Research Article
NAFLD and Alcohol-Related Liver Diseases

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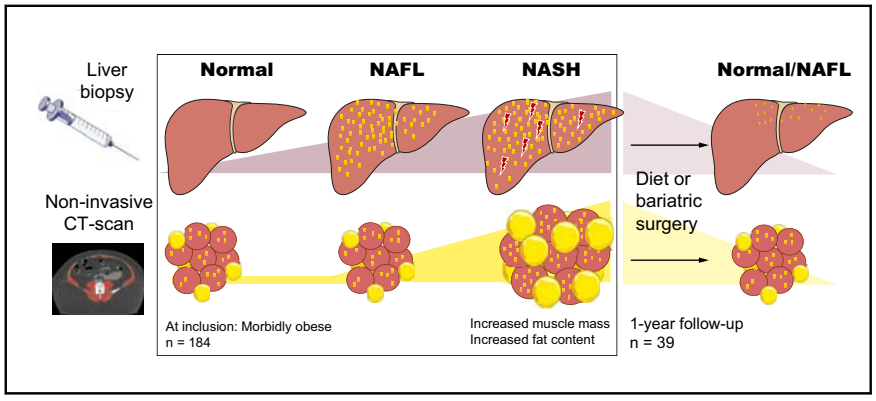
Muscle fat content is strongly associated with NASH: a longitudinal study in patients with morbid obesity

Graphical abstract



Highlights

- Muscle fat content is higher in patients with obesity and NASH than in those with obesity and NAFL.
- There is no low muscle mass in patients with obesity-associated NASH.
- Muscle fat content is strongly associated with cardinal histological features of NASH.
- NASH improvement is associated with a significant decrease of muscle fat content.

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Lay summary

The fat content in skeletal muscles is highly reflective of the severity of non-alcoholic fatty liver disease (NAFLD) in patients with morbid obesity. In particular, muscle fat content is strongly associated with non-alcoholic steatohepatitis (NASH) and decreases upon NASH improvement. These data indicate that muscle fatty infiltration could be a marker and possible pathophysiological contributor to NASH.

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Muscle fat content is strongly associated with NASH: a longitudinal study in patients with morbid obesity

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Background & Aims: Studies exploring the relationship between muscle fat content and non-alcoholic fatty liver disease (NAFLD) are scarce. Herein, we aimed to evaluate the association of muscle mass and fatty infiltration with biopsy-assessed NAFLD in patients with obesity.

Methods: At inclusion (n = 184) and 12 months after a dietary intervention (n = 15) or bariatric surgery (n = 24), we evaluated NAFLD by liver biopsy, and skeletal muscle mass index (SMI) by CT (CT-SMI) or bioelectrical impedance analysis (BIA-SMI). We developed an index to evaluate absolute fat content in muscle (skeletal muscle fat index [SMFI]) from CT-based psoas muscle density ($SMFI_{Psoas}$).

Results: Muscle mass was higher in patients with NAFLD than in those without (CT-SMI 56.8 ± 9.9 *vs.* 47.4 ± 6.5 cm^2/m^2, p <0.0001). There was no association between sarcopenia and non-alcoholic steatohepatitis (NASH). $SMFI_{Psoas}$ was higher in NASH ≥F2 and early NASH F0-1 than in NAFL (78.5 ± 23.6 and 73.1 ± 15.6 *vs.* 61.2 ± 12.6, p <0.001). A 1-point change in the score for any of the individual cardinal NASH features (*i.e.* steatosis, inflammation or ballooning) was associated with an increase in $SMFI_{Psoas}$ (all p <0.05). The association between $SMFI_{Psoas}$ and NASH was highly significant even after adjustment for multiple confounders (all p <0.025). After intervention (n = 39), NASH improvement, defined by NAFLD activity score <3 or a 2-point score reduction, was achieved in more than 75% of patients (n = 25 or n = 27, respectively) that had pre-established NASH at inclusion (n = 32) and was associated with a significant decrease in $SMFI_{Psoas}$ (p <0.001). Strikingly, all patients who had ≥11% reduction in $SMFI_{Psoas}$ achieved NASH improvement (14/14, p <0.05).

Conclusions: Muscle fat content, but not muscle mass, is strongly and independently associated with NASH. All individuals who achieved a ≥11% decrease in $SMFI_{Psoas}$ after intervention improved their NASH. These data indicate that muscle fatty infiltration could be a potential marker for (and perhaps a pathophysiological contributor to) NASH.

Lay summary: The fat content in skeletal muscles is highly reflective of the severity of non-alcoholic fatty liver disease (NAFLD) in patients with morbid obesity. In particular, muscle fat content is strongly associated with non-alcoholic steatohepatitis (NASH) and decreases upon NASH improvement. These data indicate that muscle fatty infiltration could be a marker and possible pathophysiological contributor to NASH.

Introduction

Non-alcoholic fatty liver disease (NAFLD) has become the most prevalent chronic liver disease worldwide.[1] NAFLD encompasses a spectrum of diseases ranging from non-alcoholic fatty liver (NAFL, simple steatosis), affecting ~25% of the world adult population, to non-alcoholic steatohepatitis (NASH) that leads to fibrosis and progressively to end-stage liver disease and hepatocellular carcinoma (HCC) in a subset of patients.[1] The progression of the disease is unpredictable, often silent, and its definitive diagnosis relies on a liver biopsy.[2] Non-invasive scores or imaging techniques are increasingly validated to assess steatosis or fibrosis, but cannot distinguish NASH from NAFL.[2] Even in the absence of fibrosis, patients with NASH are at an increased risk of liver disease progression, cardiovascular events and HCC.[1,3] Hence, we need additional tools to identify patients with or at-risk of NASH, irrespective of the fibrosis stage.

The concept that disrupted "muscle health" is a sign and/or a pathophysiological contributor to various diseases has gathered

Keywords: Muscle mass; muscle composition; sarcopenia; muscle fat; muscle lipid; myosteatosis; NAFLD; NASH; CT scan; obesity; liver disease; non-alcoholic steatohepatitis.
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support in recent years.[4–6] Literature suggests that low muscle mass and/or low muscle strength are associated with NAFLD severity.[7–16] However, these studies suffer from limitations, including the lack of a gold standard methodology for muscle mass evaluation (*i.e.* CT or MRI)[17] and for NAFLD staging (liver biopsy). The majority of reports come from Asian cohorts with limited data available in Western populations.[8,10,11,13] Of note, the term "sarcopenia" is often used to designate the presence of mere low muscle mass in patients with NAFLD, although there is an international consensus to include muscle strength when available.[18,19] Thus, the relationship between skeletal muscle features and NAFLD is still unclear.

A high muscle fat concentration (often referred to as myosteatosis) is linked with insulin resistance[20,21] that can itself lead to loss of muscle mass.[22] While myosteatosis has been strongly associated with cardiometabolic diseases,[23] studies exploring the relationship between myosteatosis and NAFLD are scarce.[11,13] In addition, whether evaluating absolute muscle fat content (and not concentration) could have a clinical relevance in patients with NAFLD has not been investigated.

