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

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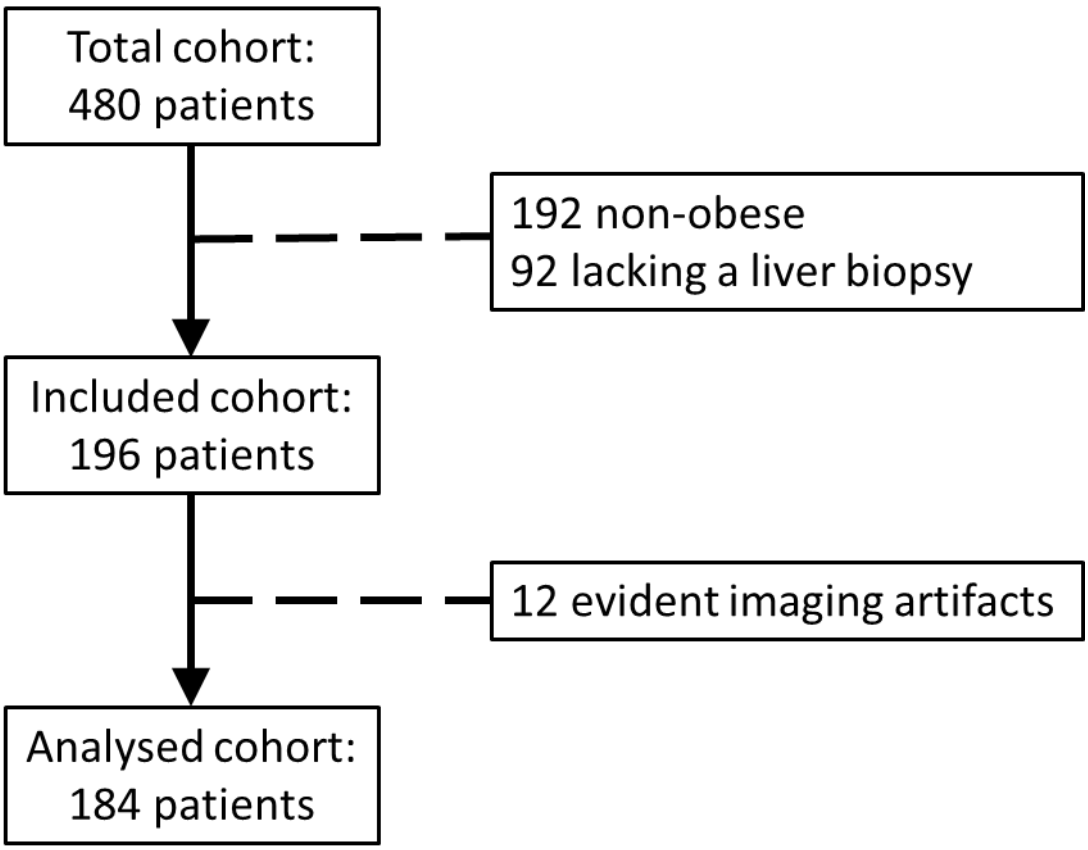

