepatol Int. 2020.

12. Wong V W, Wong G L, Chan R S, et al. Beneficial effects of lifestyle intervention in non-obese patients with non-alcoholic fatty liver disease. J Hepatol. 2018;69(6):1349-1356.

13. Petta S, Wong V W, Camma C, et al. Serial combination of non-invasive tools improves the diagnostic accuracy of severe liver fibrosis in patients with NAFLD. Aliment Pharmacol Ther. 2017;46(6):617-627.

14. Li R, Liao X H, Ye J Z, et al. Association of keratin 8/18 variants with non-alcoholic fatty liver disease and insulin resistance in Chinese patients: a case-control study. World J Gastroenterol. 2017;23(22):4047-4053.

15. Caussy C, Alquiraiash M H, Nguyen P, et al. Optimal threshold of controlled attenuation parameter with MRI-PDFF as the gold standard for the detection of hepatic steatosis. Hepatology. 2018;67(4):1348-1359.

16. Jayakumar S, Middleton M S, Lawitz E J, et al. Longitudinal correlations between MRE, MRI-PDFF, and liver histology in patients with non-alcoholic steatohepatitis: analysis of data from a phase II trial of selonsertib. J Hepatol. 2019;70(1):133-141.

17. Ye J, Wu Y, Li F, et al. Effect of orlistat on liver fat content in patients with nonalcoholic fatty liver disease with obesity: assessment using magnetic resonance imaging-derived proton density fat fraction. Therap Adv Gastroenterol. 2019;12:1756284819879047.

18. Chinese Nutrition Society. Chinese Dietary Reference Intakes (2013). Beijing: Science Press. 2013;pp.660.

19. Chinese Nutrition Society. Chinese Dietary Guidelines (2007). Beijing: The Tibet People's Publishing House. 2008;pp.205.

20. Waxman A, et al. WHO global strategy on diet, physical activity and health. Food Nutr Bull. 2004;25(3):292-302.

21. Mei C, Ge J, Zou H, et al. Chinese multidisciplinary expert consensus on the diagnosis and treatment of hyperuricemia and related diseases. Chin Med J (Engl). 2017;130(20):2473-2488.

22. Park S, Buranakitjaroen P, Chen C H, et al. Expert panel consensus recommendations for home blood pressure monitoring in Asia: the hope Asia network. J Hum Hypertens. 2018;32(4):249-258.

23. Weng J, Ji L, Jia W, et al. Standards of care for type 2 diabetes in China. Diabetes Metab Res Rev. 2016;32(5):442-458.

24. Yu K H, Chen D Y, Chen J H, et al. Management of gout and hyperuricemia: multidisciplinary consensus in Taiwan. Int J Rheum Dis. 2018;21(4):772-787.

25. Ye J, Wu Y, Li F, et al. Effect of orlistat on liver fat content in patients with nonalcoholic fatty liver disease with obesity: assessment using magnetic resonance imaging-derived proton density fat fraction. Therap Adv Gastroenterol. 2019;12:1756284819879047.

26. Shin D, Kongpaksarn K, Bohra C. Trends in the prevalence of metabolic syndrome and its components in the United States 2007-2014. Int J Cardiol. 2018;259:216-219.

27. Sogabe M, Okahisa T, Kurihara T, et al. Differences among patients with and without nonalcoholic fatty liver disease having elevated alanine aminotransferase levels at various stages of metabolic syndrome. PLoS One. 2020;15(8):e0238388.

28. Bi W R, Yang C Q, Shi Q, et al. Large-scale analysis of factors influencing nonalcoholic fatty liver disease and its relationship with liver enzymes. Genet Mol Res. 2014;13(3):5880-5891.

29. Neuman M G, Malnick S, Chertin L. Gamma glutamyl transferase—an underestimated marker for cardiovascular disease and the metabolic syndrome. J Pharm Pharm Sci. 2020;23(1):65-74.

30. Ryan M C, Itsiopoulos C, Thodis T, et al. The Mediterranean diet improves hepatic steatosis and insulin sensitivity in individuals with non-alcoholic fatty liver disease. J Hepatol. 2013;59(1):138-143.
31. Cruz M A, Cruz J F, Macena L B, et al. Association of the nonalcoholic hepatic steatosis and its degrees with the values of liver enzymes and homeostasis model assessment-insulin resistance index. Gastroenterology Res. 2015;8(5):260-264.

32. Lee D S, Evans J C, Robins S J, et al. Gamma glutamyl transferase and metabolic syndrome, cardiovascular disease, and mortality risk: the Framingham Heart Study. Arterioscler Thromb Vasc Biol. 2007;27(1):127-133.

**Figures**

**Figure 1**

Flow diagram of participant recruitment, screening and allocation.
Figure 2

The cumulative normalization rates of ALT and GGT levels after 12 months of treatment. (a) The cumulative normalization rates of ALT and GGT levels at baseline in the group with both abnormal ALT and GGT levels. (b) The relationship between the weight change ratio and ALT or GGT normalization rates at baseline in the group with abnormal levels of both ALT and GGT. (c) The cumulative normalization rate of ALT levels in the baseline ALT-only abnormal group. (d) The relationship between the weight change ratio and ALT normalization rate in the baseline ALT-only abnormal group. (e) The cumulative normalization rate of GGT levels in the baseline GGT-only abnormal group. (f) The relationship between the weight change ratio and GGT normalization rate in the baseline GGT-only abnormal group. * P < 0.05, ** P < 0.01, *** P < 0.001.
Figure 3

ROC curves for GGT and ALT predicting the return to normal levels in 341 people with abnormal GGT and ALT levels at baseline. Prediction of GGT normalization in all populations (a) and the normal ALT population (b). Prediction of ALT normalization in all populations (c) and the normal GGT population (d). Abbreviations: ALT_N: ALT decreased to normal was defined as ALT level \( \leq 40 \text{ U/L} \); HOMA-IR_N: HOMA-IR decreased to normal was defined as HOMA-IR \( \leq 2.69 \); GGT_N: GGT decreased to normal was defined as GGT level \( \leq 50 \text{ U/L} \).

Figure 4

The correlation between liver fat content determined using MRI-PDFF and GGT levels in 630 patients with NAFLD. Scatter plots of the correlation between GGT levels and the liver fat content at (a) baseline, (b) after 12 months of treatment, and (c) changes from baseline to month 12.