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Complex of Amino Acids, Choline, and B-Group Vitamins for the Improvement of Early-Stage Metabolic Dysfunction-Associated Steatotic Liver Disease

Natalija Murane[1], Klinta Luize Sprudza[2], Jelena Ivanova[3,4], Polina Zalizko[1,2,3,4*]

[1]Faculty of Medicine and Life Sciences, University of Latvia, Riga, Latvia
[2]Faculty of Medicine, Riga Stradins University, Riga, Latvia
[3]Olivia Clinic, Riga, Latvia
[4]Center of Gastroenterology, Hepatology and Nutrition, Pauls Stradins Clinical University Hospital, Riga, Latvia
Email: *polina.zalizko@lu.lv

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Abstract

Metabolic dysfunction-associated steatotic liver disease (MASLD) has emerged as the most common cause of chronic liver disease worldwide, affecting an estimated 25% of the global population. Up to 80% of individuals with hepatic steatosis remain asymptomatic and may show no biochemical abnormalities, meaning that MASLD is frequently silent until complications occur, which makes early diagnosis challenging. Lifestyle modification, including dietary changes, regular physical activity, and sustained weight reduction, is the cornerstone of MASLD management. At present, however, there is still no universally standardized pharmacological treatment for MASLD all over the world, and management continues to rely primarily on lifestyle modification. The amino acids and B-group vitamin complex is expected to be effective in treating the early stage of steatohepatosis and steatohepatitis, reducing aminotransferases levels and decreasing liver fat cell accumulation.

Keywords

MASLD, MASH, Amino Acids, B-Group Vitamins

1. Introduction

Metabolic dysfunction-associated fatty liver disease, formerly referred to as non-alcoholic fatty liver disease (NAFLD), has recently undergone a redefinition. In 2023, three major international liver associations proposed the term metabolic

dysfunction-associated steatotic liver disease (MASLD) to replace NAFLD, emphasizing the central role of metabolic dysfunction in its pathogenesis [1] [2].

MASLD has emerged as the most common cause of chronic liver disease worldwide, affecting an estimated 25% of the global population [3]. Consensus definitions describe MASLD as an umbrella term encompassing a spectrum of disorders characterized by hepatic steatosis in more than 5% of hepatocytes, in the context of metabolic risk factors such as obesity, type 2 diabetes mellitus, arterial hypertension, or hypertriglyceridemia, while excluding significant alcohol intake and other chronic liver diseases. The pathogenesis of MASLD is multifactorial, involving genetic predisposition, lifestyle influences, and metabolic disturbances. Excessive lipid accumulation within hepatocytes induces lipotoxic stress, which activates oxidative pathways and downstream signaling mediators. These signals may propagate to neighboring cells via extracellular vesicles or passive diffusion, triggering hepatocellular apoptosis or necrosis and driving progression from simple steatosis to hepatic inflammation and fibrosis [4] [5]. Epidemiological data underscore the growing clinical relevance of MASLD. The Global Burden of Disease (GBD) study highlights its rapidly increasing health and economic burden, with rising rates of mortality and disability reported across the world [6]-[9]. As expected, global prevalence parallels the obesity epidemic, and a recent meta-analysis estimated that approximately 75% of individuals with obesity are affected by MASLD [10].

MASLD encompasses a spectrum of hepatic involvement ranging from simple steatosis to its progressive form, metabolic dysfunction-associated steatohepatitis (MASH). The distinction between these stages is determined by the extent of hepatic inflammation and structural remodeling, particularly fibrosis. Early recognition of advanced fibrosis is essential for guiding clinical management, as affected patients carry a substantially higher risk of complications such as hepatocellular carcinoma (HCC) and portal hypertension-related outcomes, including oesophageal varices [11]-[15]. MASLD is defined by hepatic steatosis in the presence of metabolic dysfunction, whereas MASH represents the inflammatory and fibrotic progression of the disease. The transition to MASH is marked by hepatocellular injury, identified by characteristic histopathological features. These include lobular inflammation, typically consisting of a mixed inflammatory infiltrate of $CD4^+$ and $CD8^+$ T lymphocytes, Kupffer cell aggregates, macrophages, and polymorphonuclear leukocytes. A defining hallmark is hepatocellular ballooning degeneration, which reflects severe cellular stress and damage. Additional findings may include apoptotic bodies and Mallory-Denk bodies—eosinophilic intracytoplasmic inclusions composed of misfolded keratin intermediate filaments, ubiquitinated proteins, and heat-shock proteins—indicating disrupted cytoskeletal integrity. Together, these histological changes signal the transition from simple steatosis to an active, fibrogenic, and progressive liver disease [16]-[18].

