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【 ORIGINAL ARTICLE 】

# Type 2 Diabetes Mellitus Is a Risk Factor for Skeletal Muscle Loss in the Course of Dietary Treatment for Patients with Metabolic Dysfunction-associated Steatotic Liver Disease

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**Abstract:**

**Objective** This study assessed the impact of dietary therapy and reduced body weight on the loss of skeletal muscle in patients with metabolic dysfunction-associated steatotic liver disease (MASLD).

**Methods** This was a single-center retrospective observational study. We enrolled 129 patients with MASLD who had undergone dietary therapy at our facility. We assessed skeletal muscle mass using a bioelectrical impedance analysis at the start of dietary treatment and 12 months after the first assessment. Variables related to muscle reduction were analyzed using a logistic regression model.

**Results** One hundred and eighteen cases were analyzed, excluding those with missing data. In the muscle reduction group, there were more subjects with body weight reduction than in the control group (68% and 40%, respectively, p=0.002), and their body mass index (BMI) was decreased (-0.7 kg/m$^2$ and +0.3 kg/m$^2$, respectively, p=0.0003). There was a significant correlation between the changes in the BMI and muscle mass (R=0.48, p<0.0001). We standardized muscle mass change by dividing it by weight change to analyze the severe decrease in muscle mass compared to weight change. A logistic regression analysis revealed that type 2 diabetes mellitus (T2DM) was an independent variable related to severe skeletal muscle loss (odds ratio, 2.69; 95% CI: 1.13-6.42, p=0.03).

**Conclusion** Weight loss is associated with skeletal muscle loss during dietary treatment for MASLD. T2 DM is a risk factor for severe skeletal muscle loss.

**Key words:** metabolic dysfunction-associated steatotic liver disease, non-alcoholic fatty liver disease, sarcopenia, type 2 diabetes mellitus

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## Introduction

Non-alcoholic fatty liver disease (NAFLD) has become the leading cause of chronic liver disease, paralleling the worldwide increase in obesity. The global prevalence of NAFLD is estimated to be 24% (1). Approximately 7-30% of patients with NAFLD develop a progressive form termed non-alcoholic steatohepatitis (NASH), which is characterized by hepatic inflammation and fibrosis, potentially leading to liver cirrhosis and hepatocellular carcinoma (HCC) (2). NAFLD/NASH has recently been defined as metabolic dysfunction-associated steatotic liver disease (MASLD) and metabolic-associated steatohepatitis (MASH), with a focus

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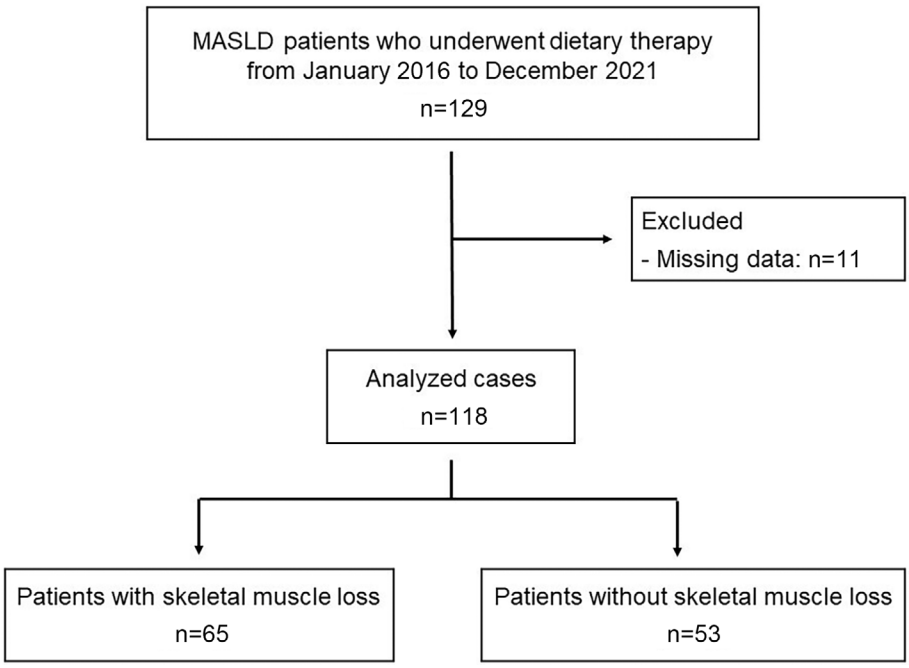


**Figure 1.** **Flowchart describing the patients analyzed in the present study.**

on the presence of metabolic dysfunction [obesity, type 2 diabetes mellitus (T2DM), hypertension, and dyslipidemia] (3). Research on the pathophysiology of steatotic liver disease has advanced worldwide in recent years. However, treatment options are still limited, and few specific treatments are available for MASLD. In Japan, the primary treatment for steatotic liver disease is weight reduction through diet and exercise therapy (4).

Sarcopenia, a common complication in chronic liver disease, especially cirrhosis, adversely impacts clinical outcomes such as survival, quality of life, development of other complications, and post-liver transplantation survival (5). Although malnutrition is a significant factor in sarcopenia, steatotic liver disease, which is primarily induced by overnutrition, may also cause sarcopenia due to disruption of the adipose-muscle-liver axis (6). Several retrospective studies have indicated that the prevalence of sarcopenia is increased in patients with NAFLD (7-9). Furthermore, NAFLD and sarcopenic obesity, reflecting a combination of sarcopenia and obesity, are highly prevalent conditions (10). Sarcopenic obesity has been reported to affect mortality in patients with chronic liver diseases (11).

We hypothesized that dietary therapy for steatotic liver disease, in which calorie restriction is a significant component, could potentially cause skeletal muscle loss. This study therefore assessed the impact of dietary therapy and reduced body weight on the loss of skeletal muscle in patients with MASLD.

