Observational Study
Prevalence of Sarcopenia by different methods in patients with Non-Alcoholic Fatty Liver Disease

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Abstract
BACKGROUND
Sarcopenia is a clinical condition associated with several liver diseases and it includes Non-Alcoholic Fatty Liver Disease (NAFLD) in its broad spectrum as steatosis, steatohepatitis and fibrosis. However, the criteria to define sarcopenia are diverse, and even those established in consensus have been discussed regarding their performance in making an accurate diagnosis.

AIM
To evaluate the prevalence of sarcopenia, through different methods, in patients with NAFLD and its association with clinical-anthropometric parameters.

METHODS
Observational study in outpatients with NAFLD. Sarcopenia was defined by the European Working Group Consensus on Sarcopenia in Older People of 2010 (EWGSOP1) and 2018 (EWGSOP2). The skeletal muscle index (SMI) was used to estimate muscle mass, handgrip strength was assessed using the dynamometer and
physical performance by a distance covered of four meters with usual walking speed. The non-invasive fibrosis scores, fibrosis-4 index (FIB-4) and Aspartate aminotransferase to platelet ratio index (APRI), were used to assess the absence and presence of fibrosis.

RESULTS
Fifty-seven individuals with NAFLD were evaluated, the mean age (SD) was 52.7 (11.3) years and 75.4% were female. Fibrosis assessed by FIB4 and APRI was observed in 3.7% and 16.6% of patients with NAFLD, respectively. The diagnosis of sarcopenia was identified only by EWGSOP1 in 3.5% of NAFLD patients, and the prevalence of probable/pre-sarcopenia was higher using the EWGSOP2 consensus, 26.3%, when compared to EWGSOP1, 1.8%. Sarcopenia defined by EWGSOP1, was associated with grade I steatosis, but without overweight (p<0.05). The association with sarcopenia and fibrosis wasn’t observed (p>0.05). EWGSOP2 shows a greater number of patients with probable sarcopenia, and who were overweight (12 (80.0%)), with a higher degree of steatosis (11 (73.3%) and presence of fibrosis (1 (6.7%), FIB4 and 3 (20.0%), APRI) compared to EWGSOP1 (1 (100%), 0 (0.0%), 0 (0.0%), FIB4 and 0 (0.0%), APRI, respectively).

CONCLUSION
The present study showed that sarcopenia in NAFLD wasn’t predominant in patients without fibrosis, by both diagnostic methods. And the prevalence of probable sarcopenia also depends on the method applied.

INTRODUCTION
Non-alcoholic fatty liver disease (NAFLD) is considered the most prevalent liver disease and affects about 25% to 30% of the world population\textsuperscript{[1]}. Among obese and/or diabetic individuals, the prevalence is even higher, reaching around 75-90% in these population\textsuperscript{[2]}. The increased prevalence in these groups may be justified by the
association of NAFLD with several metabolic disorders, including insulin resistance, inflammation and altered lipid metabolism.[8]

Sarcopenia, defined as the progressive loss of muscle mass, strength and muscle function, shares some pathophysiological mechanisms like NAFLD as insulin resistance, which is the main link between these two diseases.[3-6]

Skeletal muscle is an active endocrine organ responsible for insulin-mediated glucose elimination. Thus, muscle depletion can lead to a reduction in the primary target tissue of insulin action with consequent resistance to it.[7-8]

Sarcopenia was considered a diagnostic code muscle disease (ICD), in which low muscle strength is the main determinant for triggering the trigger of the diagnostic investigation, surpassing the low muscle mass, prioritized by the first publication of the European Working Group on Sarcopenia in Older Persons (EWGSOP) [9,10]. The primary difference between the two consensuses (EWGSOP1 for 2010 and EWGSOP2, 2018) is in the triggering criteria for the diagnostic investigation, defined as "pre-sarcopenia".

Although experts in the field accept the use of EWGSOP2, the effect on the identification of sarcopenia has raised concern. To date, all consensuses have agreed on two crucial components in defining sarcopenia, the involvement of both structural damage (low muscle mass) and impaired function (low muscle strength). However, the cut point reductions suggested in EWGSOP2 seem to have lower sensitivity.[11,12]

The present study aimed to evaluate the prevalence of sarcopenia by different diagnostic methods in patients with NAFLD, and its association with the severity of this disease.

