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Neck circumference in combination with biochemical variables as a surrogate marker of NAFLD: The FLiO study

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Abstract:

Neck circumference (NC), neck circumference to height ratio (NHtR) and neck circumference to weight ratio (NWtR) appear to be good candidates for the non-invasive management of non-alcoholic fatty liver disease (NAFLD). This study aimed to evaluate the ability of routine variables to assess and manage NAFLD in participants with obesity and NAFLD included in a 2-year nutritional intervention program. Anthropometric measurements, biochemical variables and imaging techniques were performed at different study time-points (baseline, 6, 12 and 24 months). The nutritional intervention significantly improved all anthropometric measurements as well as the glucose profile and the hepatic enzymes. NC and neck ratios combined with ALT levels and HOMA-IR showed good prediction ability for the hepatic fat content and hepatic steatosis at all the study time-points in a ROC analysis. The prediction ability of the combination panels improved when the weight loss variable was also considered. NC and neck ratios are easy anthropometric measurements that in combination with routine biochemical variables (ALT and HOMA-IR) showed good prediction ability of NAFLD. More research studies are necessary to validate the utility of these simple and easy variables as surrogate markers of NAFLD since their application could improve the prevention and management of this prevalent disease.

Keywords: Anthropometric measurements; fatty liver disease; nutritional intervention; imaging techniques; long-term follow-up; neck-to-height ratio; non-invasive diagnostic methods; neck-to-weight ratio; FLIO study; steatosis markers.

1. Introduction

Background: Non-alcoholic fatty liver disease (NAFLD) is characterized by the accumulation of fatty acids within the hepatocytes as fat vacuoles in subjects consuming little or no alcohol without other causes of liver disease [1]. This entity includes several conditions with ascending severity. The most common condition is a simple liver fat accumulation, a non-serious state called fatty liver (simple steatosis). When fat accumulation is associated with liver cell inflammation and different degrees of scarring is considered a more serious condition called non-alcoholic steatohepatitis (NASH). NASH may lead to severe liver scarring, fibrous bridges might be created (fibrosis) and in more advanced stages regenerative nodules are formed (cirrhosis). Cirrhosis occurs when the liver sustains substantial damage. Subjects at this stage may eventually require a liver transplant [2]. Moreover, hepatic cirrhosis is a potential precursor of hepatocarcinoma. Both steatosis

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and NASH are reversible and can evolve from one to another. However, when the fibrous bridges are generated, the process is irreversible.

In developed countries, particularly in Europe, the estimated prevalence of NAFLD in the general population is 20-30%, and it increases in the case of subjects with obesity or metabolic syndrome, up to 70% [2,3]. Disease progression is slow and asymptomatic: patients are not aware of the presence of the disease until it reaches an irreversible stage when the liver is unable to work properly.

In the coming years, NASH and alcoholic liver disease will become the most common causes of chronic liver disease all over the world [4]. The gold standard for diagnosing NAFLD and assessment of its severity is liver biopsy. This is an aggressive technique and has possible complications, such as bleeding, which may even endanger the patient's life. In addition, a small amount of liver parenchyma is evaluated which may not be representative of the entire liver parenchyma [5,6]. Therefore, non-invasive diagnostic methods are needed, such as radiological techniques, biomarkers, anthropometric measurements or serologic tests that may be used at the population level with low risk and cost, promoting early detection of the disease [7]. Among the imaging techniques, ultrasound can discriminate between the presence and absence of steatosis graduating its severity as mild, moderate or severe. It is a technical operator-dependent measurement, but its low cost, availability, and non-risk make it an important tool to be considered. Magnetic resonance imaging (MRI) is a technique available in most hospitals and radiology centers. It provides an objective value, comparable and reproducible. Sensitivity and specificity are high, 96% and 93% respectively [8], and can be considered the best imaging technique in the evaluation and quantification of hepatic steatosis [9].

On the other hand, several panels of biomarkers or scores, such as the Fatty Liver Index, or combinations of biochemical and anthropometric variables to use in the screening of NAFLD have been developed. In recent years it has been published that neck circumference (NC), as well as neck ratios neck-to-height ratio (NHtR) and neck-to-weight ratio (NWtR), might be effective complements for NAFLD screening and can be good indicators of the hepatic status [10,11]. Therefore, the combination of liver markers with anthropometric measurements such as NC could be a useful method for assessing noninvasively the degree of hepatic steatosis in overweight/obese patients with NAFLD to determine the prognosis, monitoring the progression of the disease and to manage an effective treatment.

Due to the above, it is essential to achieve an early diagnosis to prevent the development and progression of the disease to irreversible stages of fibrosis.

All the studies analyzed that have identified surrogate markers of NAFLD are cross-sectional studies. In this sense, the main aim of our research work tries to determine surrogate markers not only for hepatic fat prediction but also for the nutritional management of the disease. In this sense, the study's objective was to assess NC and neck ratios as easy and feasible surrogate markers to predict liver fat content in participants with NAFLD during a 2-year nutritional intervention program.