Fig. S1. Flow chart of patient selection at baseline

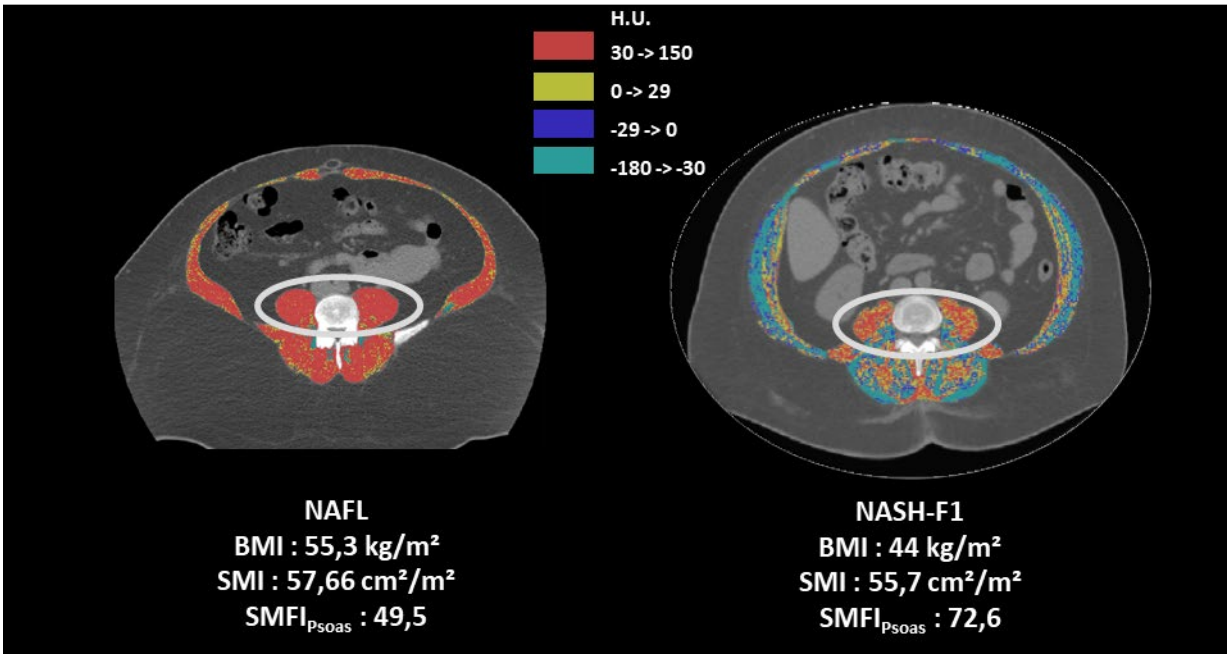

Fig. S2. Illustration of CT-based methodology to measure muscle mass and muscle fatty infiltration

Representative abdominal CT-slices at L4 level in a patient with NAFL (left) and a patient with NASH (right). Muscle area and density were semi automatically measured with Slice-O-Matic software (*see methods*). White circle indicates the both psoas muscles bilateral to the vertebra. Colors scaled on pixel densities with red ≥30 to 150 Hounsfield Unit (H.U), yellow 0 to 29 H.U, dark blue -29 to 0 H.U and turquoise -180 to -30 H.U. Additional data shown are body mass index (BMI), skeletal muscle index (SMI, *i.e*. whole muscle area at L4 divided by patient height squared, in m²), skeletal muscle fat index ($SMFI_{Psoas}$, *i.e* psoas area in cm² multiplied by 100 and divided by psoas density in H.U). Of importance, the patient with NASH-F1 was selected to illustrate a high muscle fat concentration (H.U-based color-scale) and a high muscle fat content (as calculated by the $SMFI_{Psoas}$). In our cohort, it is the muscle fat content, and not muscle fat concentration, that is strongly associated with NASH.