**MATERIALS AND METHODS**

*Study design and population*

A cross-sectional study was conducted at the Nonalcoholic Steatohepatitis Outpatient Clinic (NASH) - Federal University of Bahia, Brazil.

A consecutive and voluntary sample of patients with NAFLD was selected from January 2019 to December 2021, both sexes, aged over 18 years. The criteria for NAFLD
included presence of hepatic steatosis on abdominal ultrasound; negative history of ethanol intake (<140g of ethanol per week); exclusion of others liver disease as B and C virus infection; hemochromatosis, autoimmune hepatitis. Patients with hypothyroidism, pregnant and lactating women, those with hepatomegaly or splenomegaly, ascites, abdominal tumors and recent abdominal surgeries, or any physical limitation that compromised the anthropometric assessment also were not included.

**Abdominal ultrasound**

All patients underwent ultrasound of the upper abdomen by a single evaluator to measure intrahepatic fat, in a specialized clinic, using the Xario 100 Canon Medical Systems device®.

**Non-invasive fibrosis scores**

Non-invasive fibrosis scores were used to assess the absence and presence of fibrosis. The fibrosis-4 index (FIB-4), considering absence of fibrosis FIB-4 <1.30, indeterminate FIB-4 1.30 - 2.67 and fibrosis FIB-4 >2.67 [13, 14], for Aspartate aminotransferase to platelet ratio index (APRI), absence of fibrosis APRI ≤0.50, indeterminate APRI >0.5 - <1.49 and fibrosis APRI >1.50[15].

**Anthropometric assessment**

Anthropometric measurements were performed in duplicate by a trained, and standardized team. Body weight (kg) and height (cm) were measured with light clothes and without shoes, using a digital scale with a resolution of 100g and a stadiometer with 0.5 cm [16]. The Body Mass Index (BMI) was obtained using the formula weight (Kg) / height$^2$ (m$^2$) [17] and for better interpretation of the data, overweight and non-overweight categorizations were used. To this end, adult individuals were considered overweight when BMI ≥ 25 kg/m$^2$ and the elderly, when BMI ≥ 28 kg/m$^2$ [17, 18].

**Sarcopenia assessment**

As diagnostic criteria, the 2010 European Consensus was used (EWGSOP1) [9], which recommends muscle mass, muscle strength and physical performance for the
diagnosis, and the 2018 European Consensus (EWGSOP2)\cite{1} that uses muscle strength and muscle quantity/quality, in that order, maintaining physical performance only as a way of categorizing the severity of the disease (Table 1).

**Muscle mass**

Muscle mass was evaluated by calculating skeletal muscle mass (SMM), using the prediction equation proposed by Janssen *et al.*\cite{19}, where height is measured in cm, resistance in ohms, male = 1, female = 0, and age is measured in years.

The resistance value was obtained through bioelectrical impedance (BIA), using the tetrapolar Biodynamics®, model 450. The technique and previous procedures were performed according to Kyle *et al.*\cite{20}. From the SMM, the equation SMI calculated the skeletal muscle index (SMI) = MM/height^2\cite{19}.

Low muscle mass was defined according to the cutoff points predicted by the EWGSOP1\cite{9} (women < 6.42 kg/m^2, men < 8.87 kg/m^2) and EWGSOP2\cite{10} (women < 5.5 kg/m^2, men < 7 kg/m^2).

**Muscle strength**

Muscle strength was assessed by the maximal handgrip strength test, with a portable handheld dynamometer SAEHAN Spring Hand Dynamometer (Smedley-Type SH5002), and a 0-100 kg/force grading scale (kg/f).

Two measurements were taken on each hand, alternately, with 1 minute of rest between them. The average between each pair of measurements was obtained and the highest average obtained was considered for analysis. For EWGSOP1, low muscle strength is defined as <30kgf for men and <20kgf for women\cite{9} and for EWGSOP2, <27kgf for men and <16kgf for women\cite{10}.

**Physical performance**

Usual gait speed was measured in meters per second (m/s). The patient walked four meters in a straight and flat environment, with the usual walking speed. The test
was repeated twice, and the shortest time spent was used for analysis. Individuals with gait speed < 0.8 m/s were evaluated with reduced gait speed or poor physical performance\textsuperscript{[9,10]}.

**Statistical analysis**

For the tabulation and analysis of the data, the statistical program Statistical Package for the Social Science\textregistered (SPSS) version 20.0 was used. The results of categorical variables were expressed as absolute and relative frequency and continuous variables were expressed as mean and standard deviation. Pearson’s chi-square test was used to compare qualitative variables. Values of p<0.05 were considered statistically significant.

