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Article

Assessment of Skeletal Muscle Alterations and Circulating Myokines in MASLD Patients: A Prospective Cohort Study

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Abstract: Aim: To determine the prevalence of skeletal muscle alterations (SMA) in patients with metabolic dysfunction-associated steatotic liver disease (MASLD) and to assess the significance of circulating myokines as potential biomarkers of SMA in patients with MASLD. **Material and methods:** Measurements of both skeletal muscle strength and skeletal muscle mass parameters, anthropometric and metabolic features as well as serum levels of different myokines were performed in a cohort of 62 MASLD patients and then compared according to the stage of liver fibrosis and hepatosteatosis degree both diagnosed by transitional elastography.

Results: No significant differences were found in skeletal muscle strength and skeletal muscle mass in MASLD patients stratified according to the stage of liver fibrosis. Noteworthy, serum levels of FGF21 were significantly higher in MASLD patients with advanced hepatic fibrosis (F3-F4) than in those with lower stages of hepatic fibrosis (F0-F2) (197.49±198.27 pg/ml vs 95.62±83.67 pg/ml, p=0.049, respectively). MASLD patients with severe hepatosteatosis (S3) had significantly higher serum levels of irisin (1116.87±1161.86 pg/ml) than those with lower grades (S1-S2) (385.21±375.98 pg/ml, p=0.001). **Conclusion:** SMA were uncommon in our MASLD patients, particularly in those with lower stages of liver fibrosis. An increase in serum FGF21 was detected in patients with a higher stage of fibrosis with possible therapeutic implications in MASLD.

Keywords: skeletal muscle; myokines; MASLD; liver fibrosis; hepatosteatosis

1. Introduction

Sarcopenia, characterized by a reduction in muscle mass and muscle function in chronic diseases, and metabolic dysfunction-associated steatotic liver disease (MASLD) share a similar pathophysiology in their development, based in inflammation, insulin resistance, and physical inactivity as key factors ([1–5]. In patients with MASLD, sarcopenia is considered a potential risk factor for the development and progression of liver fibrosis. The presence of sarcopenia in this context appears to accelerate the evolution from simple fatty liver (MAFLD) to steatohepatitis (MASH) and

fibrosis ([5–9]. Sarcopenia in MASLD patients is approximately 46% in patients with fibrosis, and 25%, in those without fibrosis, although definition is heterogeneous across studies [6,9–12].

Systemic inflammation emerges as a likely common link between sarcopenia and MASH, marked by a shared proinflammatory cytokine profile [5,13–15]. These cytokines have the potential to activate hepatic stellate cells, triggering liver fibrosis, and protein catabolism (specifically myostatin), leading to muscle loss. The exact sequence of events remains elusive, whether sarcopenia instigates the activation of myostatin receptors in hepatic stellate cells, thereby contributing to liver fibrogenesis, or whether MASLD condition contributes to myostatin activation in skeletal muscle, subsequently culminating in sarcopenia. Studies are lacking for definitively establishing the cause-and-effect relationship between sarcopenia and MASLD [1,5,8]. Furthermore, the majority of published studies have focused on large Asian cohorts with a phenotype distinct from the Western population regarding fatty liver disease (“lean” MASLD with BMI<25 Kg/m²) [6,16–18].

Given the aforementioned context, the aims of the present study were firstly to determine the prevalence of skeletal muscle alterations (low muscle strength and/or low muscle mass) in patients with MASLD and secondly to measure the serum levels of myokines in the study cohort, searching for correlations with the stage of liver fibrosis and the grade of hepatosteatosis.

2. Materials and Methods

Study Population

We prospectively enrolled 62 patients who fulfilled the recently described criteria for the diagnosis of MASLD: evidence of hepatic fat accumulation (steatosis) in abdominal ultrasound and one of the following three criteria: overweight/obesity, type 2 diabetes mellitus (DM), or the presence of at least two metabolic risk abnormalities [16]. None of these patients drank alcohol more than 140g/week for males or 70g/week for females, had analytical evidence of iron and copper overload, were seropositive for autoantibodies and/or for hepatitis B virus, hepatitis C virus (HCV), and human immunodeficiency virus (HIV) or were having potentially hepatotoxic drugs. Patients treated with steatosis-inducing drugs and glucagon-like peptide 1 (GLP1) agonist were excluded.