The aim of the present study was to clarify the relationship between muscle mass, muscle fat concentration and content (as measured by CT) and NAFLD severity (as histologically assessed on liver biopsy). The analyses were performed on a large population of patients with morbid obesity (n = 184), of whom 39 also underwent a liver biopsy and CT scan ~1 year after a therapeutic intervention (n = 15 diet and n = 24 bariatric surgery).

Patients and methods

Patient cohort and characteristics

Analyses were retrospectively performed on a previously described cohort of the Antwerp University Hospital (Fig. S1).[24] Briefly, overweight or obese patients visiting the obesity clinic underwent a metabolic and a liver-specific work-up including a detailed questionnaire, a bio-impedance analysis (BIA) and a CT scan at L4 level. Patients were excluded in case of a liver disease other than NAFLD, significant alcohol consumption (>20 g/day), a history of bariatric surgery or pre-existing diabetes. Since diabetes is a specific risk factor for NASH and fibrosis and some drugs used to treat diabetes may beneficially impact NAFLD histology, introducing potential substantial confounding,[25] pre-existing diabetes (defined as an established diagnosis on previous assessment and/or active use of antidiabetic drugs) was an exclusion criterion. Metabolic assessment in non-diabetic patients included an oral glucose tolerance test to assess glycaemic control status. Patients who were *de novo* diagnosed, based on this oral glucose tolerance test, as being diabetic, were not excluded, as no prior antidiabetic treatment could have affected the baseline observations. In case of suspicion of NAFLD (based on a pre-defined combination of abnormalities of ultrasound and liver biochemistry), a liver biopsy was proposed and obtained after written consent. To prevent potential confounding from obesity-induced mechanical overload on skeletal muscles, only patients with obesity (BMI ≥30 kg/m^2; 288 out of 480 patients) were considered for further analysis. Of these 288 patients, 196 had a liver biopsy; 12 of them were excluded for obvious artefacts in CT-acquisition, such as the presence of orthopaedic material in the spine. Hence, 184 patients were considered for baseline analyses. All had a therapeutic intervention consisting of dietary counselling or bariatric surgery. Thirty-nine had a repeated liver biopsy and CT scan available for analysis after a median 14 months (IQR 12.7–15.4 months) follow-up with a median 41 days (IQR 7–56 days) between the 2 tests. The patient cohort was part of the HEPADIP protocol (Belgian registration number B30020071389) that was approved by the Ethical Committee of the Antwerp University Hospital (6/25/125). Additional approval was obtained for this retrospective analysis (16/5/46).

Liver biopsy assessment

Liver biopsy was obtained by percutaneous (35.3%) or transjugular (17.9%) approach or during surgery in those patients who underwent bariatric surgery after work-up (46.7%). For the latter, patients were included if their body weight was stable between work-up and surgery (*i.e.* those who went on a low-calorie diet and lost weight between CT scan and biopsy were excluded). After follow-up, biopsies were obtained by percutaneous (82.1%), transjugular (12.8%) or peroperative approach (5.1%, during elective cholecystectomy). All liver biopsies were scored by 2 experienced pathologists blinded for clinical data using the NASH Clinical Research Network (NASH-CRN) system.[26] NASH was defined by the presence of steatosis grade ≥1 and lobular inflammation grade ≥1 and ballooning grade ≥ 1. The following groups were defined for analysis: 1) Normal liver, absence of steatosis, ballooning, inflammation or fibrosis; 2) NAFL, steatosis ≥1 with no ballooning and/or no inflammation and fibrosis ≤F2; 3) NASH (F0,F1); 4) NASH (F2–F4).

NASH improvement was based on liver biopsy and defined as (1) a NAS <3, (2) a ≥2-point NAS reduction or (3) the resolution of ballooning with no or minimal (grade 1) inflammation after intervention.[27]

Muscle assessment

Skeletal muscle mass, density and fat indexes were calculated from the abdominal CT scan at L4, performed 21 [0–104] days before liver biopsy. We used Hounsfield unit (HU) values at the commonly accepted thresholds of −29 to +150 HU[28] to semi-automatically delineate psoas, dorsal and abdominal muscles. Muscle area and density were quantified by the Slice-O-Matic software, version 4.3 (Tomovision, Montreal, Canada) (Fig. S2). Total muscle area was normalised for stature (height (m) squared) and referred to as the CT-skeletal muscle index (CT-SMI) (cm^2/m^2). A CT-SMI <39 cm^2/m^2 in female and <50 cm^2/m^2 in male defined sarcopenia.[29] We measured the mean psoas muscle density to evaluate fat concentration in the muscle (PD, or psoas fat concentration^{-1}).[30] We then developed an index to evaluate absolute fat content in the muscle from CT-based psoas density, termed the skeletal muscle fat index ($SMFI_{Psoas}$), computed as follows: $SMFI_{Psoas}$ = 100 * [psoas area (cm^2)/psoas density (HU)] and reported as an index hereafter for simplification. Thus, the higher the muscle fat concentration, the lower the muscle density and the higher the muscle fat content the higher the $SMFI_{Psoas}$. Of note, we did not analyse dorsal muscles (quadratus lumborum, erector spinae) because, in a significant proportion of available images, the section crossed the iliac bone, potentially generating interferences for density measures. All CT images were randomly analysed by a single trained observer (MN),[31,32] blinded for clinical and liver histology data. Body composition was also assessed by BIA as previously described.[24] Muscle mass was measured and normalised on height squared (kg/m^2) and on body mass (%) to compute BIA-skeletal muscle indexes (BIA-SMI).[33]

Statistics

All data are presented as mean ± SD unless specified otherwise. Statistical analyses were performed using 2-tailed Student's or Welch's *t* test, one-way or two-way ANOVA followed by Bonferroni's *post hoc* correction when appropriate for continuous variable and by Wilcoxon signed-rank test or Kruskal-Wallis test for ordinal variables and chi-square test for categorical variables, using SPSS (v24). Multivariate analyses and table generation were performed on SPSS (v24) using binary logistic regression. All parameters were systematically checked for co-linearity. Odds ratio (ORs) were computed using exponentiation of the B coefficient. Of note, B coefficients are expressed in log-odds units and thus are influenced by variable scaling. Hence, the re-scaling in $SMFI_{Psoas}$ artificially lowered the OR in numbers but without influencing the test significance. To better reflect the magnitude of the association, we also reported ORs derived from Z-score normalized $SMFI_{Psoas}$. These latest ORs have to be interpreted in terms of a 1 SD increase. Differences were considered significant at values of p <0.05. All figures were created with GraphPad Prism 8 software.

Results

Study population

Table 1 shows the characteristics of the 184 patients with obesity at inclusion and according to NAFLD presence. The mean age of individuals was 41 years and two-thirds were females. Mean BMI was 40.2 kg/m^2. Diabetes was diagnosed during work-up in 12.1% patients. At liver biopsy, 34 had normal liver histology, 36 had NAFL and 114 had NASH. Fibrosis (≥F1) was present in 60 patients, all of whom had NASH (hence there were no cases with steatofibrosis). Several metabolic parameters differed between No NAFLD and NAFLD patients.