Conclusions: In our work we demonstrated that NC and neck ratios are easy anthropometric measurements that in combination with routine biochemical variables (ALT and HOMA-IR) showed good prediction ability of NAFLD. More research longitudinal studies are necessary to validate the utility of these simple and easy variables as surrogate markers of NAFLD since their application could improve the prevention and management of this prevalent disease.

2. Materials and Methods

This study is part of a randomized controlled trial registered as FLiO (Fatty Liver in Obesity), (www.clinicaltrials.gov, NCT03183193). It was approved by the Ethics Committee of the Universidad de Navarra, Spain on 24 April 2015 (54/2015) following the Declaration of Helsinki, and the study was conducted following the CONSORT 2010 guidelines. All subjects signed adequately the informed consent before enrollment in the study.

 doi:10.20944/preprints202211.0028.v1

Study participants

A total of 98 overweight/obese men and women (age 40-80 years old; BMI ≥ 27.5 kg/m^2 to <40 kg/m^2) were enrolled after fulfilling the inclusion criteria of the study (12). All participants underwent an ultrasound examination which confirmed the presence of steatosis and graduated its severity as low, moderate or severe.

Subjects included in the study (n=98) were randomized into two different dietary groups following the Mediterranean style to achieve significant weight loss during the 2-year nutritional intervention program. At baseline, participants were randomly assigned to the American Heart Association (AHA) or the Fatty Liver in Obesity (FLiO) group. A comprehensive assessment was carried out at baseline and the end of the study. Measurements included anthropometry, body composition by dual-energy X-Ray absorptiometry (DXA), biochemical determinations, evaluation of the liver using ultrasonography, and Magnetic Resonance Imaging (MRI). Fasting blood samples were properly collected, processed, and stored at −80 °C for further analyses. A step-based physical activity recommendation of 10,000 steps/day was given to the participants [12]. Physical activity was estimated using the validated Spanish version of the Minnesota Leisure-Time Physical Activity Questionnaire. The energy expenditure in physical activity was estimated assuming the value of 1 MET (Metabolic Equivalent for Task) = 3.5 mL/kg/min.

Variable assessment

The determination of anthropometric measurements (body weight, height, and waist circumference), body composition by DXA (Lunar iDXA, encore 14.5, Madison, WI, USA), and blood pressure (Intelli Sense. M6, OMRON Healthcare, Hoofddorp, the Netherlands) were carried out under fasting conditions at the Metabolic Unit of the University of Navarra following standardized procedures. Blood samples were collected, processed, and stored at −80 ◦C for further analyses [13]. Body Mass Index (BMI) was calculated as the body weight divided by the squared height (kg/m2). Biochemical determinations, including blood glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-c), and triglyceride (TG) concentrations were measured on an autoanalyzer Pentra C-200 (HORIBA ABX, Madrid, Spain) with specific commercial kits. Insulin was measured using specific ELISA kits (Demeditec; Kiel-Wellsee, Germany) in a Triturus autoanalyzer (Grifols, Barcelona, Spain). Insulin resistance was estimated using the Homeostasis Model Assessment Index (HOMA-IR), which was calculated using the formula elsewhere described [14]. The low-density lipoprotein cholesterol (LDL-c) levels were estimated using the following formula: LDL-c = TC − HDL-c − TG/5.

Imaging techniques

The imaging hepatic assessment was performed under fasting conditions by qualified staff at the University of Navarra Clinic. Ultrasonography (Siemens ACUSON S2000 and S3000) was carried out to determine the presence of hepatic steatosis following the previously described methodology [15].

Magnetic resonance imaging (Siemens Area 1.5 T, Erlangen Germany) was also used following the Liver Lab protocol to quantify hepatic fat, iron and volume. It consists of a DIXON screening sequence 3D in-and opposed-phase T2 weighted data acquisition with two-point Dixon reconstruction. This method offers a visual qualitative assessment of hepatic steatosis. The acquired data allow for a semiquantitative estimation of fat deposition as well as iron overload. Quantitative sequences include multi-echo T2 corrected single breath-hold spectroscopy (HISTO) reproductive values from a single voxel and multi-echo 3D gradient echo (VIBE) imaging with Dixon reconstruction and correction for T2* [2].

Statistical analyses

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The sample size was calculated considering an association between image techniques and anthropometric variables different from cero. The following formula was used for sample size calculation: $N = [(Z\alpha+Z\beta)/C]2 + 3$. Thus, considering the probability of making a type I error of 0.05, a probability of making a type II error of 0.20, and hoping to find an association between variables of r=0.30, a total of 85 subjects were needed to conduct the analysis.

The normality of the distribution of the evaluated variables was assessed by the Shapiro–Wilk test. Differences between groups were compared using Student's t-test or the Mann–Whitney U test when appropriate. The differences between the baseline and endpoint within each dietary group were analyzed by a paired Student's t-test or Wilcoxon signed-rank test when appropriate. Categorical variables were compared using a Chi-squared test. Change of variables was calculated at all the study time points (6, 12 and 24 months). Spearman correlations were performed to further explore the association between anthropometric variables (neck circumference and neck ratios) and steatosis degree and changes in the hepatic fat at the different study time points (baseline, 6, 12, 24 months). Receiver operating characteristic (ROC) curves were applied to calculate the power of prediction of a combination panel (neck circumference, ALT and HOMA) for liver fat (by MRI) and liver steatosis (by ultrasonography) at baseline, 6, 12 and 24 months. These results were validated by calculating the optimism-corrected value using Tibshirani's enhanced bootstrap method described by Harrell [16].