1.1. MASLD and Hepatocellular Carcinoma

MASLD is increasingly recognized as a major driver of hepatocellular carcinoma

(HCC) and is projected to become its leading cause in many regions worldwide. Several of its principal risk factors, including obesity, type 2 diabetes mellitus, and dyslipidemia, are independently associated with liver carcinogenesis. A distinctive feature is that MASLD and its progressive form, MASH, account for a considerable proportion of HCC cases occurring in the absence of cirrhosis. Among patients with MASLD-related cirrhosis, the incidence of HCC is estimated at 10 - 15 cases per 1000 person-years [19]. The mechanisms linking MASLD to HCC are complex and multifactorial. Macrophage infiltration into visceral adipose tissue promotes chronic low-grade inflammation, aggravates insulin resistance, and disrupts lipid homeostasis, resulting in the accumulation of lipotoxic lipid species. These toxic lipids contribute to hepatocellular injury through oxidative stress, endoplasmic reticulum stress, and inflammasome activation. The downstream consequences—including apoptosis, persistent inflammation, compensatory hepatocyte regeneration, and fibrogenesis—drive progression to cirrhosis and establish a tumor-promoting hepatic microenvironment [20].

1.2. MASLD Diagnosis

Abnormalities in lipid metabolism are the primary drivers of fatty liver disease. Up to 80% of individuals with hepatic steatosis remain asymptomatic and may show no biochemical abnormalities, meaning that MASLD is frequently silent until complications occur, which makes early diagnosis challenging [21]. The diagnosis of MASLD is established by confirming hepatic steatosis while ruling out alternative etiologies, including other chronic liver diseases, excessive alcohol use (≥30 g/day for men, ≥20 g/day for women), and secondary causes such as prolonged treatment with systemic corticosteroids, methotrexate, or tamoxifen [22]. Current consensus further defines MASLD as the presence of hepatic steatosis—identified by serum biomarkers, imaging methods, or histological evaluation—together with at least one of the following: overweight or obesity, type 2 diabetes mellitus, or metabolic dysregulation. The latter is characterized by two or more abnormalities, including central obesity, hypertriglyceridemia, low HDL-cholesterol, arterial hypertension, impaired fasting glucose, insulin resistance, or chronic low-grade inflammation [22] [23]. Ultrasonography is the preferred first-line diagnostic tool for MASLD because it is widely available, safe, and cost-effective. It reliably detects moderate-to-severe hepatic steatosis but has reduced sensitivity in cases of mild steatosis and in patients with obesity. Despite these limitations, ultrasonography is currently recommended as the first-line diagnostic approach for MASLD in both clinical and research settings [24] [25]. Serum liver enzyme levels are often used to help identify patients at higher risk of advanced disease, such as MASH or fibrosis. However, advanced disease cannot be reliably excluded in certain patient groups—particularly those with type 2 diabetes mellitus—even when enzyme levels are within the normal range [26]. Following a diagnosis of MASLD, the critical clinical question is whether significant liver damage is present. Although alanine aminotransferase (ALT) testing is commonly performed in patients

with chronic liver disease, ALT levels show a poor correlation with both MASH and fibrosis, and more than half of MASLD patients may have normal ALT values. At present, no single biomarker has proven sufficiently accurate for diagnosing MASH, but several non-invasive tools for fibrosis assessment have been extensively validated. Given that fibrosis is the histological feature most strongly associated with adverse liver-related outcomes, current practice emphasizes fibrosis evaluation as a central component of MASLD management [27]. In patients with abnormal fibrosis scores, more specific non-invasive tests of fibrosis should be performed. Specific fibrosis tests can generally be divided into blood biomarkers (e.g. Enhanced Liver Fibrosis [ELF] test, FibroTest and FibroMeter) and imaging biomarkers (ultrasound elastography and magnetic resonance elastography) [25]. Clinicians should be mindful of situations in which non-invasive assessments of MASLD may be less reliable. Both the Fibrosis-4 (FIB-4) index and the MASLD fibrosis score show reduced sensitivity for advanced fibrosis in patients under 40 years of age and reduced specificity in those over 65 years [28]. Moreover, non-hepatic factors such as systemic inflammation, metabolic comorbidities, or thrombocytopenia of other causes may also compromise accuracy. In addition, falsely elevated liver stiffness measurements on ultrasound or magnetic resonance elastography can occur in patients who have recently eaten, or in the presence of conditions such as active hepatitis, biliary obstruction, congestive heart failure, or amyloidosis [29]. For these reasons, results of non-invasive tests should always be interpreted in the context of the overall clinical picture, and where uncertainty remains, confirmed with more specific tests or liver biopsy. Non-invasive assessment of advanced fibrosis in MASLD typically involves three categories of tools. Clinical scoring algorithms include the MASLD fibrosis score, Fibrosis-4 (FIB-4) index, and the aspartate aminotransferase (AST) to platelet ratio index (APRI). Serum biomarker-based panels, such as the Enhanced Liver Fibrosis (ELF) test, FibroMeter, FibroTest, and HepaScore, have also been validated. Imaging modalities frequently used for this purpose include transient elastography (TE), magnetic resonance elastography (MRE), acoustic radiation force impulse (ARFI) imaging, and supersonic shear wave elastography [30]. Liver biopsy continues to be regarded as the gold standard in the evaluation of MASLD. It remains the only method that can consistently differentiate MASLD from MASH and accurately stage the degree of fibrosis. Since current imaging modalities lack sufficient accuracy for diagnosing MASH, biopsy retains its key role in the assessment of histological changes in the liver [15].

