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ABOUT COVER
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Observational Study

Prevalence of sarcopenia using different methods in patients with non-alcoholic fatty liver disease

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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, using different methods, in patients with NAFLD, and its association with clinical-anthropometric parameters.

METHODS
This was an observational study of 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 was used to estimate muscle mass, handgrip strength was assessed using the dynamometer and physical performance by walking a distance of four meters at usual walking speed. The non-invasive fibrosis scores, fibrosis-4 (FIB-4) index 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 FIB-4 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 at 26.3%, when compared to 1.8% with EWGSOP1. Sarcopenia defined by EWGSOP1, was associated with grade I steatosis, but without overweight (P < 0.05). An association between sarcopenia and fibrosis was not observed (P > 0.05). EWGSOP2 showed 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%), FIB-4 and 3 (20.0%), APRI] compared to EWGSOP1 [1 (100%), 0 (0.0%), 0 (0.0%), FIB-4 and 0 (0.0%), APRI, respectively].

CONCLUSION
The present study showed that sarcopenia in NAFLD was not predominant in patients without fibrosis, by both diagnostic methods. In addition, the prevalence of probable sarcopenia also depends on the method applied.

Key Words: Non-alcoholic fatty liver disease; Sarcopenia; Muscle strength; Physical performance; Liver fibrosis

Core Tip: In Non-Alcoholic Fatty Liver Disease (NAFLD), sarcopenia has been associated with the presence and severity of the disease. However, the diagnostic criteria for sarcopenia are still under evaluation and undergoing constant changes. In patients with NAFLD, sarcopenia was not common, but a higher prevalence of probable sarcopenia was observed by the most current European Working Group Consensus on Sarcopenia in Older People, 2018. This increased sensitivity to the possible early stage of sarcopenia may be an opportunity for accurate and early interventions in this population, preventing the development of sarcopenia and the worse evolution of NAFLD.

INTRODUCTION
Non-alcoholic fatty liver disease (NAFLD) is considered the most prevalent liver disease and affects approximately 25% to 30% of the world population[1]. Among obese and/or diabetic individuals, the prevalence is even higher, reaching around 75%-90% in these populations[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[3].

Sarcopenia, defined as the progressive loss of muscle mass, strength and muscle function, shares some pathophysiological mechanisms with NAFLD such 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, in which low muscle strength is the main determinant for triggering diagnostic investigation, surpassing 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 2010 and EWGSOP2 2018) is in the triggering criteria for 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 using 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 including both sexes aged over 18 years with NAFLD was selected from January 2019 to December 2021. The criteria for NAFLD included the presence of hepatic steatosis on abdominal ultrasound; negative history of ethanol intake (< 140 g of ethanol per week); exclusion of other liver diseases such as hepatitis B and C virus infection; hemochromatosis, and 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 were excluded.

**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), considered the absence of fibrosis to be FIB-4 < 1.30, indeterminate FIB-4 1.30–2.67 and fibrosis FIB-4 > 2.67[13,14]. For the Aspartate aminotransferase to platelet ratio index (APRI), absence of fibrosis was 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 100 g and a stadiometer with 0.5 cm[16]. The body mass index (BMI) was obtained using the formula weight (kg)/height² (m²)[17] and for better interpretation of the data, overweight and non-overweight categorizations were used. To this end, adult individuals were considered overweight when the BMI ≥ 25 kg/m² and in the elderly, when BMI ≥ 28 kg/m²[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)[10] 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[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, using the tetrapolar Biodynamics®, model 450. The technique and previous procedures were performed according to Kyle et al[20]. From the SMM, the equation skeletal muscle index (SMI) calculated the SMI = MM/height²[19].

Low muscle mass was defined according to the cutoff points predicted by the EWGSOP1[9] (women < 6.42 kg/m², men < 8.87 kg/m²) and EWGSOP2[10] (women < 5.5 kg/m², men < 7 kg/m²).

**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 min 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 < 30 kgf for men and < 20 kgf for women[9] and for EWGSOP2, < 27 kgf for men and < 16 kgf for women[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, at their 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.
Table 1 Classification of the diagnosis of sarcopenia according to the European Working Group on Sarcopenia in Older People, 2010 and 2018

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>EWGSOP1</th>
<th>EWGSOP2</th>
</tr>
</thead>
<tbody>
<tr>
<td>No sarcopenia</td>
<td>MM + MS + PP adequate</td>
<td>MQ + MS + P adequate</td>
</tr>
<tr>
<td>Pre-sarcopena/Probablesarcopenia</td>
<td>MM insufficient</td>
<td>MS insufficient</td>
</tr>
<tr>
<td>Sarcopenia</td>
<td>MM + (MS or PP)insufficient</td>
<td>MS + MQ insufficient</td>
</tr>
<tr>
<td>Severe sarcopenia</td>
<td>MM + MS + PP insufficient</td>
<td>MS + MQ + P insufficient</td>
</tr>
</tbody>
</table>


Statistical analysis
For tabulation and analysis of the data, the statistical program Statistical Package for the Social Sciences® (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. 84.2% of patients were overweight, 63.1% had grade II and III steatosis. Hepatic fibrosis by FIB-4 was observed in 3.7% of the NAFLD patients and in 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 were not overweight ($P = 0.027$ and $P = 0.003$, respectively). However, no association was observed between sarcopenia and fibrosis, either by FIB-4 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 adult women, who had the highest degrees of hepatic steatosis, but 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 cases of NAFLD with probable sarcopenia, who were overweight, had a higher degree of steatosis and the presence of fibrosis.