Clinical and Laboratory Assessment

Clinical examination included a detailed interview with special emphasis on both alcohol intake and medications use, history of known diabetes and arterial hypertension, as well as measurements of weight, height, blood pressure and waist and hip perimeters. Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared. A BMI≥30 kg/m² was defined as obesity. After a 12-hour overnight fast, venous blood samples of each participant were obtained to test serum levels of liver enzymes, metabolic parameters and autoantibodies using routine laboratory methods. In addition, plasma insulin was determined by a chemiluminescent microparticle immunoassay (ARCHITECT insulin; Abbot Park, IL USA). IR was calculated by the homeostasis model assessment (HOMA) method [17]. Metabolic syndrome (MetS) was defined according to the ATP III criteria [18]. Antibodies against HCV and HIV as well as hepatitis B surface antigen were tested by immunoenzymatic assays (Murex, Dartford, UK).

Liver Ultrasound Imaging

Liver was examined using an ultrasound medical device (Aplio MX Canon, Madrid, Spain) with a probe frequency of 3.5 MHz after overnight fasting. Liver steatosis was defined by the presence of at least two abnormal findings by liver ultrasound including diffusely increased echogenicity of the liver with liver echogenicity stronger than kidney, and either attenuation of ultrasound signal of the liver compared with the diaphragm or the echogenic walls of the portal veins were less visible. The preperitoneal adipose tissue was assessed by its special relationship with unfavorable metabolic factors in this population group [19,20].

Transient Elastography and Controlled Attenuation Parameter

The FibroScan® (Echosens, Paris, France) was used to carry out transient elastography (TE) to assess liver stiffness (fibrosis) along with controlled attenuation parameter (CAP) measurements to estimate steatosis as previously detailed [21]. Only values with at least 10 valid measurements, a success rate of at least 60%, and an interquartile range-to-median ratio of < 30% were considered reliable, as suggested by previous studies [22]. In addition, patients in whom TE was measured while the AST or ALT > 5 × ULN was present, were excluded from the analysis due to possible exaggerated TE values as previous studies demonstrated [23]. In this study, the defined thresholds to stratify fibrosis stages were as follows: F2>8.2 kPa, F3>9.6 kPa and F4 >10.7 kPa. [24]. To define the grades of steatosis by CAP, the thresholds used were: S1: 260 dB/m, S2: 285 dB/m, and S3: 294 dB/m [21].

Measurement of Hand Grip Strength

Hand grip strength was measured using an electronic Jamar dynamometer (Kern Map 80K1, Germany). Cut-off points were established as recommended by the 2018 European consensus on definition and diagnosis of sarcopenia [25], considering < 27 kg as low strength for men and < 16 kg as low strength for women.

Measurement of Appendicular Skeletal Muscle Mass

Appendicular skeletal muscle mass (ASMM) was assessed by using a dual-frequency bioelectrical impedance device (Akern Nutrilab™, Pisa, Italia). Cut-off points were established as recommended by the 2018 European consensus on definition and diagnosis of sarcopenia [25], considering <20 kg as low appendicular skeletal muscle mass for men and <15 kg as low appendicular skeletal muscle mass for women.

Measurement of Physical Performance

Physical performance measurements include the Liver Frailty Index (LFI), incorporating grip strength, time to rise from a chair, and balance test [26,27]. LFI was calculated using the formula: $LFI = (-0.330 \times \text{sex-adjusted grip strength}) + (-2.529 \times \text{number of chair stands per second}) + (-0.040 \times \text{balance time}) + 6$. The cut-off point for low performance was considered when $LFI \geq 4.5$.

Measurement of Myokines

Circulating levels of distinct myokines were tested in serum samples from all MASLD patients studied by using the Multiplex Kit (HMYOMAG-56K, Millipore, Merk) following manufacturer's instructions. At least 50 beads per variable were examined in the Bio-Plex suspension array system 200 (Bio-Rad Laboratories). Raw data (median fluorescence intensity, MFI) were evaluated using Bio-Plex Manager Software 6.2 (Bio-Rad Laboratories) and MFI from samples were interpolated to a standard curve using a five-parameter logistic (5-PL) equation. The intra- and inter-assay coefficients of variation were lower than 10%.