## Materials and Methods

### Patients

This was a single-center retrospective observational study. We retrospectively enrolled 129 patients with MASLD who underwent dietary therapy at Tohoku University Hospital between January 2016 and December 2021. Data from 118 cases were analyzed, excluding cases with missing data (Fig. 1). Dietary therapy was provided by dietitians registered by The Japan Dietetic Association following the Japanese guidelines for NAFLD, aiming to reduce body weight by 7% (4). The calorie intake was set at 30 kcal/kg/day based on the weight calculated from a body mass index (BMI) of 22-25, with carbohydrates in 50-60% and fat in 20-25%. Interviews with dietitians were conducted, and caloric intake was evaluated every six months.

Informed consent was obtained using the opt-out method. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki, as reflected in *a priori* approval by the Ethics Committee of Tohoku University Graduate School of Medicine (APPROVAL NUMBER: 2021-1-755, 2023-1-922).

### Key variables

We diagnosed MASLD in subjects with ultrasound-defined hepatic steatosis based on current consensus criteria (3). T2DM was defined as a fasting glucose concentration ≥126 mg/dL on 2 separate occasions, a casual blood glucose concentration ≥200 mg/dL on 2 separate occasions, or hemoglobin A1c ≥6.5% with a fasting glucose concentration ≥126 mg/dL.

**Table 1. The Characteristics of Patients in the Present Study.**

| | n=118 | |
|---|---|---|
| Age, years | 55 | (20) |
| Gender | | |
| Men | 77 | (65%) |
| Women | 41 | (35%) |
| BMI, kg/m$^2$ | 29 | (7.5) |
| SMI, kg/m$^2$ | | |
| Men | 11 | (1.8) |
| Women | 9 | (2.6) |
| Liver cirrhosis | 12 | (10%) |
| Hepatocellular carcinoma | 3 | (3%) |
| Sarcopenia (baseline) | 0 | (0%) |
| Sarcopenia (after 1 year) | 0 | (0%) |
| Body weight reduction | 65 | (55%) |
| Skeletal muscle reduction | 65 | (55%) |
| Diabetes mellitus | 28 | (24%) |
| Medication related to metabolic dysfunction | | |
| Metformin | 8 | (7%) |
| DPP-4 inhibitors | 12 | (10%) |
| Thiazolidines | 1 | (1%) |
| GLP-1 agonists | 2 | (2%) |
| SGLT2 inhibitors | 11 | (9%) |
| Statins | 11 | (9%) |
| Fibrates | 3 | (3%) |
| Others | 10 | (8%) |
| Total bilirubin, mg/dL | 0.8 | (0.5) |
| Alanine aminotransferase, IU/L | 61 | (59) |
| Albumin, g/dL | 4.3 | (0.4) |
| Hemoglobin A1c, % | 6.1 | (1.0) |
| Type 4 collagen, ng/mL | 233 | (84) |
| FIB-4 index | 1.7 | (1.0) |

BMI: body mass index, SMI: skeletal muscle mass index, DPP-4: dipeptidyl peptidase-4, GLP-1: glucagon-like peptide-1, SGLT2: sodium-glucose transport protein 2, FIB-4: fibrosis-4

Categorical variables are shown as N (%).

Continuous variables are shown as the median (interquartile range).

We assessed the patients' body composition using a bioelectrical impedance analysis (BIA). This analysis was performed per protocol using a direct segmental multifrequency analyzer (InBody 720; InBody, Seoul, South Korea). The patients were asked to stand barefoot on the floor electrodes and hold both hand electrodes with the shoulder abducted and arms straightened to ensure no contact between the arms and torso. The analyzer used 6 measurement frequencies (1, 5, 50, 250, 500, and 1000 kHz), with an applied current of 80 μA (±10 μA). Muscle mass calculated by the BIA method was adjusted by dividing the square of the height to calculate the skeletal muscle mass index (SMI). The cutoff values of sarcopenia used in the present study were 7.0 kg/m$^2$ in men and 5.4 kg/m$^2$ in women based on the recommendation of the Asian Working Group for Sarcopenia (12) and the Japan Society of Hepatology (13). We assessed skeletal muscle mass using BIA at the start of dietary therapy and 12 months after the first assessment.

Liver cirrhosis was diagnosed based on imaging findings (presence of varices, liver deformity, and splenomegaly) and serological findings. HCC was diagnosed using computed tomography (CT) and tumor markers. Blood samples were collected after overnight fasting. The fibrosis-4 (FIB-4) index was derived from aspartate aminotransferase, alanine aminotransferase (ALT), platelet count, and age to predict advanced fibrosis (14).

### Statistical analyses

Patient characteristics are reported as the median (interquartile range) or n (%). Continuous variables were compared between the two groups using unpaired *t*-tests. Pearson's chi-squared test was used to compare categorical variables. Comparisons of continuous variables among multiple groups were performed using Tukey's test. For categorical variables, comparisons among multiple groups were performed using the chi-squared test with Bonferroni correction. A logistic regression analysis was used to identify the factors related to skeletal muscle mass loss.

The JMP Pro 17 software program (SAS Institute, Cary, USA) was used for all statistical analyses. Statistical significance was set at $p<0.05$.

## Results

### Patients' characteristics in this study and effects of dietary therapy on the liver function in MASLD patients

One hundred and twenty-nine MASLD patients >18 years old who underwent dietary therapy were enrolled in the study. After excluding 8 subjects due to a lack of data on the BIA, we analyzed 118 subjects in the present study (Table 1). The median age of the patients was 55 (range: 19-80) years old, predominantly men (65%). No patient was diagnosed with sarcopenia based on skeletal muscle mass at baseline. The rate of patients whose body weight decreased 12 months after starting dietary therapy was 55%, and the degree of body weight change was -0.45 kg (Fig. 2A). At baseline, the median ALT level was 61 IU/L, indicating liver injury. In subjects with reduced body weight, ALT levels were significantly decreased (Fig. 2B), and the amount of weight change and ALT level change showed a correlation (Fig. 2C). This suggests that weight loss through dietary therapy in patients had a positive effect on the liver function.