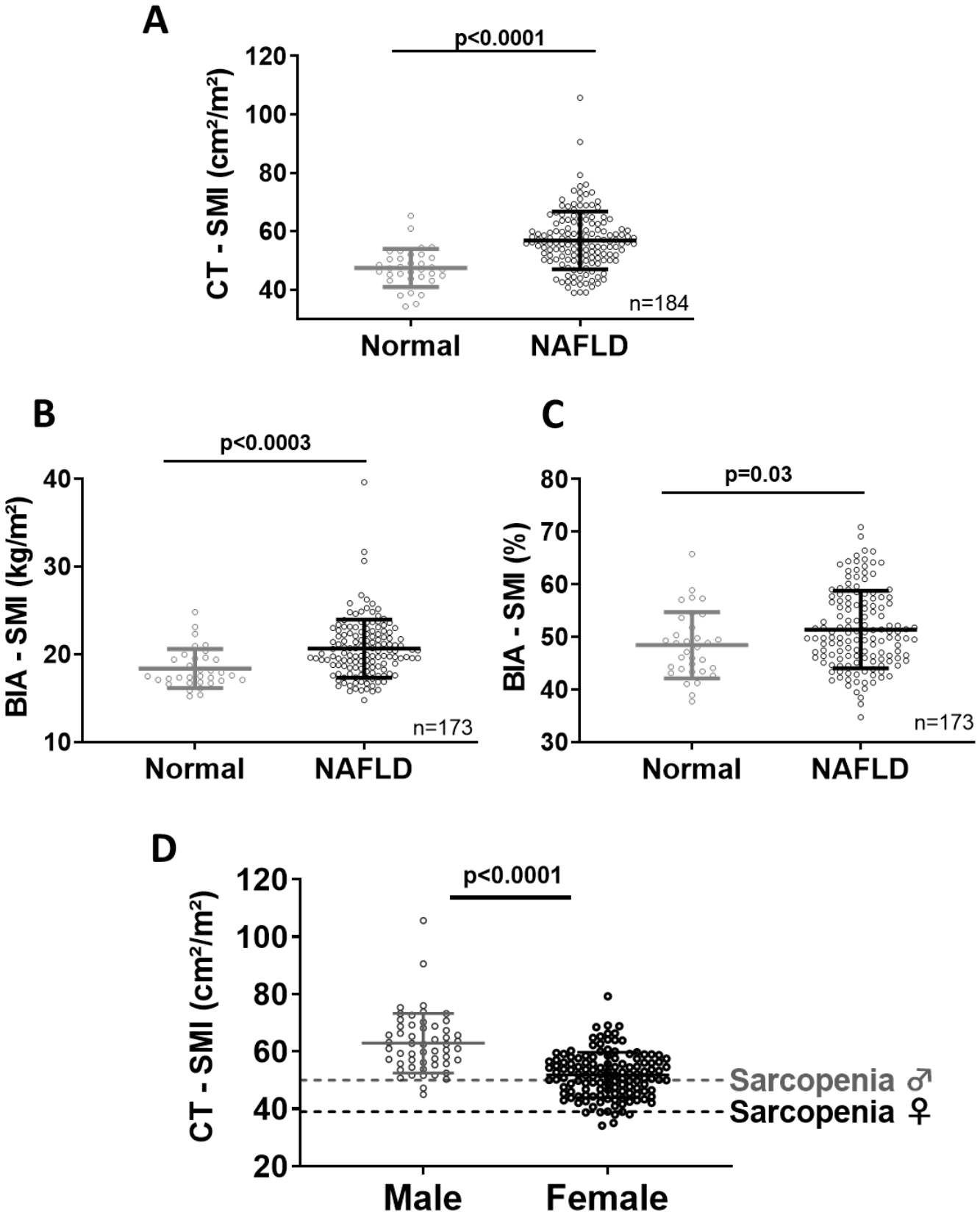

Fig. S3. NAFLD is associated with higher muscle mass than normal liver

Skeletal muscle mass index evaluated with (A) CT, (B) BIA (height scaling) and (C) BIA (weight scaling) in patients with NAFLD and those with normal liver. (D) Skeletal

muscle mass index in male and female patients. Dashed line: sex-specific cut-off for sarcopenia. All data are mean ± SD. Student t test.