Statistical Analysis

Qualitative variables are presented as absolute (number, n) and relative (percentage, %) frequencies. Quantitative variables are expressed as measures of central tendency (mean) and dispersion (standard deviation). Qualitative data between groups were compared by Pearson's -test or Fisher exact test as appropriate. Quantitative variables were analysed using t test for normal distributional data (Kolmogorov Smirnov test) and equal variances (Levene's test), or Mann-Whitney U test for no normal distribution or heterocedasticity variances. Significance was set at a value of $P < 0.05$. Statistical analysis was performed using the SPSS 26.0 statistical software (SPSS Inc., IBM, Armonk, NY).

Ethical Considerations

This study was carried out in agreement with the Declaration of Helsinki and with local and national laws. All subjects enrolled voluntarily gave their written consent to participate in the study and the Ethics Committee of Human Research of the La Princesa University Hospital in Madrid, Spain, which is the reference ethics committee for all institutions involved in the present study, approved the study procedures (RRN 4645, 21/10/2021).

3. Results

Characteristics of the Study Population

Table 1 shows the demographic, anthropometric and clinical features of MASLD patients studied, stratified according to the stage of liver fibrosis measured by TE. The study cohort was largely comprised by middle-aged adults (60.7 ± 10.9 years) and 54.8% were women. About the elastography values, 44 patients were compatible with F0-F2 liver fibrosis stages and 18 patients with F3-F4 stages (6.01 ± 1.67 kPa and 15.35 ± 7.36 , $p < 0.001$, respectively). Noteworthy, according to CAP elastography values, the majority of patients included in our study cohort had severe steatosis (S3; $n=54$). To highlight, no significant differences were observed in terms of age and sex as well as in the prevalence of obesity and diabetes in MASLD patients related to the stage of liver fibrosis. In addition, both hand grip strength and appendicular skeletal muscle mass were similar in MASLD patients with low fibrosis stages (F0-F2) and in those with advanced fibrosis (F3-F4). Regarding the value of Liver Frailty index (LFI), pre-frailty was prevalent in both groups ($n=36$ (81.8%) in $<F3$ vs $n=14$ (77.8%) in $>F3$, $p=0.564$). Impedance measurement values and peritoneal adipose tissue did not show statistical significance between both groups according to elastography values (Table 1).

Table 1. Characteristics of the study population according to the stage of liver fibrosis by transient elastography ($n=62$).

Features	F0-F2 (N=44)	F3-F4 (N=18)	P-value
Age (years)	59.36 ± 11.36	63.17 ± 10.81	0.230
Men, n (%)	23 (56.1)	11 (61.1)	0.780
Body mass index (kg/m ²)	32.32 ± 4.49	30.72 ± 4.30	0.203
Diabetes, n (%)	16 (39.0)	10 (55.6)	0.257
HOMA-IR score	5.16 ± 3.12	5.27 ± 3.95	0.909
CAP (dB/m)	323.64 ± 48.35	327.50 ± 31.49	0.756
Liver elastography (kPa)	6.01 ± 1.67	15.35 ± 7.36	<0.001
Hand grip strength (kg)			
Men	33.62 ± 6.02	32.19 ± 5.45	0.523
Women	20.18 ± 4.69	17.00 ± 2.15	0.100
ASMM (kg)	22.66 ± 5.21	22.44 ± 6.20	0.887
	3.94 ± 0.43	4.11 ± 0.39	0.173
LFI (a.u)	2 (4.5)	0 (0)	

Robust, n (%)	36 (81.8)	14 (71.8)	
Pre-fragile, n(%)	4 (9.1)	3 (16.7)	
Fragile, n(%)			
	0.84 ± 0.41	0.98 ± 0.64	0.334
Peritoneal adipose tissue (cm²)			

Data are shown as mean ± SD or as number of cases (%). F0-F2, lower stages of liver fibrosis, F3-F4, higher stages of liver fibrosis, CAP, controlled attenuation parameter; ASMM, appendicular skeletal muscle mass, LFI, Liver Frailty index.