### Weight loss was an independent factor related to skeletal muscle loss

Table 2 compares the subjects with skeletal muscle loss (n =65) and without skeletal muscle loss (n=53). There were no significant differences in the median age and sex between these groups. Although there was a tendency for more men to be in the muscle mass reduction group than in the group without skeletal muscle loss, there was no significant

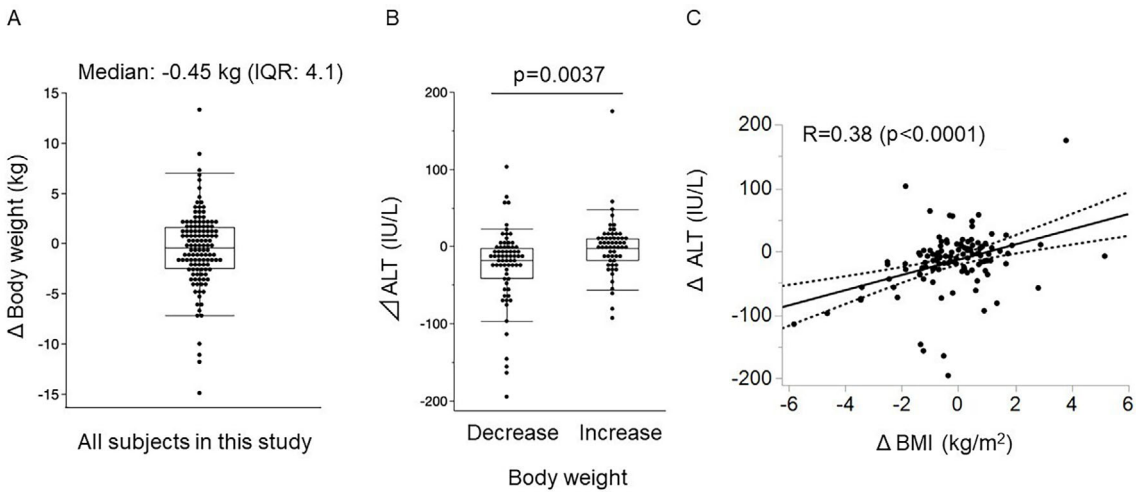


**Figure 2. The association between weight reduction and the value of alanine aminotransferase during dietary therapy in MASLD patients. (A) The median value of the change of body weight in all subjects in this study. (B) The change in ALT in patients with a body weight decrease versus an increase. The box plots present the minimum score, first (lower) quartile, median, third (upper) quartile, and maximum score. (C) Peason correlation plot with regression line and 95% confidence interval demonstrating the correlation between the changes in the BMI and ALT levels. IQR: interquartile range, ALT: alanine aminotransferase, BMI: body mass index, MASLD: metabolic dysfunction-associated steatotic liver disease**

difference in the change in SMI value between men and women (Supplementary material 1A). There was no significant difference in the change in the SMI between young and elderly patients, but the p value was relatively low (p=0.06) (Supplementary material 1B). We also examined whether or not the change in the SMI differed according to the baseline SMI (cutoff: median). We found no significant differences in SMI changes according to the baseline SMI (Supplementary material 1C). No significant differences in metabolic medication status were found between the two groups; for SGLT 2 inhibitors, the change in SMI was compared but was not significantly different (Supplementary material 1D). The skeletal muscle mass reduction group included more subjects with body weight reduction (68% and 40%, respectively, p=0.002) and a decreased BMI (-0.7 kg/m$^2$ and +0.3 kg/m$^2$, respectively, p=0.0003) than the group with increased skeletal muscle mass. There was a significant correlation between the changes in the BMI and SMI (R=0.48, p<0.0001) (Fig. 3A). A logistic regression model revealed that body weight reduction was a variable relative to skeletal muscle loss (odds ratio: 3.19, 95% CI: 1.50-6.81, p=0.002) (Table 3). These results indicate that body weight reduction was a significant factor for skeletal muscle loss in patients with MASLD who underwent dietary therapy.

### *T2DM was a risk factor for severe skeletal muscle loss in MASLD patients*

We found a significant association between the changes in body weight and muscle mass. Therefore, we analyzed changes in muscle mass in relation to weight changes and examined the risk factors associated with a substantial reduction in muscle mass relative to body weight change. In this study, we classified the patients as follows: Group 1 consisted of those who experienced an increase in both body weight and muscle mass; Group 2 included those who gained weight but lost muscle mass; Group 3 comprised individuals who lost weight but gained muscle mass or maintained muscle mass relative to the weight loss (ΔSMI/|ΔBMI| > median); and Group 4 consisted of those who lost weight with a severe reduction in muscle mass compared to the amount of weight loss (ΔSMI/|ΔBMI| <median) (Fig. 4). The median ΔSMI/|ΔBMI| was determined only from the body weight and SMI loss groups and was calculated to be -0.16.

Supplementary material 2 presents the characteristics of each group. Groups 2 and 4 were considered to have a severe decline in muscle mass relative to the changes in body weight. Apart from the changes in the BMI, however, no significant differences were observed between Groups 2 and 4. Therefore, we analyzed these groups collectively as the "severe muscle loss group" and conducted subgroup analyses for each individual group.

The ranges of each group are shown on the correlation diagram of ΔBMI and ΔSMI (Fig. 3B). Table 4 shows the characteristics of the severe and non-severe muscle loss groups. There were no differences in the number of participants with weight reduction or BMI change. In the severe muscle loss group, there were significantly more cases with a decrease in the SMI, and the SMI value also significantly decreased. In addition to the items related to skeletal muscle mass, only the presence of T2DM was significantly higher in the severe muscle loss group (36% and 17%, respectively; p=0.02). A logistic regression analysis was conducted to elucidate the factors related to severe muscle loss (Table 5).