Higher muscle mass, rather than sarcopenia, associates with NAFLD in patients with obesity

We evaluated muscle mass with CT-SMI.[18] Patients with NAFLD had a higher muscle mass than those with No NAFLD had (Fig. S3A–C). Only 8 out of 184 patients (4.3%, of whom 6 female) were sarcopenic (Fig. S3D). Sex-stratification confirmed that patients with NAFLD had a higher or similar – rather than a lower – muscle mass than patients with No NAFLD, irrespectively of the method used to assess muscle mass (Fig. S4). Among the 8 patients with sarcopenia according to the pre-defined cut-off, 6 had normal liver histology and 2 had NAFLD (1 NAFL and 1 NASH). We found plausible confounding factors for 2 patients without NAFLD (2/6), but not for those with NAFLD (0/2) (Table S1). Thus, sarcopenia is uncommon in obese patients with NAFLD.

NAFLD is strongly associated with a high muscle fat content

We measured PD on CT images (Fig. S2). Fat being less dense than lean tissue, a decreased density reflects a higher tissue fat concentration. Muscles as well as the liver represent ectopic locations for lipid storage. Therefore, we anticipated a higher lipid concentration in muscles (or myosteatosis) in patients with NAFLD. Unexpectedly, PD was significantly higher, indicating a lower fat concentration in psoas, in patients with NAFLD than in those without NAFLD (p = 0.04) (Fig. 1A). We then used the novel

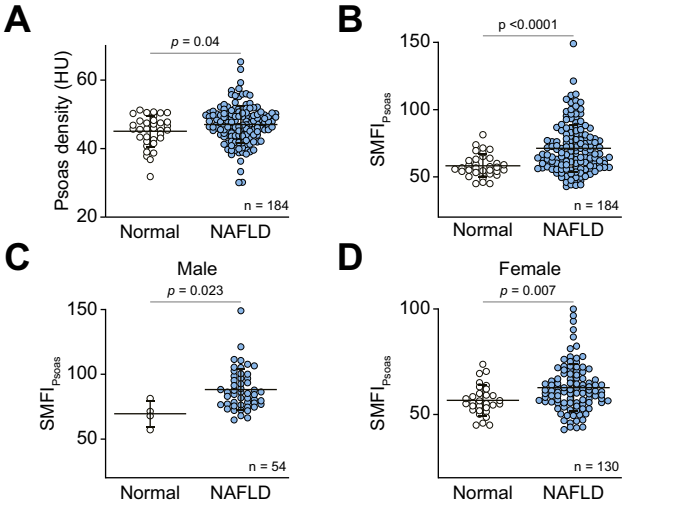

Fig. 1. Muscle fat content is higher in patients with NAFLD irrespective of sex. (A) Psoas density in patients with NAFLD and those with normal liver. (B), (C) and (D) Psoas fat index (*i.e.* the inverse of the psoas muscle density per psoas muscle area) in respectively all, male and female patients with NAFLD and those with normal liver. All data are mean ± SD. Student's *t* test. NAFLD, non-alcoholic fatty liver disease; $SMFI_{Psoas}$, skeletal muscle fat index of the psoas muscle.

Table 1. Baseline characteristics of study population.

	Total population (n = 184)	No NAFLD (n = 34)	NAFLD (n = 150)	*p* value no NAFLD *vs.* NAFLD
Age (year)	41 ± 6	40 ± 5	41 ± 6	n.s.
Female sex (%)	70.6 (130/184)	88.2 (30/34)	66.6 (100/150)	**0.013**
BMI (kg/m^2)	40.2 ± 6	38.3 ± 3.8	40.6 ± 6.3	**0.039**
ALT (U/L)	45 ± 27	32 ± 15	48 ± 28	**0.001**
AST (U/L)	31 ± 19	25 ± 11	32 ± 21	**0.049**
GGT (U/L)	42 ± 31	34 ± 21	44 ± 33	n.s.
Total cholesterol (mg/dl)	205 ± 37	202 ± 38	206 ± 37	n.s.
Triglycerides (mg/dl)	159 ± 75	142 ± 72	163 ± 75	n.s.
Hb1Ac (%)	5.6 ± 0.56	5.44 ± 0.27	5.66 ± 0.6	**0.044**
HOMA-IR	4.23 ± 4.26	3.22 ± 3.98	4.47 ± 4.31	n.s.
De novo diabetes (%)	10.8%	5.8%	12%	n.s.
Liver histology (Normal/NAFL/NASH)	34/36/114	34/0/0	0/36/114	**<0.001**
Stage of fibrosis (0/1/2/3/4)	124/31/20/8/1	34/0/0/0/0	90/31/20/8/1	**<0.001**

Data presented as mean ± SD. Student's *t* test was performed for continuous variables and chi-square test for categorial variables. Values in bold denote statistical significance. ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma glutamyltransferase; Hb1Ac, glycated haemoglobin; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis.

skeletal muscle fat index ($SMFI_{Psoas}$) (*see methods*) to evaluate absolute fat content in the psoas. The $SMFI_{Psoas}$ was significantly higher in patients with NAFLD when compared to controls (71.2 ± 17.6 *vs.* 52.2 ± 8.4, p <0.0001) (Fig. 1B) and this relationship remained significant when patients were stratified by sex (Fig. 1C–D). Thus, muscle fat content is higher in patients with NAFLD, as demonstrated by the $SMFI_{Psoas}$.

Muscle mass increases according to NAFLD spectrum

Patients were stratified in 4 groups according to NAFLD severity assessed on liver biopsy (Normal liver, NAFL, NASH F0-1 and NASH ≥F2) (Table 2). Several clinical and anthropometrical parameters differed between these categories. Patients with NASH ≥F2 had higher BMI, larger visceral fat area, higher serum alanine aminotransferase (ALT), aspartate aminotransferase and gamma glutamyltransferase levels, glycated haemoglobin and higher insulin resistance (as measured by homeostatic model assessment for insulin resistance [HOMA-IR]). Muscle mass (measured with SMI) was higher in patients with NAFL than in those with normal liver and increased even further in patients with NASH, with the highest values in NASH ≥F2 (Table 2). When stratified for sex (Fig. S4C), muscle mass significantly increased with disease severity in females, and a similar trend was observed in males. Thus, muscle mass is higher in patients with NASH compared to those with NAFL or normal liver.

Muscle fat content is significantly higher in NASH and is strongly related to cardinal histological features, independently from sex

Patients with normal liver histology and those with NAFL had similar $SMFI_{Psoas}$, hence NAFL was not associated with increased muscle fat content in our cohort (Table 2). By contrast, $SMFI_{Psoas}$ was significantly higher in patients with NASH than in those with NAFL or normal liver histology. Remarkably, among the large set of biological and anthropometrical data, $SMFI_{Psoas}$ is the only parameter that is significantly different between "early NASH" (*i.e.* NASH F0,1) and NAFL (Table 2).

$SMFI_{Psoas}$ was comparable in patients with NASH, irrespectively of the fibrosis stage, we therefore grouped patients with NASH F0,1 and those with ≥F2 (hereafter designated as NASH) and compared them to those with NAFL or normal liver. We stratified $SMFI_{Psoas}$ values according to sex-specific quartiles. The proportion of patients with NASH was significantly higher among those with high $SMFI_{Psoas}$ (quartile 4) compared to those with low $SMFI_{Psoas}$ (quartile 1) (Fig. 2A). Accordingly, the proportion of patients with normal liver or NAFL was lower in those within the high $SMFI_{Psoas}$ quartile. This relationship was independent of sex (Fig. 2A,B). We then tested the association between $SMFI_{Psoas}$ and individual histological features of NASH (*i.e.* steatosis, inflammation, ballooning) and fibrosis in the entire cohort. In any score category, a 1-point increase significantly increased the $SMFI_{Psoas}$ (p <0.05) (Fig. 3C–F), but additional points did not cause further increase in $SMFI_{Psoas}$. This relationship was independent of sex (Fig. S5A–D). Thus, muscle fat content is higher in patients with NASH, irrespectively of the degree of disease activity (*i.e.* steatosis, inflammation and ballooning sub-score) or fibrosis stage.