Serum Levels of Myokines in MASLD Patients

A wide set of myokines (n=14) were determined in serum samples of MASLD patients (Tables 2 and 3). Regarding the stage of liver fibrosis, according to elastography, FGF21 was the only myokine that showed a significant increase in MASLD patients with advanced fibrosis (F3-F4) with respect to those with lower stages of liver fibrosis (F0-F2) (197.49±198.27 pg/ml vs 95.62±83.67 pg/ml, p=0.049, Table 2). On the other hand, as shown in Table 3, MASLD patients with severe steatosis (S3) had significantly higher serum levels of irisin (1116.87±1161.86 pg/ml) than those with lower grades of hepatosteatosis (385.21±375.98 pg/ml, p=0.001).

Table 2. Serum myokine levels according to the stage of liver fibrosis by transient elastography (n=62).

Features	F0-F2 (N=44)	F3-F4 (N=18)	p value
Apelin (pg/mL)	155.79 ± 123.40	109.44 ± 66.48	0.138
Fractalkine (pg/mL)	511.46 ± 273.69	438.57 ± 249.46	0.333
BDNF (pg/mL)	8234.10 ± 6468.53	5858.89 ± 3471.89	0.147
Erythropoietin (pg/mL)	2822.21 ± 1209.41	2471.33 ± 1039.91	0.286
Osteonectin (ng/mL)	693.27 ± 203.90	622.93 ± 329.70	0.311
LIF (pg/mL)	6.18 ± 7.25	4.23 ± 3.98	0.825
IL-15 (pg/mL)	4.66 ± 4.76	3.72 ± 2.56	0.991
Myostatin/GDF8 (pg/mL)	1148.66 ± 2135.60	889.09 ± 1361.17	0.850
FABP3 (pg/mL)	2067.59 ± 716.21	1941.87 ± 937.38	0.569
Irisin (pg/mL)	1016.74 ± 1110.38	1036.43 ± 1169.30	0.950
FSTL-1 (pg/mL)	7368.05 ± 4937.52	8009.91 ± 9363.92	0.726
Oncostatin M (pg/mL)	10.98 ± 7.45	11.17 ± 7.45	0.927
IL-6 (pg/mL)	3.17 ± 3.92	2.59 ± 2.72	0.661
FGF21 (pg/mL)	95.62 ± 83.67	197.49 ± 198.27	0.049

F0-F2, lower stages of liver fibrosis, F3-F4, higher stages of liver fibrosis, BDNF, brain derived neurotrophic factor; LIF, leukemia inhibitory factor; IL-15, interleukin 15; GDF8, growth differentiation factor 8; FABP3, fatty acid binding protein 3; FSTL, follistatin-like 1; IL-6, interleukin 6; FGF21, fibroblast growth factor 21.

Table 3. Serum myokine levels according to the grade of hepatosteatosi s by CAP (n=62).

Features	<S3 (N=8)	S3 (N=54)	p value
Apelin (pg/mL)	121.56 ± 155.93	145.42 ± 104.90	0.576
Fractalkine (pg/mL)	427.32 ± 270.19	499.63 ± 267.74	0.479
BDNF (pg/mL)	6999.39 ± 4117.58	7625.28 ± 6073.10	0.780
Erythropoietin (pg/mL)	2314.56 ± 1846.11	2780.46 ± 1200.06	0.295
Osteonectin (ng/mL)	690.85 ± 177.12	670.19 ± 256.11	0.827
LIF (pg/mL)	3.09 ± 2.20	5.99 ± 6.84	0.407
IL-15 (pg/mL)	2.88 ± 1.72	4.61 ± 4.46	0.405
Myostatin/GDF8 (pg/mL)	420.22 ± 797.45	1170.05 ± 2037.86	0.314
FABP3 (pg/mL)	2216.38 ± 729.02	2003.64 ± 791.00	0.477
Irisin (pg/mL)	385.21 ± 375.98	1116.87 ± 1161.86	0.001
FSTL-1 (pg/mL)	6256.33 ± 5857.72	7746.70 ± 6571.10	0.547
Oncostatin M (pg/mL)	10.83 ± 7.17	11.07 ± 7.48	0.934
IL-6 (pg/mL)	1.09 ± 1.02	3.29 ± 3.76	0.051
FGF21 (pg/mL)	88.20 ± 76.65	130.67 ± 140.63	0.437

S3, highest grade of hepatosteatosi s, BDNF, brain derived neurotrophic factor; CAP, controlled attenuation parameter ; LIF, leukemia inhibitory factor; IL-15, interleukin 15; GDF8, growth differentiation factor 8; FABP3, fatty acid binding protein 3; FSTL, follistatin-like 1; IL-6, interleukin 6; FGF21, fibroblast growth factor 21.