**RESULTS**

**Characteristics of the population studied**

The study included fifty-seven patients with NAFLD. A total of 75.4% were female, and the ages ranged between 26 and 73 years, mean (SD) of 52.7 (11.3) years. Overweight was present in 84.2% of patients, 63.1% had grade II and III steatosis. Hepatic fibrosis by Fib4 was observed in 3.7% of the NAFLD patients and 16.6% by APRI.

**Prevalence of EWGSOP1 vs EWGSOP2 Sarcopenia**

The diagnosis of sarcopenia in these NAFLD patients was identified only by EWGSOP1, in 3.5% patients. The prevalence of probable/pre-sarcopenia was higher when using the EWGSOP2 consensus when compared to EWGSOP1, 26.3% vs 1.8% of patients with NAFLD.

When evaluated separately by the items that define sarcopenia, most patients had preserved muscle mass and physical performance, both by EWGSOP1 and EWGSOP2. However, it is observed that the number of people with preserved muscle strength was higher by the EWGSOP2, 71.9%, when compared to those evaluated by the EWGSOP1, 47.4% (Table 2).
Prevalence of sarcopenia according to BMI, degrees of steatosis and fibrosis

Patients with NAFLD diagnosed with sarcopenia by EWGSOP1 presented grade I steatosis and non-overweight (P = 0.027 and P = 0.003 respectively). However, no association was observed between sarcopenia and fibrosis, either by FIB4 or APRI (p>0.05) (Table 3).

By EWGSOP2, no association was observed between probable-sarcopenia and degree of steatosis or probable-sarcopenia and excess weight (p>0.05). However, using this method, a greater number of patients with probable sarcopenia and who were overweight, with a higher degree of steatosis and presence of fibrosis were observed (Table 3).

DISCUSSION

This sample of NAFLD patients was composed mostly of overweight adults’ women, who presented highest degrees of hepatic steatosis, however without fibrosis. The diagnosis of sarcopenia was identified by EWGSOP1 criteria, and the EWGSOP2 algorithm identified probable sarcopenia or pre-sarcopenia major. According to the EWGSOP2, we observed more frequent cases of NAFLD with probable sarcopenia, who presented overweight, higher degree of steatosis and the presence of fibrosis.

Due to the wide variety of methods available, the identification of sarcopenia undergoes variation, and consequently discrepancies are observed between the prevalence of sarcopenia when applying the EWGSOP1 and EWGSOP2 criteria \(^{21}\). A systematic review, which compared the prevalence of sarcopenia in the geriatric population based on these two consensuses found 6.2% to 35.3% sarcopenic by EWGSOP1 and 3.2% to 26.3% by EWGSOP2\(^{22}\). Some studies have shown that EWGSOP1 seems to have great sensibility to identify individuals at higher risk for health outcomes\(^{12,21,23}\). However, the sensitivity assessment of both methods has been, in its entirety, applied in geriatric populations associated with other diseases.
Sarcopenia has been associated with increased incidence and risk for NAFLD [24,25]. Despite the divergence of methods used to identify sarcopenia in several studies, and considering the specific characteristics of each population, the literature shows a positive association between sarcopenia and NAFLD, with a significant prevalence in this population [24, 26, 27].

The association of steatosis with fibrosis in patients with sarcopenia evaluated by scores FIB4 and APRI [26-28]. The currently available scores, including FIB4 and APRI, have presented some limitations to diagnose fibrosis. There are also difficult to define a cutoff point capable of differentiating between the absence of fibrosis or the presence of advanced fibrosis in NAFLD. In general, predictive fibrosis scores have a good Negative Predictive Value to exclude advanced fibrosis with low Positive Predictive Value. Therefore, these scores can be safely used for basal risk stratification to exclude advanced and nonexistent fibrosis. There is an interval considered undetermined or gray area. In these cases, other methods such as liver biopsy may be necessary for the diagnosis of fibrosis[14, 29].

Different methods have been used for the diagnosis of sarcopenia in these studies, among them, the ratio between appendicular skeletal muscle mass and BMI with different cut-off points, and fibrosis identified through liver biopsy and/or non-invasive markers.