Due to the wide variety of methods available, the identification of sarcopenia varies, and consequently discrepancies are observed between the prevalence of sarcopenia when applying the EWGSOP1 and EWGSOP2 criteria. A systematic review, which compared the prevalence of sarcopenia in the geriatric population based on these two consensus found 6.2% to 35.3% had sarcopenia by EWGSOP1.

speed or poor physical performance [9,10].

speed or poor physical performance [9,10].
Table 2 Variables for the definition of sarcopenia by the European Working Group of Sarcopenia in Older People, 2010 and 2018

<table>
<thead>
<tr>
<th>Variable</th>
<th>EWGSOP1</th>
<th>EWGSOP2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle mass, n (%)</td>
<td>Adequate</td>
<td>54 (94.7)</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>3 (5.3)</td>
</tr>
<tr>
<td>Muscle strength, n (%)</td>
<td>Adequate</td>
<td>27 (47.4)</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>30 (52.6)</td>
</tr>
<tr>
<td>Physical performance, n (%)</td>
<td>Adequate</td>
<td>48 (84.2)</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>9 (15.8)</td>
</tr>
</tbody>
</table>

EWGSOP1: European Working Group of Sarcopenia in Older People, 2010; EWGSOP2: European Working Group of Sarcopenia in Older People, 2018.

Table 3 Association of sarcopenia with the classification of body mass index, degrees of steatosis and presence of fibrosis (Pearson’s chi-square test)

<table>
<thead>
<tr>
<th>Variable</th>
<th>EWGSOP1</th>
<th>EWGSOP2</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI, n (%)</td>
<td>No excess weight</td>
<td>7 (13.0)</td>
</tr>
<tr>
<td></td>
<td>Overweight</td>
<td>47 (87.0)</td>
</tr>
<tr>
<td>Degrees of steatosis, n (%)</td>
<td>Grade I</td>
<td>18 (33.3)</td>
</tr>
<tr>
<td></td>
<td>Grade II-III</td>
<td>36 (66.7)</td>
</tr>
<tr>
<td>FIB-4</td>
<td>No fibrosis</td>
<td>49 (96.1)</td>
</tr>
<tr>
<td></td>
<td>Fibrosis</td>
<td>2 (3.9)</td>
</tr>
<tr>
<td>APRI</td>
<td>No fibrosis</td>
<td>42 (82.4)</td>
</tr>
<tr>
<td></td>
<td>Fibrosis</td>
<td>9 (17.6)</td>
</tr>
</tbody>
</table>

EWGSOP1: European Working Group of Sarcopenia in Older People, 2010; EWGSOP2: European Working Group of Sarcopenia in Older People, 2018; BMI: Body mass index; FIB-4: Fibrosis-4 index; APRI: Aspartate aminotransferase to platelet ratio index.

3.2% to 26.3% by EWGSOP2[22]. Some studies have shown that EWGSOP1 seems to have great sensitivity in identifying 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 an increased incidence and risk of 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 was evaluated by scores FIB-4 and APRI[26-28]. The currently available scores, including FIB-4 and APRI, have some limitations in the diagnosis of fibrosis. There is also difficulty in defining 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 cutoff 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 consensuses (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 demonstrated 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 the early stages of sarcopenia when compared to the analysis of muscle mass[35].

These study results suggest that prioritization of muscle strength by EWGSOP2 may allow greater identification of early cases of sarcopenia in individuals with NAFLD. The measurement of muscle strength is easy to perform and of low cost, which is a positive factor for clinical applicability when compared with the measurement of muscle mass. From a clinical viewpoint, 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 studies to investigate the impact of using the two most frequently 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 varies depending on the sensitivity of the method applied. In addition, due to the pathophysiological association of sarcopenia with NAFLD, it is important to identify the best method for early detection of loss of muscle function in this population.

It is also possible to identify 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 questioned in relation to their diagnostic accuracy.

Research motivation
Previous studies demonstrated 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, using 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 showed 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. In addition, 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 is essential in NAFLD patients.

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FOOTNOTES
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Conflict-of-interest statement: All the authors have no conflicts of interest to declare.

Data sharing statement: Technical appendix, statistical code, and dataset available from the corresponding author at email address raquelrocha2@yahoo.com.br. Participants gave informed consent for data sharing. No additional data are available.

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L-Editor: Webster JR
P-Editor: Liu JH
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19. Fernandes LV, Paiva AEG, Silva ACB, de Castro IC, Santiago AF, de Oliveira EP, Porto LCJ. Prevalence of sarcopenia according to EWGSOP1 and EWGSOP2 in older adults and their associations with unfavorable health outcomes: a