1.3. MASLD Treatment

Lifestyle modification, including dietary changes, regular physical activity, and sustained weight reduction, is the cornerstone of MASLD management. Current evidence demonstrates that weight loss is the most effective intervention for improving histopathological features of the disease. Achieving and maintaining weight reduction not only promotes regression of liver injury but also lowers the

risk of cardiovascular disease and type 2 diabetes mellitus. A weight loss of at least 10% has been shown to induce resolution of MASH and to improve fibrosis by at least one stage [31].

1.4. Pharmacological Approaches in MASLD

Several potential therapies have been extensively investigated over the past decades for the treatment of MASLD or its advanced stages. According to 2024 EASL Clinical Practice Guidelines the incidence of clinical and subclinical hypothyroidism appears to be higher in individuals with MASLD or MASH relative to age-matched controls, and low thyroid function is associated with more severe outcomes. In March 2024 resmetirom was approved for the treatment of non-cirrhotic MASH (mostly fibrosis stages 2 and 3). Resmetirom is an orally active, liver-directed, thyroid hormone receptor agonist with high selectivity for the β1 receptor.

At present, however, there is still no universally standardized pharmacological treatment for MASLD all over the world, and management continues to rely primarily on lifestyle modification.

Glucagon-like peptide-1 receptor agonists (GLP-1 RAs) are incretin-based agents used in the treatment of type 2 diabetes mellitus, acting through stimulation of the GLP-1 receptor to enhance glucose-dependent insulin secretion. In addition to their glucose-lowering effects, GLP-1 RAs induce clinically significant weight loss, typically averaging 3 - 5 kg, and improve insulin sensitivity. GLP-1 receptors have been identified in both animal models and human hepatocytes, and their activation has been shown to reduce hepatic steatosis by enhancing insulin signaling, alleviating lipotoxicity, and improving mitochondrial function [32]-[35].

Sodium–glucose cotransporter-2 (SGLT-2) inhibitors are a novel class of oral antidiabetic agents that lower plasma glucose by selectively inhibiting the SGLT-2 protein in the kidney. This transporter is predominantly expressed on the epithelial cells of the S1 segment of the renal proximal convoluted tubule, where it mediates glucose reabsorption. By blocking this pathway, SGLT-2 inhibitors promote glycosuria and thereby lower blood glucose levels in an insulin-independent manner, providing effective glycemic control together with additional metabolic benefits [32] [33]. Beyond diabetes management, SGLT-2 inhibitors are of growing interest in MASLD, where they may improve hepatic steatosis, reduce body weight, and enhance insulin sensitivity, with several trials demonstrating favorable effects on liver fat content and metabolic parameters [36]-[38].

L-ornithine L-aspartate (LOLA) is a stable salt of the amino acid's ornithine and aspartate, traditionally used in the management of hepatic encephalopathy because of its ammonia-lowering properties. Clinical trial data now suggest that LOLA also exerts hepatoprotective effects in patients with MASLD and MASH. Reported benefits include reductions in serum aminotransferases, particularly ALT, decreased triglyceride levels, and improvements in liver-to-spleen CT atten-

uation ratios. These effects are believed to result from the conversion of LOLA into glutamine, L-arginine, and glutathione (GSH). Both glutamine and GSH provide antioxidant and cytoprotective activity by counteracting oxidative stress and lipid peroxidation in experimental MASLD/MASH models, while L-arginine enhances hepatic microcirculation through stimulation of nitric oxide synthesis [39].

2. Materials and Methods

2.1. Study Population Description

This was a prospective observational pilot study. The study protocol was reviewed and approved by the Ethics Committee (Approval No.: 5-1/238/2018). Written informed consent was obtained from all participants prior to enrollment, in accordance with the Declaration of Helsinki.

The study included patients' males and females over 18 years of age, who were newly diagnosed with steatosis and/or steatohepatitis and diagnosis was established during the first visit to a gastroenterologist. Also were included patients with an existing diagnosis (steatohepatosis and/or steatohepatitis) who have not achieved recovery and/or have shown no improvement in liver enzyme levels and/or ultrasound findings. This study did not include a control group. As a result, improvements observed during the 30-day period cannot be attributed solely to the intervention, since natural variation, regression to the mean, or concurrent lifestyle changes may also have contributed. This limits the ability to draw firm causal inferences, and future randomized controlled trials will be required to confirm these findings.

MASLD and steatohepatitis are strongly associated with metabolic syndrome. About 90% of MASLD patients have more than one sign of metabolic syndrome, and 33% have three or more signs. Thus, comorbid conditions should be considered, where patients with steatosis may also exhibit insulin resistance, abdominal obesity, dyslipidemia, elevated triglycerides, high blood pressure, and other conditions.

Excluded from the study were patients with uncontrolled diabetes (HbA1c > 6.5%), uncontrolled hypertension (>150 mmHg), viral hepatitis, autoimmune liver diseases, severe uncontrolled chronic and autoimmune diseases, genetic or metabolic liver diseases.