The association between muscle fat content and NAFLD/NASH is robust and independent from metabolic confounders

As $SMFI_{Psoas}$ is strongly associated with NASH and distinguishes NAFL from NASH, we used multivariate analysis to evaluate whether $SMFI_{Psoas}$ was independently associated with NAFLD and/or NASH (Table 3). We tested the association between $SMFI_{Psoas}$ and NAFLD among the total population (n = 184) and between $SMFI_{Psoas}$ and NASH among the NAFLD population (n = 150, patients with normal liver excluded). The $SMFI_{Psoas}$-associated ORs per SD increase for NAFLD in the total population and for NASH in NAFLD were respectively 3.57 (95% CI 1.87–6.83, p <0.001) and 2.95 (95% CI 1.68–5.20, p <0.001) when unadjusted. This association remained significant when multiple confounders were considered: age, sex, BMI, systolic blood pressure, smoking and diabetes status, triglycerides, cholesterol levels, and ALT levels (all p <0.025). To adjust for possible confounders for ectopic lipid deposition in skeletal muscle, we included specific muscle insulin resistance index (OGIS[34]), HOMA-IR and visceral fat area in our models. Again, the relationship between $SMFI_{Psoas}$ and NAFLD/NASH remained highly significant (Table 3). Therefore, muscle fat content assessed with $SMFI_{Psoas}$ is robustly associated with NAFLD and specifically with NASH in our cohort.

Muscle fat content is not independently associated with liver fibrosis in patients with NAFLD

To evaluate the parameters associated with liver fibrosis in patients with NAFLD, we used a multivariate model in which we compared patients with NAFLD and no fibrosis with those with fibrosis (Table S2). Only ALT levels (OR 1.03; 95% CI 1.01–1.05; p = 0.01) were significantly associated with liver fibrosis.

Muscle fat content decreases with weight loss and associates with NAFLD improvement

After intervention, patients (n = 39) lost weight and had improved metabolic parameters (Table 4). Most of them improved pre-established NASH, whether defined by a NAFLD activity score (NAS) <3 or a ≥2-point NAS reduction after intervention. Weight loss was associated with a decrease of muscle mass and muscle fat content (Table 4). Interestingly, despite weight loss, $SMFI_{Psoas}$ remained high in patients who still had NASH (n = 8) on follow-up when compared to those without (n = 31) (Fig. S6A), with values comparable to those at inclusion for similar (no)-NAFLD categories (Table 2, Fig. 2B). Likewise, a 1-point increase in the score for any individual histological NASH feature (*i.e.* steatosis, inflammation, ballooning) was associated with a significantly higher $SMFI_{Psoas}$ (Fig. S6B–C). Bariatric surgery was more effective at reducing body weight and achieving NASH improvement than dietary intervention alone (Table S3). However, the decrease of $SMFI_{Psoas}$ was not statistically greater after bariatric surgery when compared to dietary intervention (Table S3). There was no correlation between $SMFI_{Psoas}$ change and BMI or HOMA-IR improvement (Fig. S7A–B).

NASH improvement is associated with a significant decrease of $SMFI_{Psoas}$

Since the nature of the intervention and the magnitude of weight loss had no significantly different effect on $SMFI_{Psoas}$, we

Table 2. Clinical and anthropometrical characteristics of patients stratified according to NAFLD histological scoring.

	No NAFLD	NAFL	NASH+F1	NASH+F2+F3+F4	ANOVA statistics	*Post hoc* (Bonferroni)
Sex (female/male; n)	30/4	31/5	56/31	13/14	n/a	
Age (year)	39.8 ± 5.1	41.2 ± 5.0	41.4 ± 6.3	41.1 ± 5.9	0.567	n/a
Weight (kg)	106.20 ± 10.98	115.15 ± 21.03	114.32 ± 19.34	132.73 ± 22.66	<0.001	ccc ## §§§
Height (cm)	166.68 ± 6.23	167.61 ± 6.23	169.98 ± 8.68	174.39 ± 11.13	0.002	cc ##
BMI (kg/m^2)	38.3 ± 3.8	41.0 ± 7.0	39.5 ± 5.8	43.6 ± 6.2	0.002	cc §
Fat mass (kg)	54.0 ± 9.4	59.3 ± 16.6	54.4 ± 14.4	63.3 ± 11.3	0.014	cc §
Fat mass %	51.6 ± 6.3	50.9 ± 7.3	47.8 ± 7.5	47.7 ± 6.6	0.021	n/a
Fat free mass (kg)	51.2 ± 8.4	55.9 ± 10.4	58.8 ± 11.5	70.2 ± 17.0	<0.001	bb ccc ## §§
Systolic blood pressure (mmHg)	123.06 ± 14.50	126.79 ± 15.01 (34)	128.10 ± 13.84 (86)	131.56 ± 16.33	0.144	n/a
Albumin (g/dl)	4.19 ± 0.38	4.41 ± 0.43 (35)	4.46 ± 0.41 (79)	4350 ± 0.40 (23)	0.007	bb c
ALT (U/L)	31.71 ± 14.97	34.83 ± 20.65 (35)	45.56 ± 23.78	72.63 ± 32.77	<0.001	b ccc ### §§§§
AST (U/L)	24.76 ± 11.45	24.49 ± 8.46 (35)	25.22 ± 11.00	53.95 ± 35.95	<0.001	ccc ### §§§
ALP (U/L)	89.35 ± 35.10	80.89 ± 19.27	81.33 ± 22.95	87.48 ± 24.33	0.32	n/a
GGT (U/L)	34.26 ± 20.68	36.81 ± 21.26	41.66 ± 25.17	60.85 ± 54.55	0.004	cc # §
Creatinine (mg/dl)	0.77 ± 0.10	0.80 ± 0.11	0.84 ± 0.19	0.86 ± 0.15	0.075	n/a
CK18 (U/L)	217.46 ± 127.16 (13)	180.88 ± 94.03 (13)	202.80 ± 133.15 (34)	635.06 ± 572.11 (10)	<0.001	cc ### §§§
CRP (mg/dl)	0.98 ± 0.83	0.75 ± 0.68	0.88 ± 0.98(83)	0.71 ± 0.50	0.56	n/a
HbA1c (%)	5.44 ± 0.27	5.59 ± 0.33	5.57 ± 0.45	6.03 ± 1.06 (26)	<0.001	ccc ## §§
HOMA-IR	3.22 ± 3.98 (31)	3.25 ± 1.91 (33)	4.32 ± 4.95 (72)	6.59 ± 3.96 (24)	0.011	c #
OGIS (muscle insulin sensitivity)	417.00 ± 76.23 (31)	391.47 ± 83.57 (34)	362.96 ± 78.12 (80)	325.40 ± 68.83 (25)	<0.001	bb cccc #
Total cholesterol (mg/dl)	201.85 ± 37.62	205.56 ± 42.66	205.72 ± 36.15	204.70 ± 34.46	0.964	n/a
HDL (mg/dl)	53.71 ± 13.53	49.25 ± 15.11	47.04 ± 13.79	39.93 ± 7.27	0.001	cc #
TG (mg/dl)	141.74 ± 71.91	141.75 ± 73.58	166.02 ± 76.29	181.33 ± 67.46	0.073	n/a
Calculated LDL (mg/dl)	117.10 ± 32.90	125.62 ± 36.64 (35)	125.45 ± 34.12	128.53 ± 30.53	0.552	n/a
Vitamin D (ng/ml)	23.21 ± 9.56	27.91 ± 22.56 (33)	22.55 ± 14.85 (86)	19.77 ± 10.33 (26)	0.206	n/a
Skeletal muscle index (cm^2/m^2)	47.41 ± 6.53	52.95 ± 9.55	56.84 ± 8.13	62.06 ± 13.06	<0.001	bbb ccc ##
Psoas muscle density (HU)	45.07 ± 4.55	47.29 ± 5.28	46.54 ± 5.27	48.20 ± 5.52	0.108	n/a
$SMFI_{Psoas}$	58.21 ± 8.43	61.16 ± 12.6	73.13 ± 15.62	78.47 ± 23.57	<0.001	bbb ccc *** ###
Visceral fat area (cm^2)	144.67 ± 56.57	206.17 ± 76.47	213.88 ± 68370	262.78 ± 86.72	<0.001	aa bbb ccc # §