**Table 2. The Comparison between the Groups of MASLD Patients with and without Skeletal Muscle Mass Loss after 12 Months of Dietary Therapy.**

| | Skeletal muscle mass | | | | | |
|---|---|---|---|---|---|---|
| | Decrease n=65 | | Increase n=53 | | p value | |
| Age, years | 55 | (22) | 55 | (24) | 0.3 | |
| Gender, men/women | 27 (42%)/38 (58%) | | 14 (26%)/39 (74%) | | 0.08 | |
| BMI, kg/m$^2$ | 29 | (9.6) | 29 | (7.2) | 0.4 | |
| SMI, kg/m$^2$ | | | | | | |
| Men | 11 | (1.9) | 11 | (1.6) | 0.4 | |
| Women | 9.2 | (1.6) | 8.7 | (1.7) | 0.08 | |
| Liver cirrhosis | 7 | (11%) | 5 | (9%) | 0.8 | |
| Hepatocellular carcinoma | 1 | (2%) | 2 | (4%) | 0.4 | |
| Sarcopenia | 0 | (0%) | 0 | (0%) | N/A | |
| Body weight reduction | 44 | (68%) | 21 | (40%) | 0.002 | * |
| BMI change, kg/m$^2$ | -0.7 | (1.7) | 0.3 | (1.4) | 0.0003 | * |
| SMI change, kg/m$^2$ | -0.2 | (0.3) | 0.2 | (0.3) | <0.0001 | * |
| Diabetes mellitus | 19 | (29%) | 9 | (17%) | 0.1 | |
| Medication related to metabolic dysfunction | | | | | | |
| Metformin | 4 | (6%) | 4 | (8%) | 0.8 | |
| DPP-4 inhibitors | 5 | (8%) | 7 | (13%) | 0.3 | |
| Thiazolidines | 0 | (0%) | 1 | (2%) | 0.2 | |
| GLP-1 agonists | 0 | (0%) | 2 | (4%) | 0.1 | |
| SGLT2 inhibitors | 7 | (11%) | 4 | (8%) | 0.5 | |
| Statins | 5 | (8%) | 6 | (11%) | 0.5 | |
| Fibrates | 1 | (2%) | 2 | (4%) | 0.4 | |
| Others | 6 | (9%) | 4 | (8%) | 0.7 | |
| Total bilirubin, mg/dL | 0.8 | (0.6) | 0.8 | (0.3) | 0.3 | |
| Alanine aminotransferase, IU/L | 58 | (71) | 65 | (50) | 0.5 | |
| Albumin, g/dL | 4.3 | (0.4) | 4.4 | (0.4) | 0.4 | |
| Hemoglobin A1c, % | 6.1 | (0.9) | 6.1 | (1.2) | 0.1 | |
| Type 4 collagen, ng/mL | 143 | (76) | 137 | (30) | 0.3 | |
| FIB-4 index | 1.7 | (1.0) | 1.9 | (1.1) | 0.5 | |

MASLD: metabolic dysfunction-associated steatotic liver disease, BMI: body mass index, SMI: skeletal muscle mass index, DPP-4: dipeptidyl peptidase-4, GLP-1: glucagon-like peptide-1, SGLT2: sodium-glucose transport protein 2, FIB-4: fibrosis-4

*: p value <0.05.

Categorical variables are shown as n (%).

Continuous variables are shown as the median (interquartile range).

The presence of T2DM was a variable related to the severe muscle loss group (odds ratio: 2.69, 95% CI: 1.13-6.42, p=0.03). We performed a multivariable analysis for T2DM, including age, liver cirrhosis, fibrosis markers (type 4 collagen and FIB-4 index), and biochemical markers related to the liver function (total bilirubin and albumin) (Table 6). T2DM was a significant variant associated with the severe muscle loss group in the multivariate analysis (odds ratio, 3.11; 95% CI: 1.24-7.83, p=0.02).

In the subgroup analysis, we conducted logistic regression analyses to examine the factors associated with Groups 2 and 4 in both the weight gain and weight loss cohorts. In the weight gain cohort, T2DM was a significant variable related to skeletal muscle loss (Group 2) in the univariate (odds ratio: 4.46, 95% CI: 1.01-20.66, p=0.048) and multivariate analyses (odds ratio: 6.35, 95% CI: 1.0-46.0, p=0.045) (Supplementary material 3). Furthermore, T2DM was a significant variable for severe weight loss (Group 4) in the weight loss cohort in the univariate (odds ratio: 3.48, 95% CI: 1.08-11.21, p=0.036) and multivariate analyses (odds ratio: 3.55, 95% CI: 1.05-12.03, p=0.041) (Supplementary material 4).

The characteristics of patients with and without T2DM are shown in Supplementary material 5. Although there was no significant difference in the number of subjects with SMI reduction, the SMI was significantly decreased in the T2DM group (p=0.04). The distribution of T2DM patients is shown as red dots in Fig. 3B. Among patients with increased body weight, the change in the SMI was lower in patients with T 2DM than in those without it (Fig. 5A). Furthermore, among patients with decreased body weight, the change in the SMI relative to the BMI was lower in patients with T2 DM than in those without it (Fig. 5B). Similarly, we analyzed changes in the SMI compared to changes in fat mass.