2.2. List of Methods and Technical Equipment

Diagnosis is based on the EASL Clinical Practice Guidelines for MASLD management. Diagnostic methods include liver enzyme measurements via blood tests and liver ultrasound, which help differentiate MASLD from steatohepatitis. The gold standard for diagnosing steatohepatitis is a previously conducted histological liver biopsy.

Hepatic steatosis was graded by abdominal ultrasound using standard semi-quantitative criteria based on hepatic echogenicity compared with renal cortex,

visualization of intrahepatic vessels, and clarity of the diaphragm. Steatosis was categorized as:

Grade 0 (none): Normal liver echotexture, with echogenicity similar to renal cortex.

Grade 1 (mild): Slight, diffuse increase in fine echoes in the hepatic parenchyma with normal visualization of diaphragm and intrahepatic vessel borders.

Grade 2 (moderate): Moderate, diffuse increase in fine echoes with slightly impaired visualization of intrahepatic vessels and diaphragm.

Grade 3 (severe): Marked increase in fine echoes with poor or no visualization of intrahepatic vessels, diaphragm, and posterior right lobe of the liver.

Primary non-invasive tests were included as liver enzyme measurements (AST, ALT, gamma-glutamyl transferase (GGT)). Normal AST is <37 U/L, normal ALT is <41 U/L. Tests were performed at E. Gulbis Laboratory, so their reference ranges were applied. A normal AST/ALT ratio was close to 1. Blood samples were taken on Day 1, Day 15, and Day 30.

Ultrasound is an effective method for diagnosing steatohepatosis, still at least 33% steatosis is required for detection via ultrasound. Exams were performed on Day 1 and Day 30.

Each participant received two boxes (30 sachets) of Hepastrong AMINO forte. One box contains 15 powder sachets for oral use. Each patient took one sachet daily in the morning with or after breakfast, dissolved in 100 - 150 ml of water, for 30 consecutive days.

Each sachet contains L-Ornithine: 1500 mg, Choline bitartrate: 500 mg, L-Arginine: 200 mg, L-Methionine: 200 mg, Vitamin B6: 3 mg, Vitamin B9: 200 μg, Vitamin B12: 2.5 μg.

During the 30-day intervention period, participants did not receive any additional lifestyle or dietary counseling. They were instructed to maintain their usual diet, physical activity, and daily routines throughout the study.

The initial results were clustered and coded in the MS Excel spreadsheet. The representative data values for the intervention group were 28 patients. No data was excluded. The baseline participants' characteristics of data processing in IBM Statistical Package of Social Sciences 29.0.0.0. (SPSS) were in the form of descriptive statistics (age, gender, education level, smoking status, alcohol consumption, comorbidities, menopausal status, ultrasonographic steatosis stage (USG SS), body mass index (BMI) distribution, and liver enzymes at baseline). A normality test for continuous variables was carried out to determine whether a parametric or a nonparametric test would be performed for data generation. Shapiro-Wilk normality test demonstrated that variables age and BMI are normally distributed, 0.535 and 0.052, in contrast to liver enzyme variables, <0.001.

3. Results

From all 28 participants 10 were men (35.7%). Evaluation on participants' groups can be found in **Table 1**.

Table 1. Descriptive characteristics of study group.

Baseline descriptive characteristics of all participants (n = 28)		
Mean ± SD [25., 50., 75. percentile for non-normal distribution] or number in %		
Age, mean (SD)		52.54 (14.5)
Gender, n (%)		
	woman	18 (64.3)
	man	10 (35.7)
Education level, n (%)		
	secondary school	7 (25)
	higher education	16 (57.1)
	secondary specialized	5 (17.9)
Smoking status (yes/no), n (%)		5 (17.9)/23 (82.1)
Alcohol consumption, n (%)		
	every day	1 (3.6)
	once a week or more often	4 (14.3)
	once a month or more often	8 (28.6)
	less than once a month	10 (35.7)
	does not drink alcohol	5 (17.9)
Comorbidities, n (%)		
	none	6 (21.4)
	high blood pressure	5 (17.9)
	dyslipidemia	10 (35.7)
	high BP and dyslipidemia	4 (14.3)
	high BP, DM, and dyslipidemia	1 (3.6)
	DM and dyslipidemia	2 (7.1)
Menopausal status (yes/no), n (%)		11 (39.3)/7 (25)
Ultrasonographic steatosis stage, n (%)		
	initial/mild fatty liver	15 (53.6)
	moderate fatty liver	13 (46.4)
BMI (kg/m^2), mean (SD)		29.94 (6)
*BMI distribution, n (%)		
	normal weight	5 (17.9)
	overweight	14 (50)
	obesity class I	2 (7.1)
	obesity class II	5 (17.9)
	obesity class III	2 (7.1)

Continued

Measured ASAT1 (U/L)	37.46 (25.55) [22; 26; 47]
Measured ALAT1 (U/L)	49.43 (36.6) [24.5; 39.5; 64]
Measured GGT1 (U/L)	39 (27.36) [19.5; 31.5; 45]

SD—standard deviation; BP—blood pressure; DM—diabetes mellitus; BMI—Body Mass Index; *underweight – <18.5 kg/m^2; normal weight – ≥18.5 to 24.9 kg/m^2; overweight – ≥25.0 to 29.9 kg/m^2; obesity class I – 30.0 to 34.9 kg/m^2; obesity class II – 35.0 to 39.9 kg/m^2; obesity class III – ≥40 kg/m^2 (also referred to as severe or extreme obesity).