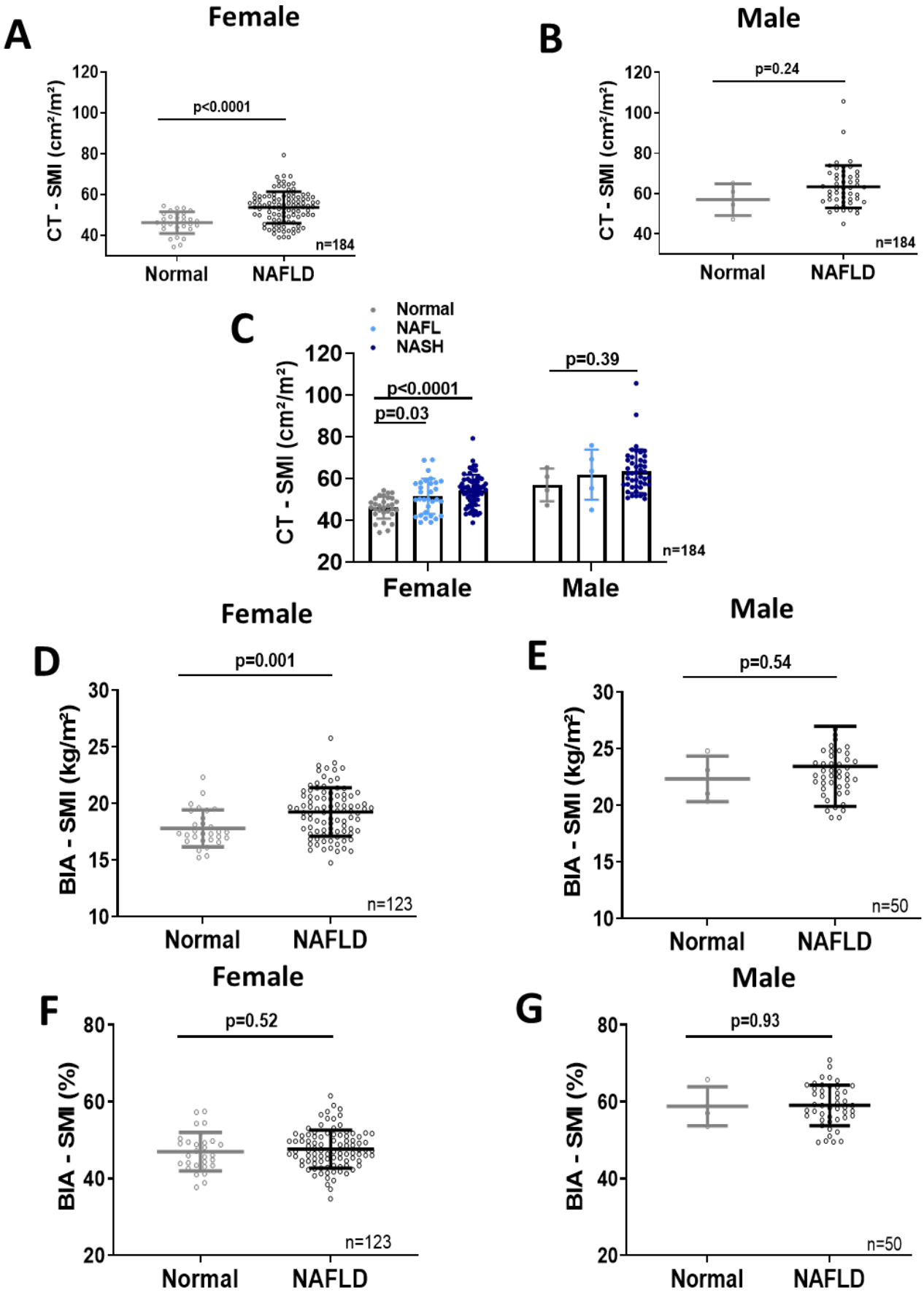

Fig. S4. NAFLD is not associated with lower muscle mass in patients with obesity irrespectively from sex

Skeletal muscle mass index evaluated with CT (A), (B) and (C); BIA (height scaling) (D), (E); and BIA (weight scaling) (F), (G) in patients with NAFLD and those with normal liver histology stratified by sex. All data are mean±SD. Student t test.