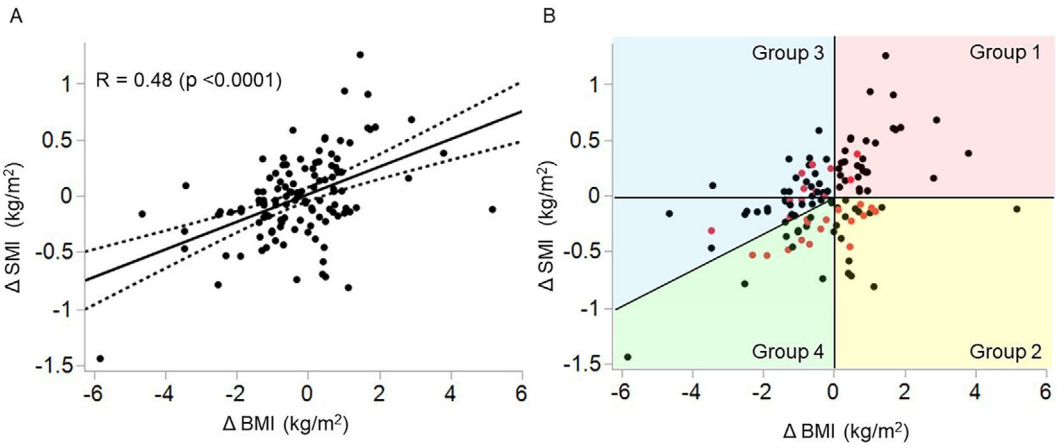


**Figure 3.** **Correlation diagram of ΔBMI versus ΔSMI highlighting each group in this study and patients with type 2 diabetes mellitus. (A) Peason correlation plot with a regression line and 95% confidence interval demonstrating the correlation between the change in the BMI and SMI. (B) The range of each group in this study is shown on the correlation diagram of the ΔBMI and ΔSMI. The severe muscle loss group is defined as the group whose SMI was decreasing while body weight was increasing (Group 2) and the group with a larger decrease in muscle mass (ΔSMI/|ΔBMI| <median) among the weight loss group (Group 4). The range of Group 2 is shown in yellow, and the range of Group 4 is shown in green. Group 1, which consists of those who had an increase in both body weight and muscle mass, is shown in red. Group 3, which includes subjects who lost weight but gained muscle mass or maintained muscle mass relative to the weight loss, is shown in blue. In addition, patients with type 2 diabetes mellitus are indicated with red dots. SMI: skeletal muscle mass index, BMI: body mass index**

**Table 3.** **The Logistic Regression Analysis for Variables Related to Skeletal Muscle Loss in Patients with MASLD.**

| | Univariate model | | | |
|---|---|---|---|---|
| | Odds ratio | | p value | |
| Age, ≥65 years | 1.22 | (0.48-3.13) | 0.7 | |
| Gender, men | 1.98 | (0.90-4.34) | 0.08 | |
| Liver cirrhosis | 1.16 | (0.35-3.88) | 0.8 | |
| Hepatocellular carcinoma | 0.40 | (0.035-4.52) | 0.4 | |
| Body weight reduction | 3.19 | (1.50-6.81) | 0.002 | * |
| Diabetes mellitus | 2.02 | (0.83-4.94) | 0.1 | |
| Medication related to metabolic dysfunction | | | | |
| Metformin | 0.80 | (0.19-3.38) | 0.8 | |
| DPP-4 inhibitors | 0.55 | (0.16-1.84) | 0.3 | |
| Thiazolidines | N/A | | | |
| GLP-1 agonists | N/A | | | |
| SGLT2 inhibitors | 1.48 | (0.41-5.35) | 0.5 | |
| Statins | 0.65 | (0.19-2.27) | 0.5 | |
| Fibrates | 0.40 | (0.0035-4.52) | 0.4 | |
| Others | 1.25 | (0.33-4.67) | 0.7 | |
| Total bilirubin, ≥1.0 mg/dL | 1.31 | (0.5-3.04) | 0.2 | |
| Alanine aminotransferase, ≥30 IU/mL | 1.27 | (0.47-3.48) | 0.6 | |
| Albumin, <4.0 g/dL | 0.52 | (0.18-1.47) | 0.2 | |
| Type 4 collagen, ≥140 ng/mL | 0.58 | (0.26-1.30) | 0.18 | |
| FIB-4 index, ≥2.67 | 1.18 | (0.48-2.92) | 0.7 | |

MASLD: metabolic dysfunction-associated steatotic liver disease, DPP-4: Dipeptidyl peptidase-4, GLP-1: glucagon-like peptide-1, SGLT2: sodium-glucose transport protein 2, FIB-4: fibrosis-4.

*: p value <0.05.