(n) of dataset as follows (unless denoted in table between brackets): No NAFLD (34), NAFL (36), NASH-F0,1 (87), NASH-F2,3,4 (27).
One-way ANOVA with a = No-NAFLD *vs.* NAFL, b = No-NAFLD *vs.* NASH-F0,1, c = No-NAFLD *vs.* NASH-F2,3,4, * = **NAFL *vs.* NASH-F0,1**, # = NAFL *vs.* NASH-F2,3,4, § = NASH-F0,1 *vs.* NASH-F2,3,4, n.a. = not applicable.
1x, 2x and 3x symbol = p <0.05; p <0.01 and p <0.001. All data are mean ± SD.
ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CK18, cytokeratin 18; CRP, C-reactive peptide; GGT, gamma glutamyltransferase; Hb1Ac, glycated haemoglobin; HOMA-IR, homeostatic model assessment for insulin resistance; HU, Hounsfield unit; NAFL, non-alcoholic fatty liver; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; $SMFI_{Psoas}$, skeletal muscle fat index of the psoas muscle; TG, triglyceride.

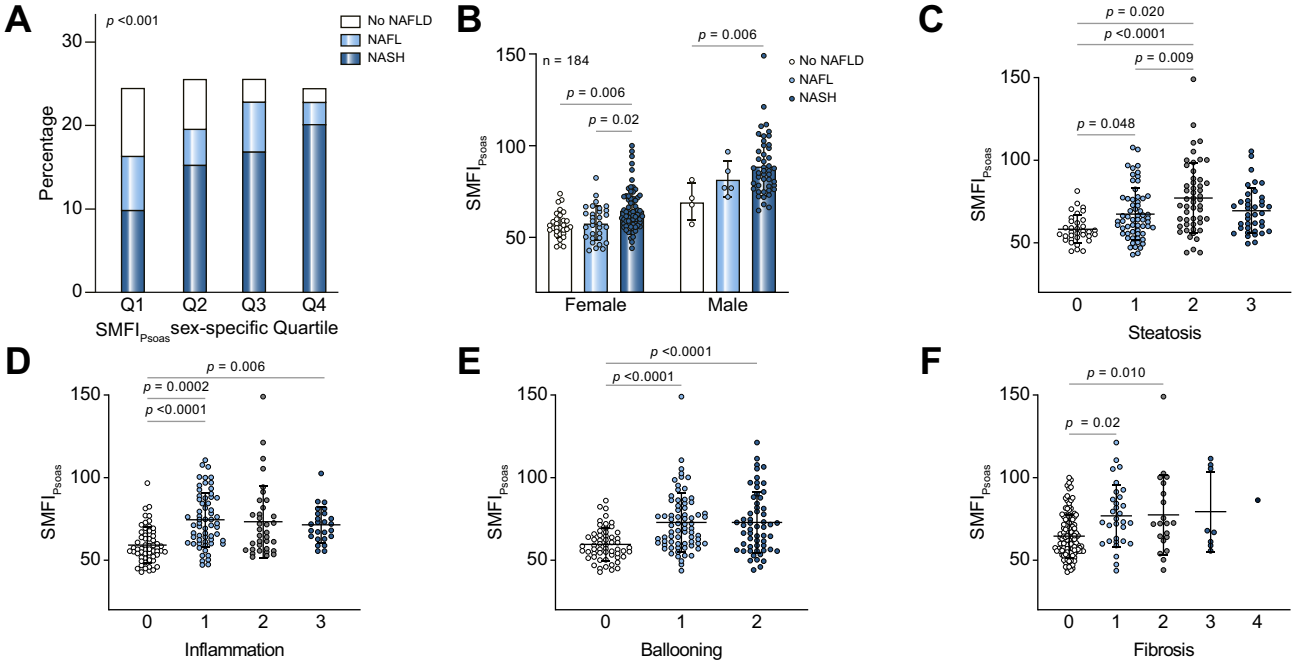

Fig. 2. Muscle fat content is higher in patients with NASH than in those with NAFL and normal liver and is significantly associated with histological NASH characteristics. (A) Relative proportion of patients with normal liver (No NAFLD), NAFL and NASH according to $SMFI_{Psoas}$ value stratified in sex-specific quartiles (chi-square test, p <0.0001). (B) Sex-specific $SMFI_{Psoas}$ values in patients stratified according to liver histology (two-way ANOVA, Interaction p = 0.45, Row factor p <0.0001 and Column factor p = 0.0002). (C), (D), (E) and (F) $SMFI_{Psoas}$ values according to NASH-CRN histological sub-score for steatosis, inflammation, ballooning and fibrosis (one-way ANOVA). All data are mean ± SD. NAFL, non-alcoholic fatty liver; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; $SMFI_{Psoas}$, skeletal muscle fat index of the psoas muscle.

analysed the data irrespective of intervention. NASH improvement was associated with a significant decrease of $SMFI_{Psoas}$ (Fig. 3A,B). This was mostly driven by the resolution of steatosis and inflammation (Fig. 3C–F). Compared to those without NASH improvement, patients who improved NASH lost muscle mass (Fig. S8A,B) with no change in muscle density (Fig. S8C,D). Hence, the latter experienced a decrease- in their absolute muscle fat content. This was effectively reflected by a significantly decreased $SMFI_{Psoas}$ (Fig. 3A,B). We computed operating characteristics of relative change in $SMFI_{Psoas}$ to predict NASH improvement and found an optimal cut-off (Youden Index) of 11% reduction in $SMFI_{Psoas}$, similar for both endpoints (*i.e.* NAS <3 or ≥2-point NAS reduction). This cut-off had a 56% sensitivity (95% CI 37–73%) and a 100% specificity (95% CI 65–100%) for the prediction of NASH improvement.