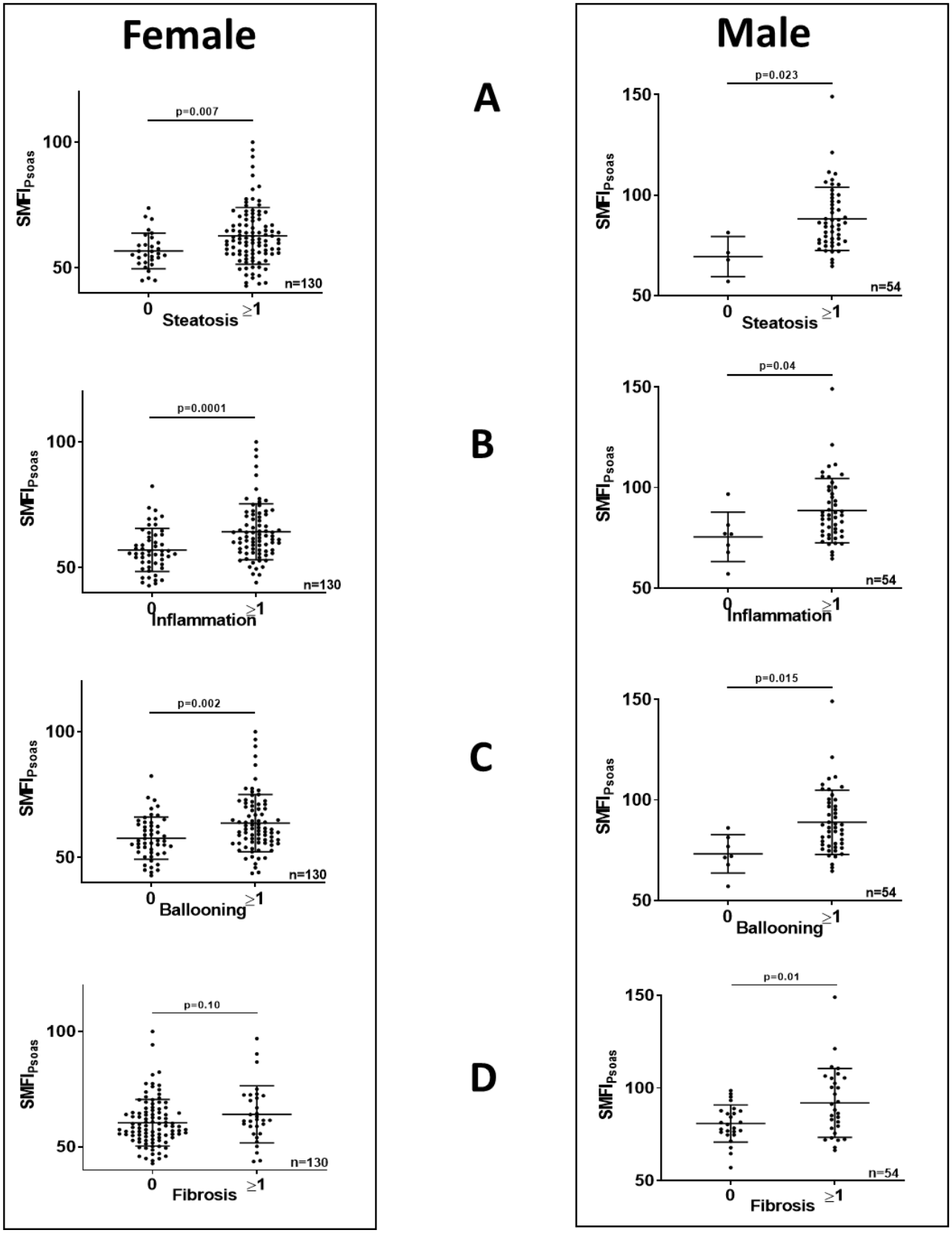

Fig. S5. $SMFI_{Psoas}$ is associated with key histological NASH features independently from sex

(A), (B), (C) and (D) Sex-stratified $SMFI_{Psoas}$ values according to NASH CRN histological sub-score for steatosis, inflammation, ballooning and fibrosis (Student t test). All data are mean ± SD