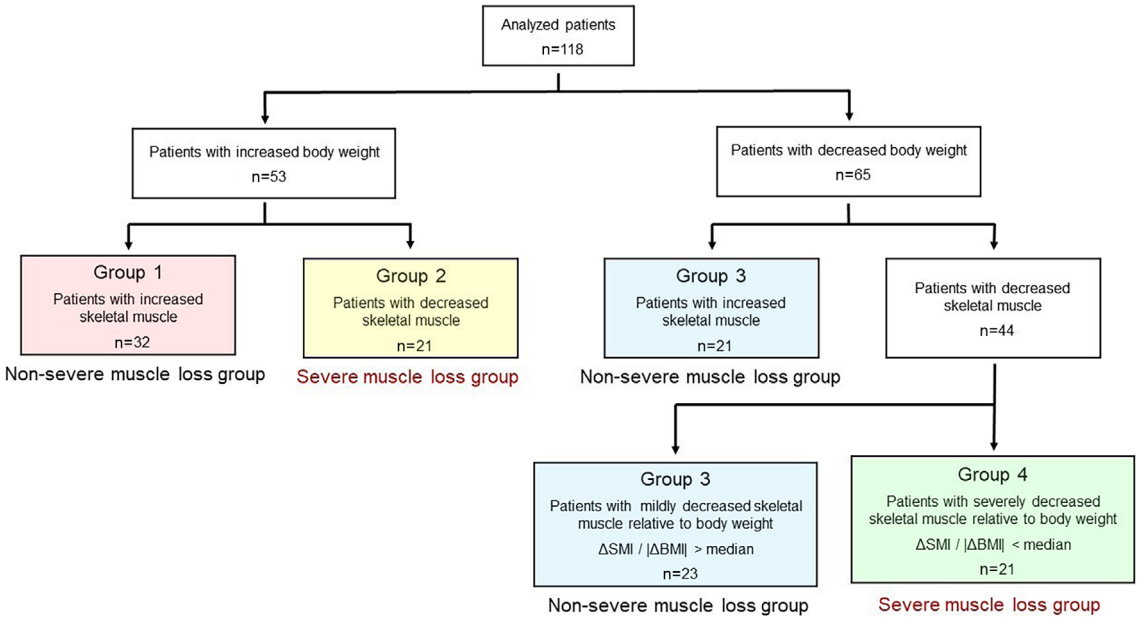


**Figure 4. The flowchart describing the categorized patients to analyze the severe muscle loss group. The patients in the present study were classified as follows: Group 1 included individuals who experienced an increase in both body weight and muscle mass; Group 2 included those who gained weight but lost muscle mass; Group 3 included individuals who lost weight but gained muscle mass or maintained muscle mass relative to weight loss (ΔSMI/|ΔBMI| > median); and Group 4, included individuals who lost weight with a significant reduction in muscle mass compared to the amount of weight lost (ΔSMI/|ΔBMI| <median). Groups 2 and 4 were defined as the severe muscle loss groups. SMI: skeletal muscle mass index, BMI: body mass index**

We found no significant difference in the change in muscle mass in the subgroup with increased fat mass (Supplementary material 6A), whereas the subgroup with decreased fat mass showed a significant decrease in the SMI change relative to fat mass (Supplementary material 6B). Supplementary material 7 presents a comparison between the severe and non-severe muscle loss groups in T2DM patients. Although type 4 collagen levels were higher in the severe muscle loss group, a logistic regression analysis did not identify this as a significant risk factor (Supplementary material 8).

## Discussion

In this study, we hypothesized that dietary therapy, an essential treatment for patients with MASLD to reduce their body weight, might affect skeletal muscle loss during weight reduction. We conducted a single-center retrospective observational study to elucidate the effects of body weight reduction due to dietary treatment on skeletal muscle mass in patients with MASLD. Weight loss was significantly associated with decreased skeletal muscle mass. Furthermore, T2 DM is a predictor of greater muscle loss relative to body weight change in patients with MASLD.

The pathogenesis of sarcopenia is multifactorial; hyperammonemia (15), increased autophagy (16), proteasomal activity (17), myostatin (18), and an impaired mitochondrial function (15) play essential roles in sarcopenia. Regarding nutritional factors, protein-energy malnutrition, especially the lack of branched-chain amino acids (BCAAs), is a crucial mechanism in the progression of sarcopenia. We previously reported that BCAAs were significantly associated with the progression of sarcopenia during HCC therapy (19). In liver cirrhosis, BCAAs are known to contribute to improving insulin resistance in muscles, the liver, and adipose tissue, and the BCAA leucine exerts a protein-synthesizing effect by activating the mammalian target of rapamycin pathway (20). In Japan, it has been reported that the combination of BCAA administration and exercise (walking) is helpful for the management of sarcopenia in patients with cirrhosis (21). In our study on the amino acid profile in patients with chronic liver disease, we reported that, in steatotic liver disease, BCAA levels decrease rapidly with a decline in the liver reserve capacity compared to other etiologies (22). As mentioned above, protein-energy malnutrition is associated with sarcopenia progression. However, the primary treatment for MASLD, which is increasingly prevalent worldwide, is diet restriction to reduce body weight. Therefore, we considered it necessary to analyze the changes in muscle mass during weight reduction for MASLD treatment and to analyze which patient groups should practice care with regard to reductions in muscle mass.

First, we investigated the impact of weight change on the liver function. In patients who experienced weight loss, ALT levels decreased, and a correlation was observed between changes in the BMI and ALT levels. Consistent with previ-

**Table 4. The Characteristics of Patients in the Severe Muscle Loss Group and the Non-severe Muscle Loss Group.**

| | Severe muscle loss group n=42 | | Non-severe muscle loss group n=76 | | p value | |
|---|---|---|---|---|---|---|
| Age, years | 56 | (17) | 54 | (21) | 0.7 | |
| Gender, men/women | 16 (38%)/26 (62%) | | 25 (33%)/51 (67%) | | 0.6 | |
| BMI, kg/m$^2$ | 29 | (4.9) | 29 | (8.0) | 0.4 | |
| SMI, kg/m$^2$ | | | | | | |
| Men | 11 | (1.7) | 11 | (2.0) | 0.6 | |
| Women | 9.2 | (1.4) | 9.1 | (1.7) | 0.8 | |
| Liver cirrhosis | 6 | (14%) | 6 | (8%) | 0.3 | |
| Hepatocellular carcinoma | 1 | (2%) | 2 | (3%) | 0.9 | |
| Sarcopenia | 0 | (0%) | 0 | (0%) | N/A | |
| Body weight reduction | 21 | (50%) | 44 | (58%) | 0.4 | |
| BMI change, kg/m$^2$ | 0.007 | (1.4) | -0.3 | (1.8) | 0.8 | |
| Skeletal muscle mass reduction | 42 | (100%) | 23 | (30%) | <0.0001 | * |
| SMI change, kg/m$^2$ | -0.2 | (0.3) | 0.2 | (0.3) | <0.0001 | * |
| Diabetes mellitus | 15 | (36%) | 13 | (17%) | 0.02 | * |
| Medication related to metabolic dysfunction | | | | | | |
| Metformin | 3 | (7%) | 5 | (7%) | 0.9 | |
| DPP-4 inhibitors | 4 | (10%) | 8 | (11%) | 0.9 | |
| Thiazolidines | 0 | (0%) | 1 | (1%) | 0.5 | |
| GLP-1 agonists | 0 | (0%) | 2 | (3%) | 0.3 | |
| SGLT2 inhibitors | 5 | (12%) | 6 | (8%) | 0.5 | |
| Statins | 4 | (10%) | 7 | (9%) | 0.9 | |
| Fibrates | 1 | (2%) | 2 | (3%) | 0.9 | |
| Others | 4 | (10%) | 6 | (8%) | 0.8 | |
| Total bilirubin, mg/dL | 0.8 | (0.6) | 0.8 | (0.4) | 0.9 | |
| Alanine aminotransferase, IU/L | 56 | (76) | 65 | (51) | 0.7 | |
| Albumin, g/dL | 4.3 | (0.4) | 4.4 | (0.3) | 0.5 | |
| Hemoglobin A1c, % | 6.1 | (1.0) | 6.1 | (1.1) | 0.3 | |
| Type 4 collagen, ng/mL | 153 | (91) | 137 | (40) | 0.07 | |
| FIB-4 index | 1.8 | (13) | 1.7 | (1.0) | 0.5 | |