4. Discussion

We studied the influence of muscle mass in patients with MASLD and its correlations with circulating levels of myokines. The diagnosis of MASLD was performed by abdominal ultrasound and transitional elastography together with inclusion of clinical, metabolic and laboratory variables defined in previous studies [19,29]. All groups were quite homogeneous with very similar clinical and metabolic variables.

Firstly, body composition was analyzed in our cohort. Surprisingly, muscle strength and skeletal muscle mass in MASLD patients were not related to the stage of liver fibrosis and the grade of hepatosteatosi s. However, despite the homogeneity in ASMM of both groups, we did observe greater fragility, determined by LFI, in patients with a higher stage of liver fibrosis.

Previous reports have shown a heterogeneous prevalence of sarcopenia between 26-46% in relation to liver fibrosis [8,30]. Several factors could explain our differences with published data. Firstly, sarcopenia definition is heterogeneous among studies and furthermore, our study cohort had a low prevalence of patients with advanced fibrosis (n=18).

Subsequently, the circulating levels of myokines were determined to explore potential correlations with skeletal muscle alterations in our study cohort.

A significant increase in FGF21 was found in MASLD patients with advanced hepatic fibrosis (F3-F4). FGF21 behaves as an anti-obesity and anti-diabetic hormone, causing a reduction in liver fat content, fibrosis and inflammation. Its serum concentration seems to correlate well with intrahepatic levels and has been described to be increased in the context of obesity and carbohydrate consumption as a compensatory effect [30–32]. In the absence of this cytokine or the loss of its action, lipotoxicity worsens, developing greater liver inflammation and fibrosis. Our data suggest that high serum concentrations in patients who show greater liver fibrosis may translate into a state of resistance to FGF21 itself. Similarly to insulin resistance, this elevation of FGF21 in the group with greater liver

fibrosis may be linked to worse metabolic parameters, with a higher tendency towards diabetes, and specifically poor functional performance in both the LFI and hand-grip strength, as well as a greater trend towards frailty, despite the absence of a clear loss of skeletal muscle mass.

Likewise, previous studies have proposed that administration of analogues or a modified FGF21 could play an important role in the treatment of liver disease due to fat deposition, and there are studies with several of them in development [28,33,34]. Therefore, it will be important to determine those patients who will present the least response to them.

Furthermore, higher levels of irisin were found in MASLD patients with severe hepatosteatosis (S3). Irisin appears to act as a connection between skeletal muscle tissue and adipose tissue, interacting with myostatin present in muscle to regulate muscle mass and also acting on glucose metabolism and insulin sensitivity. It exerts an autocrine function in adipose tissue as an adipokine, being secreted by subcutaneous fatty tissue in greater quantities than visceral fat. Irisin seems to be related to an unfavorable metabolic state, showing higher levels in patients who develop metabolic syndrome [35,36]. In obesity, the greatest release occurs from adipose tissue [37], this corresponds to our data since higher levels were found in those patients with higher CAP and BMI. Until today available data on irisin are contradictory and more studies are needed to define its role.

Our study has some limitations. The sample had a low prevalence of severe hepatic disease that limited the statistical power. Moreover, patients included in the study were patients referred to the hepatology clinic for study due to alteration of the liver profile and determine the prognosis of the metabolic process, which may have caused a certain degree of selection bias.

5. Conclusions

Serum levels of myokines, such as irisin and FGF21, were significantly higher in MASLD patients with severe steatosis and in those with advanced liver fibrosis, respectively. However, correlation between skeletal muscle alterations and the stage of liver fibrosis in MASLD patients was not found. Further clinical studies including larger number of MASLD patients are needed in order to accurately determine the relationship between skeletal muscle abnormalities and the clinical outcome of MASLD patients.