To evaluate differences in liver enzymes (ASAT (aspartate aminotransferase), ALAT (alanine aminotransferase), GGT (gamma-glutamyl transferase)) during the intervention period, the Wilcoxon signed-rank test was applied. Statistically significant Two-Sided p difference refers to the level of 0.05. Significant changes were observed in ALAT reduction from baseline to day 15 ($Z = -2.442$, $p = 0.015$) and from baseline to day 30 ($Z = -2.259$, $p = 0.024$), GGT was significantly decreased between day 15 and day 30 ($Z = -2.132$, $p = 0.033$) and from baseline to day 30 ($Z = -2.127$, $p = 0.033$). Schematic changes can be found in Figure 1.

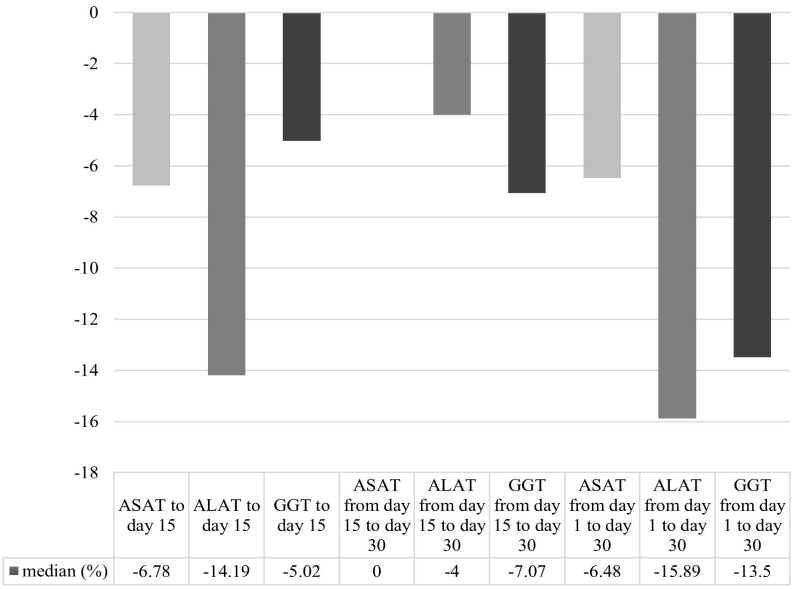

Figure 1. Enzyme changes during the intervention.

The median ALAT change from baseline to day 15 shows a decline of 14.19% with a range from – 70.09% to 50%. The median ALAT change from baseline to day 30 shows a decline of 15.89% with a range from – 82.24% to 143.75%. The median GGT from day 15 to day 30 shows a drop of 7.07%, with a range of – 63.16% to 98.92%. Moreover, the median GGT from day 1 to day 30 shows a drop of 13.5%, with a range of – 63.16% to 96.81%. The given data are supported by

additional IQRs (Table 2).

Table 2. Liver enzymes changes in IQRs.

Liver enzyme	Q1 (25th pctl.)	Q2 (50th pctl.)	Q3 (75th pctl.)
ASAT to day 15	−19.7	−6.78	11.71
ALAT to day 15	−25.08	−14.19	9.68
GGT to day 15	−21.25	−5.02	5.8
ASAT from day 15 to day 30	−6.14	0	0
ALAT from day 15 to day 30	−13.31	−4	8.29
GGT from day 15 to day 30	−24.63	−7.07	5.93
ASAT from day 1 to day 30	−29.66	−6.48	8.9
ALAT from day 1 to day 30	−31.65	−15.89	14.09
GGT from day 1 to day 30	−35.9	−13.5	5.21

During the intervention period, liver enzymes showed positive correlations. Spearman's rank (Spearman's rho) nonparametric correlation test was applied. A strong correlation was observed between ASAT and ALAT ($r = 0.802$, $p < 0.001$), while moderate positive correlations were observed between ASAT and GGT ($r = 0.416$, $p = 0.027$) and between ALAT and GGT ($r = 0.563$, $p = 0.002$).

Chi-Square test and Fisher's Exact Test were performed to determine associations between gender and liver enzymes. Statistically significant Two-Sided p difference refers to the level of $p < 0.05$. Significant association was noted between gender and ALAT level ($p = 0.048$). The men population was more likely to have elevated ALAT at baseline ($p = 0.048$), ALAT at day 30 ($p = 0.004$), and GGT at day 30 ($p = 0.013$).