BMI: body mass index, SMI: skeletal muscle mass index, DPP-4: Dipeptidyl peptidase-4, GLP-1: glucagon-like peptide-1, SGLT2: sodium-glucose transport protein 2, FIB-4: fibrosis-4

*: p value <0.05.

Categorical variables are shown as n (%).

Continuous variables are shown as the median (interquartile range).

ous reports, weight loss positively affected the liver function in patients with MASLD. Next, we compared the clinical characteristics of the groups with decreased and increased skeletal muscle mass. In this study, although there was no significant difference, patients over 65 years old showed a trend toward a decline in the SMI (p=0.06), suggesting that elderly people might tend to lose skeletal muscle during dietary therapy. Furthermore, no significant differences were found in the medication status of the metabolism-related drugs. However, the small number of patients in this study makes it difficult to determine whether or not these drugs affect muscle mass. To elucidate these points, we need to increase the number of cases for further analyses. Variables showing a significant difference between the two groups were the amount of BMI change and the number of subjects with body reduction. In addition, a strong correlation was observed between the amount of skeletal muscle change and change in the BMI. A logistic regression analysis also showed that weight loss was associated with skeletal muscle loss. These findings suggest that weight loss is significantly associated with a decrease in skeletal muscle mass in patients with MASLD.

As mentioned above, we observed the following key findings: 1) body weight reduction is beneficial for the pathological condition of MASLD, and 2) there is a strong correlation between body weight and muscle mass changes. This suggests that although weight loss is beneficial for the liver function in MASLD patients, it often results in a concomitant loss of muscle mass. Given these findings, it is crucial to investigate the risk factors associated with disproportionate loss of muscle mass relative to body weight reduction. To identify patients at an increased risk of excessive muscle mass loss during weight reduction, we proposed the "severe muscle loss group," which accounts for this disparity.

**Table 5. The Univariate Analysis for Variables Related to the Severe Muscle Loss Group.**

| | Univariate model | | | |
|---|---|---|---|---|
| | Odds ratio | | p value | |
| Age, ≥65 years | 1.04 | (0.39-2.73) | 0.9 | |
| Gender, men | 1.23 | (0.57-2.75) | 0.6 | |
| Liver cirrhosis | 1.94 | (0.59-6.46) | 0.8 | |
| Hepatocellular carcinoma | 0.90 | (0.079-10.26) | 0.9 | |
| Body weight reduction | 0.73 | (0.34-1.55) | 0.4 | |
| Diabetes mellitus | 2.69 | (1.13-6.42) | 0.03 | * |
| Medication related to metabolic dysfunction | | | | |
| Metformin | 1.09 | (0.25-4.82) | 0.9 | |
| DPP-4 inhibitors | 0.89 | (0.25-3.17) | 0.9 | |
| Thiazolidines | N/A | | | |
| GLP-1 agonists | N/A | | | |
| SGLT2 inhibitors | 1.58 | (0.45-5.51) | 0.5 | |
| Statins | 1.04 | (0.29-3.77) | 0.9 | |
| Fibrates | 0.90 | (0.079-10.26) | 0.9 | |
| Others | 1.23 | (0.33-4.62) | 0.8 | |
| Total bilirubin, ≥1.0 mg/dL | 0.71 | (0.29-1.74) | 0.3 | |
| Alanine aminotransferase, ≥30 IU/L | 0.64 | (0.23-1.78) | 0.4 | |
| Albumin, <4.0 g/dL | 0.51 | (0.16-1.68) | 0.2 | |
| Type 4 collagen, ≥140 ng/mL | 0.77 | (0.33-0.78) | 0.5 | |
| FIB-4 index, ≥2.67 | 0.69 | (0.26-1.84) | 0.5 | |

BMI: body mass index, SMI: skeletal muscle mass index, DPP-4: Dipeptidyl peptidase-4, GLP-1: glucagon-like peptide-1, SGLT2: sodium-glucose transport protein 2, FIB-4: fibrosis-4

*: p value <0.05.

**Table 6. The Multivariate Analysis of Variables Related to the Severe Muscle Loss Group.**

| | Multivariate model | | | |
|---|---|---|---|---|
| | Odds ratio | | p value | |
| Age, ≥65 years | 1.17 | (0.40-3.43) | 0.8 | |
| Liver cirrhosis | 3.43 | (0.93-12.6) | 0.06 | |
| Diabetes mellitus | 3.11 | (1.24-7.83) | 0.02 | * |
| Total bilirubin, ≥1.0 mg/dL | 0.76 | (0.29-1.97) | 0.6 | |
| Albumin, <4.0 g/dL | 0.46 | (0.13-1.64) | 0.2 | |
| Type 4 collagen, ≥140 ng/mL | 0.77 | (0.31-1.95) | 0.6 | |
| FIB-4 index, ≥2.67 | 0.67 | (0.22-2.00) | 0.5 | |

FIB-4: fibrosis-4

*: p value <0.05.