$SMFI_{Psoas}$ reduction is strongly associated with histological improvement of NASH

We stratified patients according to $SMFI_{Psoas}$ reduction (≥ or <11%) after intervention and evaluated histological changes (Fig. 4A). All patients improved steatosis, inflammation and ballooning, but patients with ≥11% $SMFI_{Psoas}$ reduction had a significantly greater improvement in inflammation and ballooning. Fibrosis improvement was not explained by $SMFI_{Psoas}$ change (Fig. 4A). We evaluated the proportion of patients who achieved NASH improvement or complete resolution of any histological feature of NASH (Fig. 4B). Remarkably, 100% of patients who had ≥11% $SMFI_{Psoas}$ reduction achieved NASH improvement (14/14) and steatosis or inflammation resolution (16/16). When patients were stratified according to the latest clinical trial guidelines for NASH resolution,[27] 16/32 (50%) resolved pre-established NASH (Fig. S9A). The proportion of patients that resolved NASH was twice as high in those that had ≥11% $SMFI_{Psoas}$ reduction (Fig. S9A).

Discussion

Herein, we developed a novel index ($SMFI_{Psoas}$) to evaluate muscle fat content in patients with obesity and found that it was strongly and specifically associated with NASH, and distinguished patients with NASH from those with NAFL. After a therapeutic intervention (*i.e.* diet or bariatric surgery), the association between $SMFI_{Psoas}$ and NASH was recapitulated. Furthermore, $SMFI_{Psoas}$ decreased more in patients with significantly improved histological markers of NASH. Strikingly, all patients who reduced $SMFI_{Psoas}$ by ≥11% achieved NASH improvement. The relationship was independent from fibrosis severity, whether at inclusion or after intervention. Our data thus suggest that muscle fat content could specifically reflect NASH-defining histological features (*i.e.* steatosis, inflammation and ballooning). We also found that sarcopenia was uncommon in patients with NAFLD and that muscle mass increased with NAFLD severity.

Muscle fat infiltration, often referred to as myosteatosis, is commonly associated with poor "muscle health".[20] CT-based HU value is considered as a valid surrogate for muscle fatty infiltration.[18,30] Low muscle CT-density, reflecting a high fat concentration, is a well-established prognostic indicator in patients with end-stage liver disease.[5] Further, in a seminal paper, Kitajima and colleagues[11] suggested an association between myosteatosis and NASH severity in patients with NAFLD (n = 208).

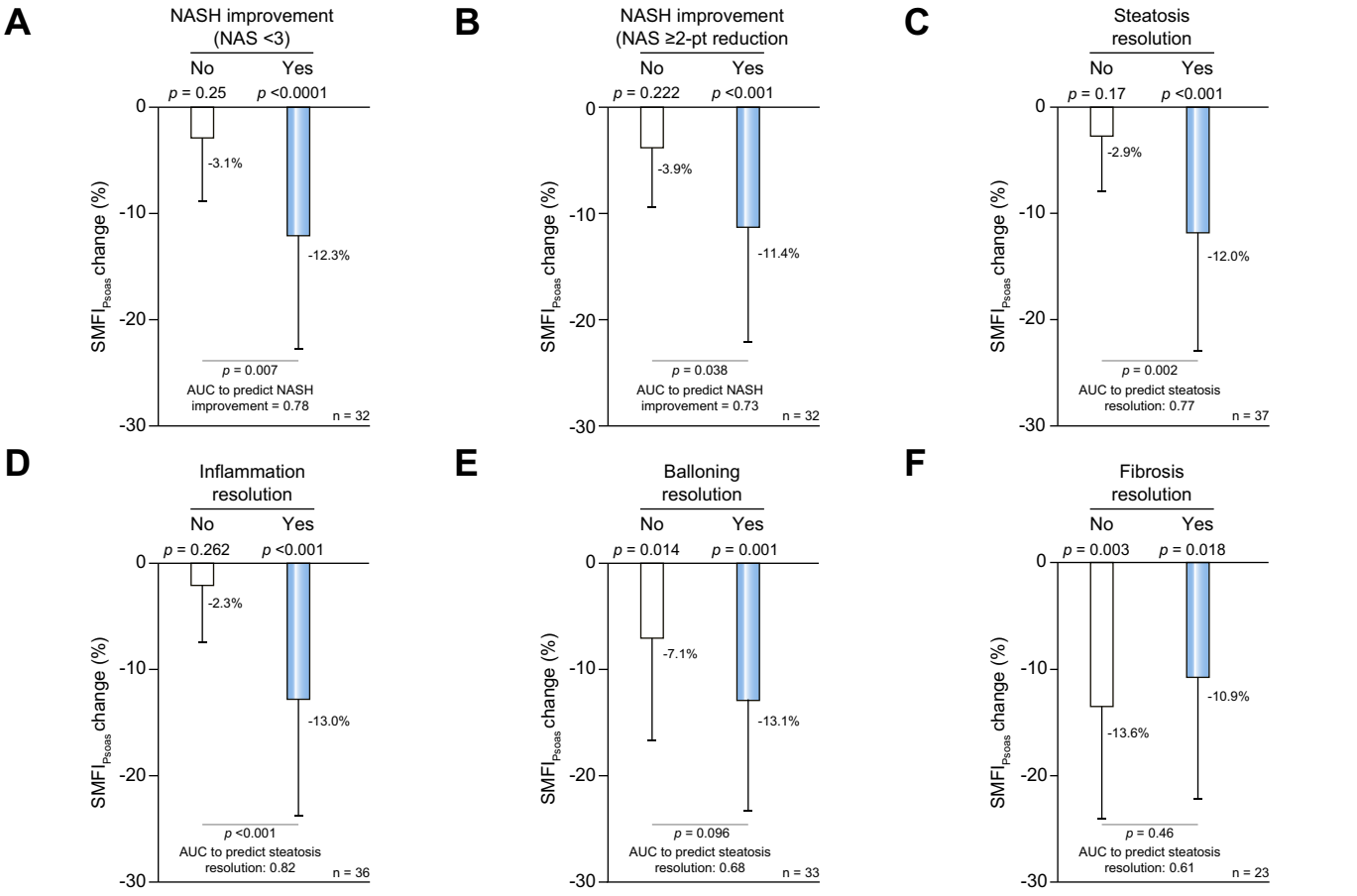

Fig. 3. NASH improvement is associated with a significant decrease of $SMFI_{Psoas}$. Relative $SMFI_{Psoas}$ decrease after intervention in patients stratified according to (A) and (B) NASH improvement and resolution (score = 0) of (C) steatosis, (D) inflammation (E) ballooning and (F) fibrosis. Intra-group differences evaluated with Paired *t* test (*p* value denoted on top of each sub-column). Inter-group differences evaluated with Student's *t* test (equal variance) or Welch's *t* test (unequal variance) and denoted behind bars. For (A) and (B), patients without NASH at inclusion were excluded. For (C), (D), (E) and (F), patients with a respective score of 0 at inclusion were excluded. n = 23-37. All data are mean ± SD. NASH, non-alcoholic steatohepatitis; $SMFI_{Psoas}$, skeletal muscle fat index of the psoas muscle.