Author Contributions: For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used “Conceptualization, AGR and YRM; methodology, CEFG and VB; data curation, CEFG, EFY, MC, AB, VB, MC, LGM, BMB and MSN; formal analysis, CEFG; investigation, CEFG, EFY, YRM, MC, AB, VB, MC, LGM, MLGB, BMB, MSN and MJB; resources, AGR and CGM; writing—original draft preparation, YRM, CEFG, AGR and CGM; writing—review and editing, CEFG, MC, VB, MC, LGM, MLGB, BMB, MSN, MJB and CGM; visualization, YRM, CEFG and CGM; supervision, YRM, AGR and CGM; project administration, AGR and YRM; funding acquisition, YRM. All authors have read and agreed to the published version of the manuscript.”

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board (or Ethics Committee) of La Princesa University Hospital (RRN 4645, 21/10/2021).“

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: We encourage all authors of articles published in MDPI journals to share their research data. In this section, please provide details regarding where data supporting reported results can be found, including links to publicly archived datasets analyzed or generated during the study. Where no new data were created, or where data is unavailable due to privacy or ethical restrictions, a statement is still required. Suggested Data Availability Statements are available in section “MDPI Research Data Policies” at <https://www.mdpi.com/ethics>.

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References

1. El Sherif, O., et al., Sarcopenia in nonalcoholic fatty liver disease: new challenges for clinical practice. *Expert Rev Gastroenterol Hepatol*, 2020. 14(3): p. 197-205.
2. Merli, M. and S. Dasarathy, Sarcopenia in non-alcoholic fatty liver disease: Targeting the real culprit? *J Hepatol*, 2015. 63(2): p. 309-11.
3. Bhanji, R.A., et al., Sarcopenia in hiding: The risk and consequence of underestimating muscle dysfunction in nonalcoholic steatohepatitis. *Hepatology*, 2017. 66(6): p. 2055-2065.
4. Li, A.A., D. Kim, and A. Ahmed, Association of Sarcopenia and NAFLD: An Overview. *Clin Liver Dis (Hoboken)*, 2020. 16(2): p. 73-76.
5. Habig, G., C. Smaltz, and D. Halegoua-DeMarzio, Presence and Implications of Sarcopenia in Non-alcoholic Steatohepatitis. *Metabolites*, 2021. 11(4).
6. Koo, B.K., et al., Sarcopenia is an independent risk factor for non-alcoholic steatohepatitis and significant fibrosis. *J Hepatol*, 2017. 66(1): p. 123-131.
7. Kim, J.A. and K.M. Choi, Sarcopenia and fatty liver disease. *Hepatol Int*, 2019. 13(6): p. 674-687.
8. Mikolasevic, I., et al., Nonalcoholic Fatty Liver Disease and Sarcopenia: Where Do We Stand? *Can J Gastroenterol Hepatol*, 2020. 2020: p. 8859719.
9. Cai, C., et al., Relationship between relative skeletal muscle mass and nonalcoholic fatty liver disease: a systematic review and meta-analysis. *Hepatol Int*, 2020. 14(1): p. 115-126.
10. Issa, D., et al., Presence of sarcopenia (muscle wasting) in patients with nonalcoholic steatohepatitis. *Hepatology*, 2014. 60(1): p. 428-9.
11. Younossi, Z.M., et al., Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology*, 2016. 64(1): p. 73-84.
12. Chalasani, N., et al., The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from the American Association for the Study of Liver Diseases. *Hepatology*, 2018. 67(1): p. 328-357.
13. Nachit, M. and I.A. Leclercq, Emerging awareness on the importance of skeletal muscle in liver diseases: time to dig deeper into mechanisms! *Clin Sci (Lond)*, 2019. 133(3): p. 465-481.
14. Altajar, S. and G. Baffy, Skeletal Muscle Dysfunction in the Development and Progression of Nonalcoholic Fatty Liver Disease. *J Clin Transl Hepatol*, 2020. 8(4): p. 414-423.
15. Armandi, A., et al., The Impact of Dysmetabolic Sarcopenia Among Insulin Sensitive Tissues: A Narrative Review. *Front Endocrinol (Lausanne)*, 2021. 12: p. 716533.
16. Eslam, M., et al., A new definition for metabolic dysfunction-associated fatty liver disease: An international expert consensus statement. *J Hepatol*, 2020. 73(1): p. 202-209.
17. Son, D.H., et al., New markers in metabolic syndrome. *Adv Clin Chem*, 2022. 110: p. 37-71.
18. Grundy, S.M., et al., Definition of metabolic syndrome: Report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. *Circulation*, 2004. 109(3): p. 433-8.
19. Parente, D.B., et al., Preperitoneal fat as a non-invasive marker of increased risk of severe non-alcoholic fatty liver disease in patients with type 2 diabetes. *J Gastroenterol Hepatol*, 2018. 33(2): p. 511-517.
20. Cuatrecasas, G., et al., Ultrasound measures of abdominal fat layers correlate with metabolic syndrome features in patients with obesity. *Obes Sci Pract*, 2020. 6(6): p. 660-667.
21. Lupşor-Platon, M., et al., Diagnostic accuracy of controlled attenuation parameter measured by transient elastography for the non-invasive assessment of liver steatosis: a prospective study. *J Gastrointest Liver Dis*, 2015. 24(1): p. 35-42.
22. Jung, K.S., et al., Risk assessment of hepatitis B virus-related hepatocellular carcinoma development using liver stiffness measurement (FibroScan). *Hepatology*, 2011. 53(3): p. 885-94.
23. Castéra, L., et al., Pitfalls of liver stiffness measurement: a 5-year prospective study of 13,369 examinations. *Hepatology*, 2010. 51(3): p. 828-35.
24. Castera, L., M. Friedrich-Rust, and R. Loomba, Noninvasive Assessment of Liver Disease in Patients with Nonalcoholic Fatty Liver Disease. *Gastroenterology*, 2019. 156(5): p. 1264-1281.e4.
25. Cruz-Jentoft, A.J., et al., Sarcopenia: revised European consensus on definition and diagnosis. *Age Ageing*, 2019. 48(4): p. 601.
26. Lai, J.C., et al., The Liver Frailty Index Improves Mortality Prediction of the Subjective Clinician Assessment in Patients With Cirrhosis. *Am J Gastroenterol*, 2018. 113(2): p. 235-242.
27. Wang, C.W., et al., The Range and Reproducibility of the Liver Frailty Index. *Liver Transpl*, 2019. 25(6): p. 841-847.
28. Loomba, R., et al., Randomized, Controlled Trial of the FGF21 Analogue Pegzofermin in NASH. *N Engl J Med*, 2023. 389(11): p. 998-1008.
29. Kim, D., et al., Metabolic dysfunction-associated fatty liver disease is associated with increased all-cause mortality in the United States. *J Hepatol*, 2021. 75(6): p. 1284-1291.
30. Dushay, J., et al., Increased fibroblast growth factor 21 in obesity and nonalcoholic fatty liver disease. *Gastroenterology*, 2010. 139(2): p. 456-63.