The Wilcoxon signed-rank test was used to compare the ultrasonographic steatosis state before and after the intervention. The test revealed a significant reduction in steatosis stage compared to baseline ($Z = -3.317$, $p < 0.001$) (Figure 2).

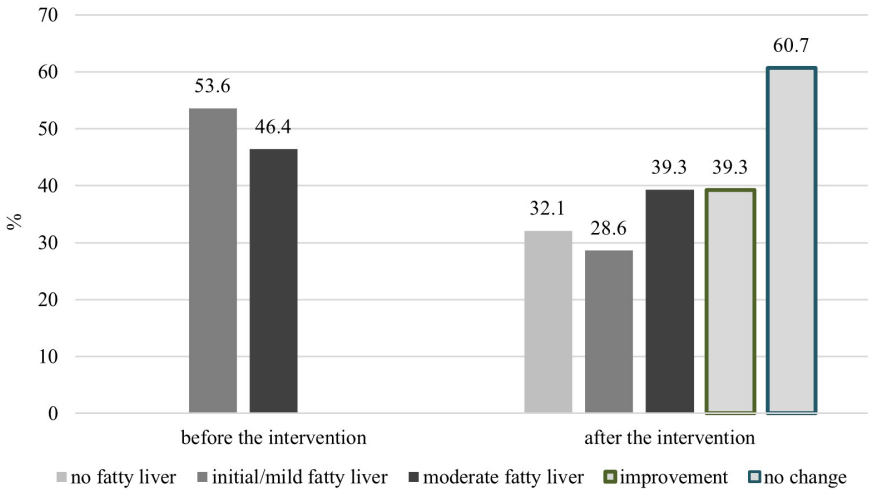

Figure 2. Ultrasonographic steatosis stage before and after the intervention.

Before the intervention, initial/mild fatty liver was recognized in 53.6% participants, moderate fatty liver in 46.4% patients. After the intervention, no fatty liver was demonstrated in 32.1% of the participants, initial/mild fatty liver count declined to 28.6%, and moderate fatty liver count fell to 39.3%. Indeed, 39.3% of participants experienced improvement in the USG stage, and 60.7% showed no change. The Wilcoxon signed-rank test confirmed a statistically significant ($Z = -3.317$, $p < 0.001$) reduction in USG stage.

Moreover, the Mann–Whitney U test was applied for dichotomous variables such as gender, menopause status, and smoking status, to explain the changes within the groups. Men suggested significantly greater changes in ultrasonographic steatosis stage compared to women ($Z = -2.322$, $p = 0.020$). Apparently, there were no significant changes in the woman subpopulations, menopausal and non-menopausal, respectively, regarding the steatosis stage. In the same way, no differences in steatosis stage changes between smokers and non-smokers were observed. To evaluate whether significant changes could be observed for variables with more than two groups as alcohol consumption and comorbidities, the Kruskal-Wallis test was performed. Statistical significance remains at the level of $p < 0.05$. No significant differences were observed in steatosis stage changes across alcohol consumption groups and between comorbidity groups.

Spearman's rank correlation was used to measure the relationship between two variables in the whole dataset. The correlation is considered to be statistically significant at the level of $p < 0.05$. Evidently, a statistically significant moderate positive correlation was observed between USG changes and BMI ($r = 0.484$, $p = 0.009$) – a higher BMI is suggested to be associated with more pronounced USG changes, respectively.

Additionally, no significant changes in the intervention period were noted, analysing liver enzyme changes and USG changes and alcohol consumption defined as yes or no. Participants were screened for alcohol consumption at baseline. Consistent with current MASLD (Metabolic dysfunction-associated steatotic liver disease) diagnostic criteria, individuals with alcohol intake above the exclusion thresholds (>30 g/day for men, >20 g/day for women) were not eligible for inclusion. The test used was the Mann-Whitney U. The results portrayed that alcohol consumption status (yes/no) did not significantly influence fatty liver changes on USG and on liver enzyme changes in the study sample. Nevertheless, the Kruskal-Wallis test, analyzing USG changes and alcohol consumption frequency, suggested that participants who consume alcohol more frequently (daily/weekly) show considerable USG changes, while minimal and non-drinkers show lower changes.

Generally, Mann-Whitney U tests in grouping USG changes and comorbidities category (yes/no) suggested that patients with comorbidities have a slightly higher average rank (14.91) for USG changes than those without comorbidities (13.00), but the difference is without significant. Particularly, patients with hypertension and DM describe slightly higher USG changes compared to patients without hy-

pertension and DM – mean rank of 17.20 and 20.00, 13.00 and 13.84. In this way, indicating no statistical significance p-value (Asymp. Sig. 2-tailed) of 0.126 and 0.148, respectively. On the contrary, dyslipidemia does not seem to be related to USG changes in the analyzed dataset – 14.91 and 14.24 in the patients without and with dyslipidemia, respectively.