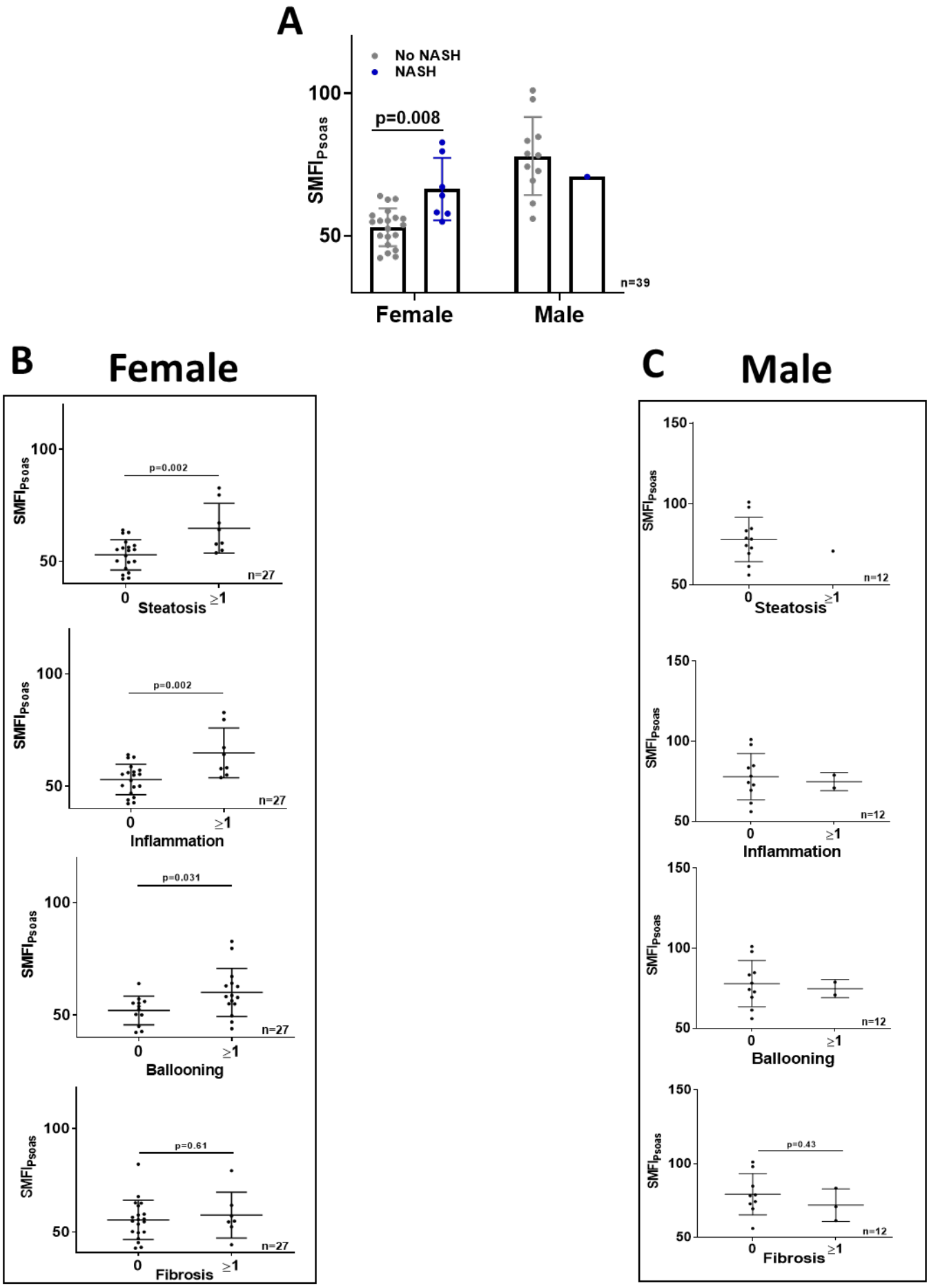

Fig. S6. After therapeutic intervention, $SMFI_{Psoas}$ remains associated with NASH and its key histological features

(A) $SMFI_{Psoas}$ in female and male patients according to NASH presence. Differences were evaluated with two-way ANOVA. Of note, since only one male patient had NASH after intervention, p values were not indicated in male patients. (B), (C) and (D) sex-stratified $SMFI_{Psoas}$ values according to NASH CRN histological sub-score for steatosis, inflammation, ballooning and fibrosis (Student's *t* test). All data are mean ± SD

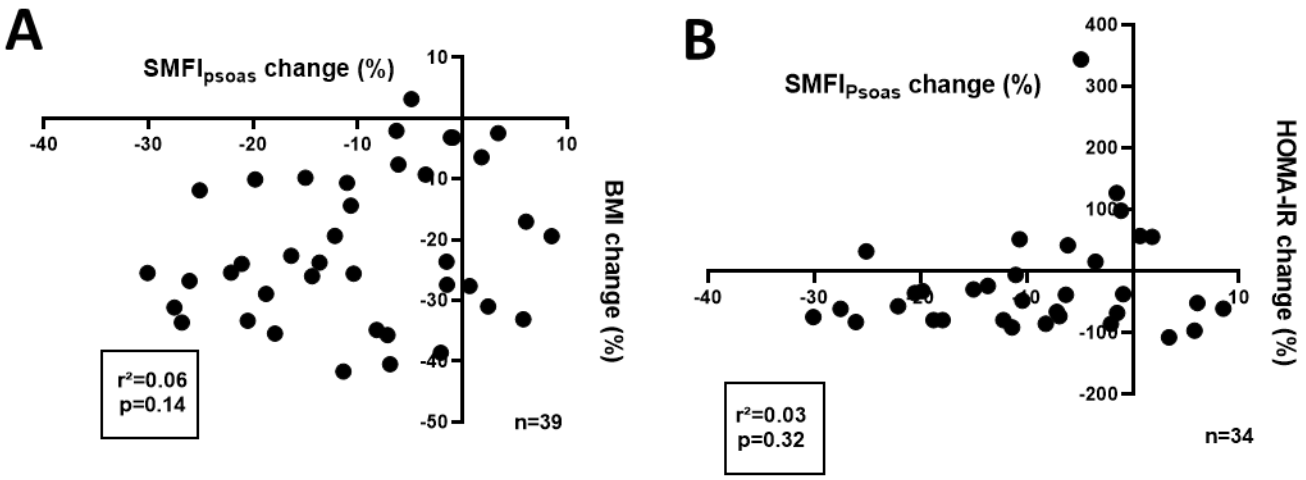

Fig. S7. BMI decrease and HOMA-IR are not correlated with $SMFI_{Psoas}$ decrease

Correlation of (A) BMI change (%) and (B) HOMA-IR change (%) with $SMFI_{Psoas}$ change (%) after therapeutic intervention. Pearson's correlation coefficient indicated on graphs.