We analyzed the risk factors associated with "severe muscle loss" and found no significant difference in weight change between the severe and non-severe muscle loss groups, with the only variable showing a significant difference being the presence of T2DM. In the logistic regression analysis, T2DM was extracted as a variable related to the severe muscle loss group. T2DM is characterized by insulin resistance, inflammation, accumulation of advanced glycation end-products, and increased oxidative stress. These characteristics can negatively affect various aspects of muscle health, including the muscle mass, strength, quality, and function, through impairments in protein metabolism, vascular and mitochondrial dysfunction, and cell death (23). A Korean retrospective study of a large number of subjects with T2DM revealed that the prevalence of sarcopenia was higher in patients with T2DM, suggesting that patients with T2DM are at risk of developing sarcopenia (24). The findings of this study are consistent with those of previous studies.

Several limitations associated with the present study warrant mention. First, changes in the muscle mass were estimated using a BIA, which, while useful for this purpose, does not provide information on the intramuscular fat content (myosteatosis) or muscle quality. The correlation between weight change and muscle mass change could be influenced by variations in fat content within the skeletal muscles, which was not analyzed in this study because our dataset did not include CT images or grip strength data, which could have provided more detailed insights into the muscle quality and composition. The sarcopenia guidelines of the Japan Society of Hepatology, Asian Working Group for Sarcopenia, and European Working Group on Sarcopenia in Older People include grip strength criteria (13, 25, 26). The absence of these measurements precludes a more comprehensive analysis of how the intramuscular fat and muscle quality may affect the observed changes in muscle mass. Therefore, although our findings suggest a strong correlation between weight change and muscle mass change, they should be interpreted with caution. The potential effects of intramuscular fat and variations in the muscle quality on this relationship remain unexplored and represent a significant

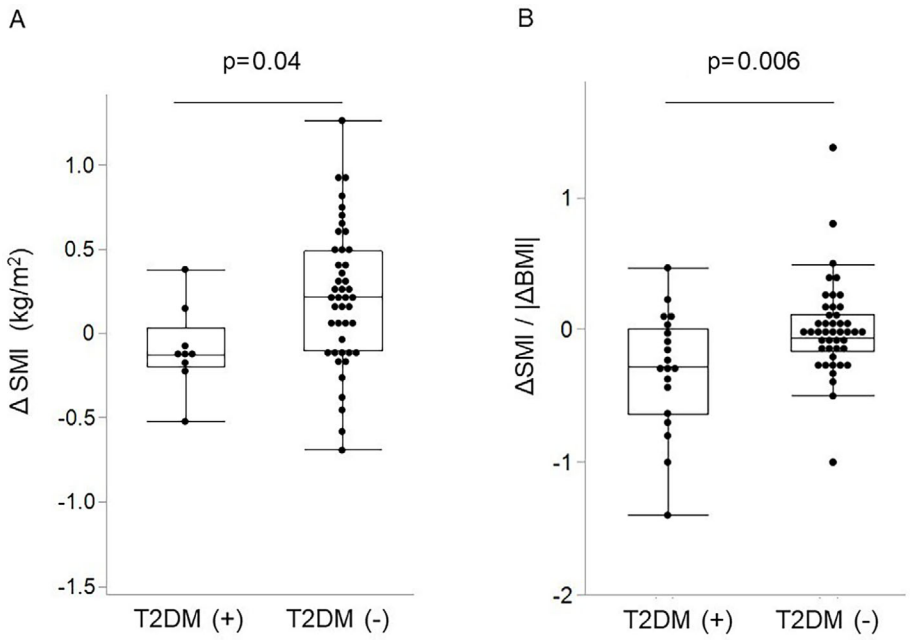


**Figure 5. The association between type 2 diabetes mellitus and severe muscle loss in MASLD patients. (A) The change in the SMI in patients with and without T2DM in the increased body weight group. (B) The change in the SMI relative to the BMI in patients with and without T2DM in the decreased body weight group. The box plots present the minimum score, first (lower) quartile, median, third (upper) quartile, and maximum score. T2DM: type 2 diabetes mellites, SMI: skeletal muscle mass index**

limitation of the present study. Future research should include more direct measures of muscle composition to fully understand the dynamics between changes in body weight and muscle mass in patients with steatotic liver disease. Second, we did not record the patients' exercise habits, intake status of each nutrient, or adherence to dietary guidelines to a consistent standard, meaning such data could not be included in this study. These items are extremely important for determining the muscle mass, and future research should be conducted to summarize them in detail. Third, this study used a single-center, retrospective design. Therefore, prospective multicenter studies are required. Finally, there were few cases of liver cirrhosis in the present study. Sarcopenia is a condition that requires particular attention in patients with cirrhosis, so it is necessary to further increase the number of cases of liver cirrhosis in future evaluations.

Despite these limitations, however, the results of this study suggest that, while weight reduction with nutritional therapy improves the liver function in patients with MASLD, it may reduce skeletal muscle mass, particularly in patients with diabetes, highlighting the need to pay attention to the decrease in skeletal muscle mass. There may be potential for exercise therapy and protein intake to be effective in such cases, although this will need to be examined in the future.

## Conclusion

This retrospective observational study revealed that weight loss is related to skeletal muscle loss in patients with MASLD during dietary therapy. In addition, T2DM is associated with a more severe risk of skeletal muscle decrease than weight changes. These findings indicate that we need to focus more on skeletal muscle changes in patients with MASLD undergoing treatment for weight reduction, especially in patients with T2DM.

**The authors state that they have no Conflict of Interest (COI).**

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