31. Maratos-Flier, E., Fatty liver and FGF21 physiology. *Exp Cell Res*, 2017. 360(1): p. 2-5.
32. Lundsgaard, A.M., et al., Circulating FGF21 in humans is potently induced by short term overfeeding of carbohydrates. *Mol Metab*, 2017. 6(1): p. 22-29.
33. Harrison, S.A., et al., FGF21 Agonists: An Emerging Therapeutic for Metabolic Dysfunction-Associated Steatohepatitis and Beyond. *J Hepatol*, 2024.
34. Ciardullo, S., et al., Advancements in pharmacological treatment of NAFLD/MASLD: a focus on metabolic and liver-targeted interventions. *Gastroenterol Rep (Oxf)*, 2024. 12: p. goae029.
35. Park, K.H., et al., Circulating irisin in relation to insulin resistance and the metabolic syndrome. *J Clin Endocrinol Metab*, 2013. 98(12): p. 4899-907.
36. Moreno, M., J.M. Moreno-Navarrete, and J.M. Fernández-Real, [Irisin: a messenger from the gods?]. *Clin Investig Arterioscler*, 2014. 26(3): p. 140-6.
37. Bernal Rivas, C., et al., Effects of exercise on irisin in subjects with overweight or obesity. A systematic review of clinical studies. *Nutr Hosp*, 2022. 39(6): p. 1389-1396.

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