Also, Tanaka *et al.*[13] (n = 632) and Chen *et al.*[35] (n = 2,249) reported a low muscle density in patients with NAFLD when compared to controls. However, the latter studies[13,35] lack a liver biopsy to refine the analysis to NASH *vs.* NAFL, and the former study[11] is limited by a suboptimal analysis, as muscle density was normalized to the subcutaneous fat density, which we now know is not invariant.[36] Contrary to the aforementioned literature, patients with NAFLD in our study had a slightly (yet

Table 3. Univariate and multivariate analysis producing ORs for NAFLD and NASH according to $SMFI_{Psoas}$.

	$SMFI_{Psoas}$ OR for NAFLD in total population (n = 184)			$SMFI_{Psoas}$ OR for NASH in NAFLD (n = 150)		
	OR per unit increase (95% CI)	OR per SD increase (95% CI)	*p* value	OR per unit increase (95% CI)	OR per SD increase (95% CI)	*p* value
Unadjusted	1.08 (1.04–1.12)	3.57 (1.87–6.83)	<0.001	1.06 (1.03–1.10)	2.95 (1.68–5.20)	<0.001
Age, sex adjusted	1.09 (1.05–1.14)	4.72 (2.03–10.98)	<0.001	1.07 (1.02–1.12)	3.26 (1.50–6.95)	0.003
Multivariate model 1	1.10 (1.04–1.17)	5.32 (2.04–13.85)	0.001	1.08 (1.03–1.14)	3.99 (1.68–9.47)	0.002
Multivariate model 2	1.11 (1.04–1.17)	5.67 (2.12–15.18)	0.001	1.08 (1.03–1.14)	3.98 (1.66–9.57)	0.002
Multivariate model 3	1.10 (1.04–1.17)	5.32 (1.93–14.65)	0.001	1.07 (1.02–1.13)	3.31 (1.37–8.00)	0.008
Multivariate model 4	1.09 (1.02–1.16)	4.25 (1.46–12.35)	0.008	1.07 (1.02–1.13)	3.32 (1.35–8.16)	0.009
Multivariate model 5	1.11 (1.04–1.18)	5.91 (2.00–17.42)	0.001	1.06 (1.01–1.12)	2.84 (1.15–7.00)	0.024
Multivariate model 6	1.11 (1.04–1.18)	5.86 (1.98–17.36)	0.001	1.06 (1.01–1.12)	2.83 (1.14–7.01)	0.025

Multivariate model 1 was adjusted for age, sex, body mass index, systolic blood pressure, smoking, diabetes.
Multivariate model 2 was adjusted for triglycerides and cholesterol levels in addition to factors included in model 1.
Multivariate model 3 was adjusted for alanine aminotransferase levels in addition to factors included in model 2.
Multivariate model 4 was adjusted for OGIS (muscle insulin sensitivity) and visceral fat area in addition to factors included in model 3.
Multivariate model 5 was adjusted for homeostatic model assessment for insulin resistance; in addition to factors included in model 3.
Multivariate model 6 was adjusted for height in addition to factors included in model 5.
NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; OR, odds ratio; $SMFI_{Psoas}$, skeletal muscle fat index of the psoas muscle.

Table 4. Effects of therapeutic intervention on skeletal muscle compartment and on NAFLD improvement.

	Baseline (n = 39)	Follow-up (n = 39)	Δ	*p* value
Age (year)		42.8 ± 5.6	–	–
Female sex (%)		69.2% (27/39)	–	–
Intervention (diet/bariatric surgery)		15/24	–	–
BMI (kg/m^2)	39.8 ± 5.5	31.0 ± 4.9	-22%	<0.001
Skeletal muscle index (cm^2/m^2)	55.8 ± 10.2	49.9 ± 8.3	-11%	<0.001
Psoas area (cm^2)	33.1 ± 8.9	28.4 ± 7.4	-14%	<0.001
Psoas density (HU)	47.0 ± 4.7	45.1 ± 4.4	-4%	0.018
$SMFI_{Psoas}$	70.5 ± 17.0	62.9 ± 14.6	-11%	<0.001
AST (U/L) (38)	31.4 ± 19.4	21.4 ± 6.6	-32%	0.003
ALT (U/L) (38)	47.3 ± 28.3	29.5 ± 11.4	-38%	<0.001
HOMA-IR (34)	4.7 ± 7.0	2.0 ± 1.5	-57%	0.032
Liver histology (Normal/NAFL/NASH)	2/5/32	30/1/8	–	<0.001
NAS score (min-max)	4.6 (0-8)	1.3 (0-6)	–	<0.001
Stage of fibrosis (0/1/2/3)	19/10/7/3	29/9/1/0	–	0.003
NASH improvement				
NAS <3		25/32 (78%)	–	–
NAS 2-point reduction		27/32 (84%)	–	–

Data presented as mean ± SD with n = 39 unless specified otherwise. Repeated Student's *t* test was performed for continuous variables and Wilcoxon signed-rank test for categorial variables. ALT, alanine aminotransferase; AST, aspartate aminotransferase; HOMA-IR, homeostatic model assessment for insulin resistance; HU, Hounsfield unit; NAFLD, non-alcoholic fatty liver disease; NAS, NAFLD activity score; NASH, non-alcoholic steatohepatitis; $SMFI_{Psoas}$, skeletal muscle fat index of the psoas muscle.

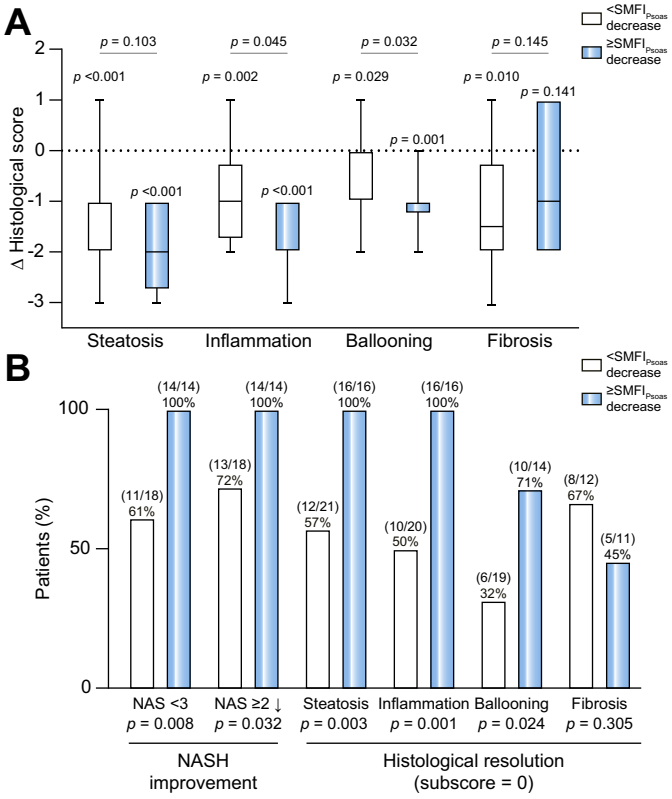

Fig. 4. $SMFI_{Psoas}$ change is strongly associated with NASH histological improvement. (A) Delta change of histological score for the different cardinal NASH features after intervention when compared to at inclusion in patients stratified according to $SMFI_{Psoas}$ decrease (< or ≥11%). Intra-group differences evaluated with Wilcoxon signed-rank test and inter-group differences evaluated with Kruskal-Wallis test. Box = IQR and whiskers = min and max. (B) Proportion of patients with NASH improvement or resolution of any NASH histological sub-score. Differences evaluated with chi-square test. n = 23-37. NASH, non-alcoholic steatohepatitis; $SMFI_{Psoas}$, skeletal muscle fat index of the psoas muscle.