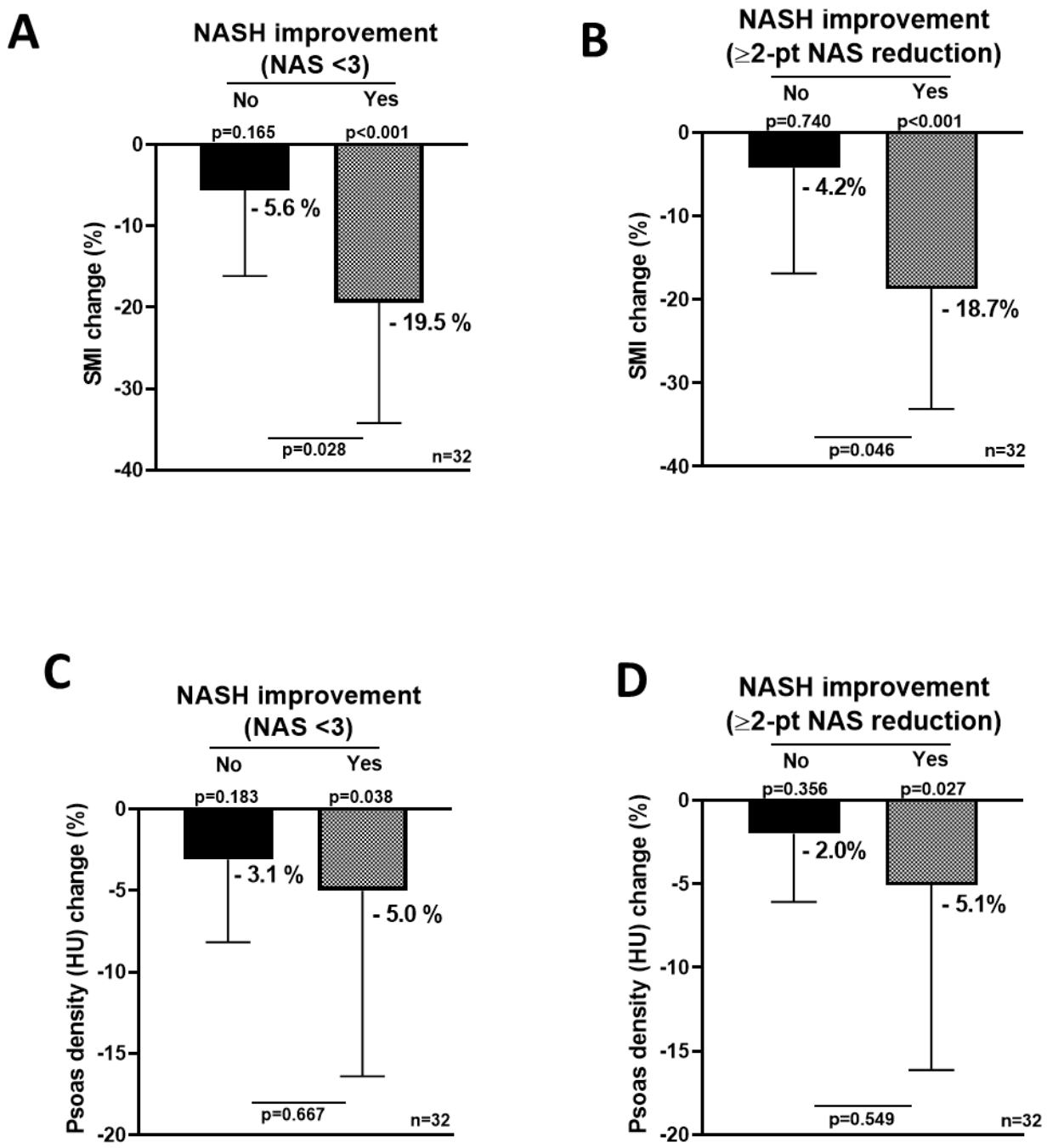

Fig. S8. NASH improvement is associated with a decreased muscle mass but not muscle density, compatible with an absolute fat content reduction

Relative (a-b) Skeletal Muscle mass index (SMI) change and (c-d) Psoas muscle density change after intervention in patients stratified according to NASH improvement. 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 *t* test (unequal variance) and denoted behind bars. Patients without NASH at baseline were excluded. All data are mean ± SD.