4. Discussion

This study demonstrates that the intervention produced significant improvements in both biochemical and imaging markers of MASLD, with reductions in ALT and GGT and a clear decrease in ultrasonographic steatosis after 30 days. These results highlight the responsiveness of hepatocellular injury and steatosis to relatively short-term interventions. The observed improvements may, at least in part, be attributed to the pharmacological action of the amino acids, choline, and B-group vitamin complex. Given the limited treatment landscape for MASLD—the leading and steadily increasing cause of chronic liver disease.

Evidence from clinical investigations indicates that L-ornithine L-aspartate (LOLA) exerts hepatoprotective effects in patients with MASLD. These effects include attenuation of elevated serum aminotransferases, particularly ALT, and reduction of serum triglyceride concentrations, both of which represent relevant biomarkers of disease activity. Experimental studies further demonstrate protective actions against oxidative stress and lipid peroxidation, processes central to the progression of MASLD [40] [41]. Beyond these clinical and experimental observations, LOLA plays an important role in ammonia (NH_3) detoxification. This activity is closely associated with normalization of fatty acid transport regulation, branched-chain amino acid (BCAA) catabolism, energy expenditure, and mitochondrial energy balance. The constituent amino acids L-ornithine and L-aspartate are not only key substrates in NH_3 detoxification but also participate in the biosynthesis of proline and polyamines. The latter metabolites are essential for DNA synthesis, cellular replication, and hepatic regeneration. While under physiological conditions L-ornithine and L-aspartate are synthesized de novo in sufficient quantities, in MASLD their exogenous administration may provide critical metabolic support [42] [43]. L-arginine, a semi-essential (functional) amino acid derived from dietary sources or endogenous metabolism, contributes additional therapeutic potential. It is involved in multiple biochemical pathways, including the synthesis of polyamines, creatine, homoarginine, and agmatine. Importantly, L-arginine serves as the precursor for nitric oxide (NO), a key signaling molecule with diverse physiological functions. As a substrate for endothelial nitric oxide synthase (eNOS), L-arginine may reduce blood pressure through endothelium-dependent vasodilation and improve tissue perfusion. Beyond vascular effects, L-arginine plays crucial roles in neurogenesis, ammonia detoxification, immune modulation, and the regulation of hormone secretion, including insulin, glucagon, and growth hormone [44] [45].

Hepastrong AMINO forte contains not only amino acids but also B-group vit-

amins (B6, B9, and B12), which may have contributed additional hepatoprotective and metabolic benefits. Vitamin B6 (pyridoxal, pyridoxine, and pyridoxamine) functions as an essential cofactor for enzymes involved in amino acid, lipid, and glucose metabolism, and by supporting these pathways, it may exert beneficial effects in the prevention of metabolic syndrome, including insulin resistance and MASLD [46] [47]. Vitamin B9, also known as folate, plays a critical role in one-carbon metabolism, which provides activated one-carbon units for biosynthetic processes such as purine and thymidine synthesis and the remethylation of homocysteine to methionine. While NAFLD/MASLD is closely linked to metabolic disorders, the association between folate status and MASLD has not yet been fully established [48]. Several hypotheses have been proposed in view of its central role in one-carbon metabolism. Folate may lower circulating homocysteine concentrations through transmethylation, thereby improving vascular and metabolic homeostasis, and it may also enhance autophagy, which could contribute to hepatocellular protection. In a recent human randomized control trial, patients with NAFLD were randomized to folic acid 1 mg/day vs placebo for 8 weeks. Folic acid prevented a rise in homocysteine but produced no statistically significant between-group improvements in ALT, steatosis grade, HOMA-IR or lipids over 8 weeks (within-group changes were reported). Furthermore, folate has been shown to protect against lead acetate-induced hepatotoxicity by reducing nuclear factor kappa B (NF-κB) and interleukin-1β (IL-1β) production, as well as lipid peroxidation–mediated cellular injury. Recent findings also suggest that folate deficiency increases lipid accumulation and leptin production in adipocytes, indicating that inadequate folate status may represent an additional risk factor for adiposity and metabolic dysfunction [49]-[51]. Multiple studies have demonstrated that vitamin B12 exists in humans in two active forms, methylcobalamin and 5′-deoxyadenosylcobalamin, both of which are primarily stored in the liver. Supplementation with vitamin B12 in patients with MASLD has been associated with improvements in serum homocysteine concentrations, fasting blood glucose, and malondialdehyde (MDA) levels, with significant benefits observed in trial groups receiving vitamin B12 [52]. Another study, which concluded that serum Vitamin B12 levels were much lower in patients with MASLD. Consequently, low Vitamin B12 levels may be associated with MASLD, especially in grade 2 to grade 3 hepatosteatosis. Among recent human trials, vitamin B12 supplementation (1000 μg/day cyanocobalamin over 12 weeks) in patients with NAFLD decreased homocysteine and showed favorable within-group trends in glucose, oxidative stress and liver steatosis, though many between-group differences were not statistically significant. Also, a 3-month micronutrient cocktail including vitamin B12 plus folate derivative (5-MTHF), choline, etc., in adults with obesity and metabolic syndrome led to significant reductions in steatosis and fibrosis measures (CAP and transient elastography) compared with placebo [53] [54]. Taken together, these findings highlight that adequate supplementation with B-group vitamins is crucial for patients with MASLD, as they contribute to the regulation of key metabolic path-

ways, reduction of oxidative stress, improvement of insulin sensitivity, and attenuation of hepatocellular injury.