significantly) increased muscle density (thus a lower fat concentration) when compared to those without NAFLD. In our entire cohort of non-critically ill patients with obesity, high body weight is associated with high muscle mass. We thus hypothesised that high muscle mass, which we believe to be a consequence of maintaining posture, influences muscle lipid concentration and in turn muscle density. Indeed, lipids are stored within myocytes or in surrounding adipocytes.[12,20] On the one hand, myocyte hypertrophy might lower lipid concentration in the sarcoplasm for a similar lipid content per cell. On the other hand, the relative ratio of adipocyte area compared to myocyte area decreases in the case of muscle hypertrophy. Hence, in the context of significant muscle hypertrophy, higher or unchanged muscle density might obscure the presence of a pathological absolute lipid content since lipid would be "diluted" in an enlarged muscle compartment. Alternatively, a high muscle fat content could enlarge muscle area, a theory not supported by the higher muscle density found in our patients with NAFLD. To overcome confounders and better reflect the absolute amount of fat in a given skeletal muscle, we developed the skeletal muscle fat index (SMFI). Hence "myosteatosis" refers to a high fat concentration in the skeletal muscle (*i.e.* a low muscle density) while SMFI reflects absolute fat content.

At variance with several reports,[7–13] we found that sarcopenia was uncommon in patients with NAFLD. Rather, muscle mass increased with NAFLD severity in the presented cohort. The lack of consensus on the definition of sarcopenia in patients with obesity, coined "sarcopenic obesity", likely explains the controversy.[37] Indeed, how to define "low muscle mass" and how to report muscle mass in patients with obesity is intensely debated.[37] CT or MRI-derived muscle mass indexes are classically scaled on height. By contrast, the muscle mass index derived from dual-energy X-ray absorptiometry and BIA data are variably scaled on height, on weight or on BMI.[18] Thus, patients with obesity are more likely to be categorized as sarcopenic when applying weight or BMI-based muscle mass scaling, as used in the majority of sarcopenia studies in NAFLD.[7–13,15,16] Moreover,

high BMI *per se* is a well-known risk factor for NAFLD.[1] Hence, to find a low relative muscle mass in patients with NAFLD is not a surprise. Data from 2 large cohorts (n = 2,761 and n = 2,551)[35,38] elegantly highlighted this paradox. When scaling muscle mass on body weight, the OR for having sarcopenia was 1.73 (95% CI 1.31–2.28) in patients with NAFLD. By contrast, when it was scaled on height, the OR for having sarcopenia was 0.63 (95% CI 0.46–0.87), supporting a lower risk for sarcopenia in NAFLD.[38] Chen *et al.*[35] reported a higher absolute muscle mass in patients with NAFLD when compared to controls (23.2 ± 6.1 *vs.* 20.8 ± 5.8 kg, p <0.0001), but a lower relative muscle mass when scaled on weight. In the present study, the intervention caused a significant weight loss. If the muscle to weight or BMI ratio was accepted as the definition of "muscle mass", we would have erroneously concluded that the intervention did not impact (or even increased) muscle mass. Hence, for discernment, we suggest that future studies in patients with NAFLD always report weight/BMI- as well as height-scaled muscle mass data. A plausible explanation for the high muscle mass seen in patients with obesity is the chronic workload and thus chronic 'training' imposed by excess body weight; this likely maintains muscle mass unless liver function is severely impaired, as in cirrhosis, which is known to cause skeletal muscle loss.[5,39] In case of significant weight loss, this "compensatory" muscle hypertrophy would no longer be needed. Our results indeed support a parallel decrease in muscle mass and muscle fat upon therapeutic intervention.

Non-invasive tools perform well to rule-in and rule-out steatosis and fibrosis,[2] but available markers to diagnose NASH – the driving force of the disease – remain elusive. Our data support that the prospective evaluation of muscle fat concentration and content has an added value to identify patients with NASH. Whether this could also be used to follow treatment response needs to be addressed by clinical trials, ideally with MRI-proton density fat fraction to quantitate fat simultaneously and precisely in the liver and muscles. We anticipate that the measure of muscle fat will be especially informative to evaluate the benefit of drugs that do not directly target liver steatosis.[40]

A causal link cannot be inferred from the present analysis; however, it is tempting to speculate that a high-fat content contributes to NAFLD progression: peripheral insulin resistance and/or perturbation of the muscle metabolism and secretome associated with muscle fat[14,41,42] may promote liver inflammation and hepatocellular injury.[6,12,14–16] Further studies in preclinical models, in which we showed that muscle fat infiltration (but not sarcopenia) was specifically associated with NASH,[43] will clarify the exact nature and directionality of the muscle-liver axis in NAFLD progression and potentially unravel new relevant therapeutic targets.

The strengths of the current study include the homogeneity of the clinical cohort, the use of gold standard methodologies (*i.e.* CT and liver biopsy), the longitudinal design and the development of a novel index to evaluate muscle absolute fat content. The limitations are: the relatively low number of male patients with normal liver or NAFL, the morphometrical characteristics of the patients, the analysis of CT scan at L4 rather than L3 (although variation have been shown to be marginal[44]), the use of cut-off values for sarcopenia established in patients without obesity[29] and the lack of muscle strength data (important for defining sarcopenia).

Taken together, our data support that a high muscle fat content, rather than a low muscle mass, is strongly and independently associated with NASH in patients with obesity and NAFLD. After a therapeutic intervention, the lowering of muscle fat content was robustly associated with histological improvement. These data pave the way for the exploration of muscle fat content as a potential marker and perhaps a pathophysiological contributor or a therapeutic target for NASH in NAFLD.

Abbreviations

ALT, alanine aminotransferase; BIA, bioelectrical impedance analysis; BIA-SMI, SMI based on BIA; CT-SMI, SMI based on CT; HOMA-IR, homeostatic model assessment for insulin resistance; HU, Hounsfield unit; NAFL, non-alcoholic fatty liver; NAFLD, non-alcoholic fatty liver disease; NAS, NAFLD activity score; NASH, non-alcoholic steatohepatitis; OR, odds ratio; PD, psoas density; $SMFI_{Psoas}$, skeletal muscle fat index of the psoas muscle; SMI, skeletal muscle index.

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Conflict of interest

The authors declare that they have no conflict of interest in relation to this work to disclose.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

M.N, W.K and I.A.L conceived and designed the study. W.K, B.O, L.V.G, L.V, A.V and S.F recruited the patients and carried out data collection in human studies. A.D scored liver biopsies. M.N, W.K, S.F and I.A.L analysed and interpreted the data. All authors critically discussed study design, data and manuscript for scientific content. M.N and I.A.L wrote the manuscript, with contribution from all authors.

Data availability statement

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

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Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2021.02.037.

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Author names in bold designate shared co-first authorship

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