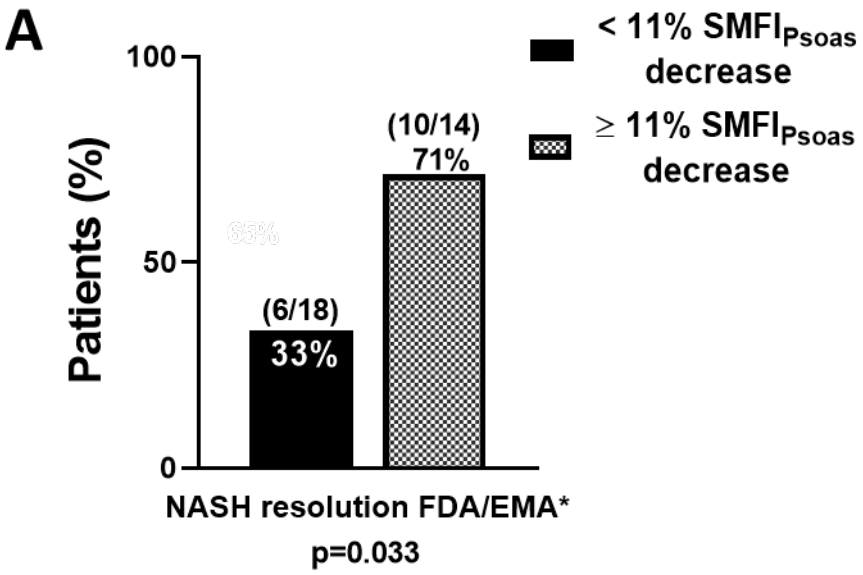

Fig. S9. Patients that had ≥11% $SMFI_{Psoas}$ reduction had a higher rate of NASH resolution as defined by EMA/FDA clinical trial endpoints

Proportion of patients that resolved NASH (according to EMA/FDA latest clinical trial endpoints) and stratified by $SMFI_{Psoas}$ decrease. Patients without NASH at baseline were excluded. Chi-squared test. N=32.

Table S1. Potential confounding for sarcopenia at inclusion

Case	Age (y)	Sex	BMI (kg/m2)	Steatosis	Ballooning	Inflammation	Fibrosis	Histology Findings	Relevant History	Potential alternative cause
1	36	F	38.9	0	0	0	0	No NAFLD	None	NO
2	45	M	32.0	0	0	0	0	No NAFLD	Different orthopaedic operations, amongst which surgery for lumbal hernia (PLIF L5-S1)	**MAYBE**
3	49	F	32.6	2	2	1	0	NASH + F0-F1	Multinodular goiter	NO
4	32	F	39.4	0	0	0	0	No NAFLD	None	NO
5	42	F	43.2	0	2	0	0	No NAFLD	None	NO
6	33	F	32.6	0	0	0	0	No NAFLD	Hydradenitis suppurativa, vertebral degeneration/arthrosis, car accident	**MAYBE**
7	47	M	50.0	2	0	2	1	NAFL	Obustrctive sleep apnœa	NO
8	39	F	35.6	0	0	0	0	No NAFLD	None	NO

Table S2. Adjusted ORs of significant fibrosis according to clinical parameters

Variables	OR (95% CI)	P value
Age	1.04 (0.95-1.12)	0.40
Sex	0.86 (0.22-3.33)	0.83
BMI	1.04 (0.95-1.14)	0.36
ALT	1.03 (1.01-1.05)	0.01
Gamma GT	1.00 (0.99-1.02)	0.69
Cholesterol	1.00 (0.99-1.01)	0.82
Triglycerides	1.00 (0.99-1.01)	0.13
Smoker	0.77 (0.45-1.33)	0.35
HOMA-IR	1.10 (0.95-1.28)	0.20
$SMFI_{Psoas}$	1.02 (0.99 -1.06)	0.22
Visceral fat area	1.00 (0.99-1.01)	0.50

Table S3. Effects of intervention on weight loss, insulin sensitivity, muscle fat content and NASH improvement

	Diet (n=15)	Intra-group p value	Bariatric surgery (n=24)	Intra-group p value	Inter-group p value
BMI change (%)	- 8.7 ± 7.1	p=0.001	- 29.5 ± 8.4	p<0.0001	p<0.001
HOMA-IR change	- 0.05 ± 2.18	N.S	- 5.06 ± 9.46	p=0.006	p=0.049
$SMFI_{Psoas}$ change (%)	- 7.4 ± 8.5%	p=0.078	- 12.7 ± 14.1%	p<0.0001	p=0.209
NASH improvement (NAS<3)	6/12 (50%)	/	19/20 (95%)	/	p=0.003
NASH improvement (≥ 2-pt NAS reduction)	5/12 (42%)	/	20/20 (100%)	/	p=0.002

Intra-group differences between baseline and follow up evaluated using Two-way repeated ANOVA. Inter-group difference evaluated using Student's *t* test or Chi-squared test.