The association between sarcopenia and NAFLD still requires further evaluation, considering the standardization and identification of the best diagnostic method for both sarcopenia and hepatic fibrosis.

Considering that sarcopenia is often not noticeable in the preliminary stages, detecting probable sarcopenia is important so that appropriate intervention can be established early [30,31]. In our population, EWGSOP2 better identified cases of probable sarcopenia when compared to EWGSOP1. In addition to the difference in cutoff points, the criteria used in the initial screening are different between the consensus (muscle mass vs. muscle strength). In a longitudinal analysis performed in the geriatric population, a higher prevalence of low muscle strength was observed [32].
The cohort study by Xia et al. [33] found that hand pressure strength is inversely associated with the incidence of NAFLD. This finding had been presented in other studies, which also pointed to a probable relationship between NAFLD and muscle strength [34,35]. Patients with NAFLD seem to be more likely to have low muscle strength when compared to controls, and a higher prevalence of NAFLD was identified in those with low muscle strength. Thus, the evaluation of muscle strength, prioritized by the EWGSOP2, in patients with NAFLD may be a more valuable parameter to identify pilot stages of sarcopenia when compared to the analysis of muscle mass [33].

This study results suggest that prioritization of muscle strength by EWGSOP2 may allow greater identification of early cases of sarcopenia in individuals with NAFLD. And the measurement of muscle strength is easy to perform and low cost, being a positive factor for clinical applicability when compared with the measurement of muscle mass. From a clinical point, early detection of cases is essential, considering that it is better to prevent skeletal muscle depletion than to try to restore it once it has progressed [36,37].

This seems to be one the first study that investigated the impact of using the two most used consensuses for the detection of sarcopenia in patients with NAFLD. Although the cross-sectional design limits the possibility of inferring causality, and the small sample size restricts the extrapolation of results to the entire NAFLD population, our results suggest a new clinical approach to sarcopenia in patients with NAFLD.

CONCLUSION

In conclusion, the prevalence of sarcopenia becomes variable depending on the sensitivity of the method applied. And due to the pathophysiological association of sarcopenia with NAFLD, it is important to identify the best method capable of early detection of loss of muscle function in this population.

It is also possible to think about identifying viable strategies to screen for sarcopenia in clinical practice, using muscle strength as a primary diagnostic indicator, as determined by the EWGSOP2. Thus, this method makes it easier to identify probable
sarcopenia in the initial screening at an outpatient level, and consequently the early
detection of cases of sarcopenia in this population.

ARTICLE HIGHLIGHTS

Research background
Sarcopenia is a clinical condition possibly associated with Non-Alcoholic Fatty Liver
Disease (NAFLD) as they share common pathophysiological mechanisms, such as
insulin resistance. The diagnostic criteria available in the literature to define sarcopenia
are diverse, and even those established in consensus have been questionable as to their
performance in the correct diagnosis.

Research motivation
Previous studies demonstrate an association between sarcopenia and NAFLD. However, the assessment of sarcopenia is performed by various diagnostic methods, which implies discrepant prevalence. The search for the best method led to the two
most used consensuses in the scientific community for the diagnosis of sarcopenia in
the population and that were not previously investigated in patients with NAFLD.

Research objectives
To evaluate the prevalence of sarcopenia, through different methods, in patients with
NAFLD, and its association with the severity of this disease.

Research methods
Sarcopenia was defined by the European Working Group Consensus on Sarcopenia in
Older People of 2010 (EWGSOP1) and 2018 (EWGSOP2). Abdominal ultrasound was
used to diagnose hepatic steatosis. The non-invasive fibrosis scores, FIB-4 and APRI,
were used to assess the absence and presence of fibrosis.

Research results
The diagnosis of sarcopenia was identified only by EWGSOP1, and the EWGSOP2 algorithm identified probable sarcopenia or pre-sarcopenia. Sarcopenia, defined by EWGSOP1, was associated with grade I steatosis, but without excess weight (p<0.05). EWGSOP2 shows a greater number of patients with probable sarcopenia, overweight, with a greater degree of steatosis and presence of fibrosis compared to EWGSOP1.

*Research conclusions*
Sarcopenia in NAFLD was not predominant in patients without fibrosis, by both consensuses. And the prevalence of probable sarcopenia, a promising early indicator of sarcopenia, was higher by the EWGSOP2 method.

*Research perspectives*
Validation of muscle strength measurement in the early identification of sarcopenia cases in NAFLD patients.

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