Interestingly, male participants in our study seemed to experience greater therapeutic benefit than females. This observation may be related to sex-specific differences in metabolic regulation, fat distribution, or adherence to treatment, although further research is required to confirm these findings. In recent years, sex-related differences have been increasingly investigated in relation to MASLD and its underlying pathogenesis [55].

Alcohol consumption frequency and the presence of comorbidities such as hypertension or diabetes showed a tendency to influence outcomes, but statistical significance was not achieved, likely due to sample size limitations.

Importantly, body mass index (BMI) demonstrated a positive correlation with the degree of steatosis improvement, underscoring the central role of metabolic burden in disease activity and treatment response. As the most widely applied anthropometric measure for assessing general and central obesity, BMI is also a well-established risk factor for MASLD [56]-[59].

The conducted study provides additional support for considerable insight into ultrasonography-based measurements regarding liver steatosis detection. There has been a statistically significant reduction in steatosis stage severity during the intervention. Evidently there may be other possible explanations. Minciuna *et al.* presented three ultrasound techniques (ShearWave Elastography (SWE), Attenuation Plane wave UltraSound, and Viscosity Plane-wave UltraSound) for the characterisation of liver steatosis. They used Vibration-controlled transient elastography (VCTE) with Controlled Attenuation Parameter (CAP) as a reference method. The VCTE technique shows a clear advantage in comparison to SWE regarding liver stiffness measurement ($p < 0.001$). A major source of SWE unreliability is due to an inhomogeneous filling of the color map. In summary, multiparametric ultrasound evaluation validates the usefulness of a single analysis in chronic liver disease patients [60].

Another statement points out that obesity is directly linked to increasing prevalence of non-alcoholic fatty liver disease. Baldini *et al.* introduced a study which aims to characterize the grade of steatosis on a micro and macro level of liver and its correlation with liver stiffness and dysfunction. This paper outlines that patients with non-alcoholic fatty liver disease present progressive increase in liver stiffness with the highest value in patients, presenting severe liver steatosis. The experiments at single cell level with Single Cell Force Spectroscopy demonstrated an increased stiffness in moderate steatosis cells, but the largest increase was proved in severe steatosis conditions. The reason is suggested to be the cytosolic lipid droplets in a single cell and steatosis grade that influences the elasticity. Mentioned assessment strategy could be a potential framework to undergo a target therapy [61].

From another point of view, artificial intelligence (AI) has received much attention in the past decade. Due to accurate initial measurements for further

method development, generated AI algorithms might yield precise results. Ultrasound elastography, used to measure liver stiffness, indicates not only challenge, such as lack of equipment, but also technical properties in patients with obesity and the viscosity as a confounding value. Viscosity factors, for example, inflammation, overload, and jaundice, might be linked to elevated liver stiffness. To improve the overall performance, a U-Net for the liver surface segmentation could be investigated and for classification - ResNet-50. This method represents a valuable approach in segmenting the left lobe liver contours. Moreover, it seems to be useful in presence and absence identification of contour irregularities in the left lobe of the liver and also an AI-based prediction was explained of liver fibrosis by image analysis in MASLD patients [62].

Further data collection would be needed to determine exactly how pharmacological agents affect MASLD in different populations and to determine the cofounders. One of the limitations of this study is the relatively small study group for evaluation of influence factors and correlations. For instance, some associations predicted e.g. between comorbidities, alcohol consumption, smoking status, and USG changes may reflect low confidence rather than a true absence of association. The restricted use of the cohort could explain why the findings can be limited in generalizing to broader populations e.g. the calculation made may not address the variability of liver enzyme responses or USG changes in larger populations. The authors cannot rule out that analyses by gender, comorbidities, or lifestyle factors due to reduced sample size per group might have influenced liver enzyme changes and/or USG changes. Despite limitations mentioned, observed results suggest a valuable hypothesis generation. Consequently, a larger, multicenter study is needed to confirm these preliminary findings, highlighting associations between gender and liver enzyme changes, BMI and USG changes, and the effects of reduced alcohol consumption.

Together, these results support the concept that metabolic factors are critical drivers of MASLD progression and should remain key targets in therapeutic strategies.

This study was exploratory and the sample size was determined by feasibility (available participants during the study period) rather than by formal a priori power calculation. As a result, the study may be underpowered to detect small to moderate effects, and the findings should be interpreted as preliminary and hypothesis-generating. Confirmation in larger, adequately powered studies is warranted.

5. Conclusion

The amino acids and B-group vitamin complex is expected to be effective in treating the early stage of steatohepatosis and steatohepatitis, reducing AST/ALT levels and decreasing liver fat cell accumulation. Further studies are necessary in order to evaluate standardized treatment of MASLD.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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