Analyses were carried out using Stata version 12.0 software (StataCorp, College Station, TX, USA). All p-values presented are two-tailed and were considered statistically significant at $p < 0.05$.

3. Results

A total of 98 overweight/obese participants began the nutritional intervention, 76 reached the 6-month visit, 72 the 12-month visit and 58 completed the nutritional intervention program (Figure 1).

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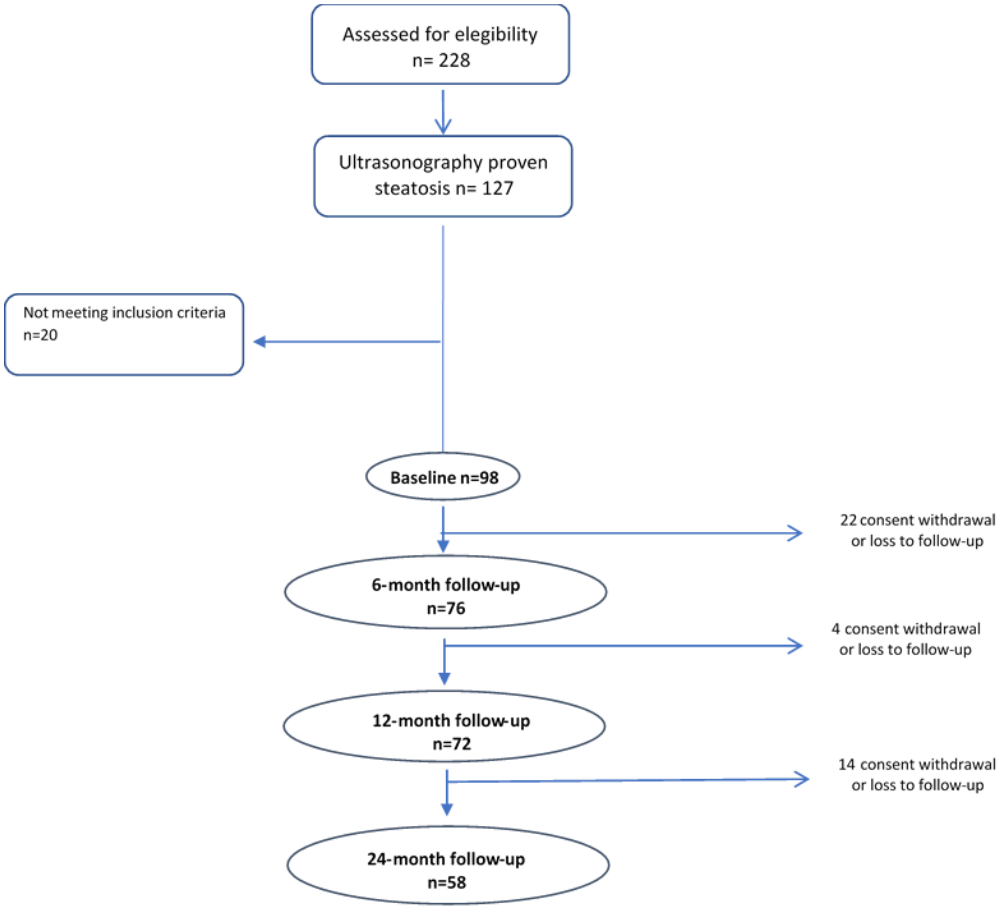

Figure 1: Flowchart of the nutritional intervention

Both diets improved anthropometric, biochemical and hepatic variables during the intervention with no relevant differences between dietary groups as demonstrated by Marin-Alejandre et al., 2021 [13]. Both dietary strategies improved anthropometric variables, biochemical and imaging parameters after 6, 12 and 24 months of the nutritional intervention. Thus, data from dietary groups were joined together to promote statistical power to carry out the aim of the study. The effect of the nutritional intervention program was significant on anthropometric variables (body weight, BMI, waist circumference) and body composition (total body fat and visceral fat content) (Table 1). Neck circumference (NC) and neck-to-height ratio (NHtR) were significantly decreased after 6 and 12 months of intervention, however, no significant differences were observed after the 24-month follow-up. Neck-to-weight ratio (NWtR) significantly increased during all study time points (Table 1). Glucose profile was significantly improved (glucose, insulin and HOMA-IR) after the 24-month follow-up program while lipid profile did not significantly change from baseline values (Table 1). Regarding hepatic status, ALT and GGT significantly decreased during the intervention while AST value was not modified. Hepatic fat and hepatic volume were significantly improved during the intervention (Table 1).

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Variables	Study time-points				
	Basal (n=98)	6M (n=76)	12M (n=72)	24M (n=58)	p-mixed model
Anthropometric variables					
Weight (kg)	94.9±13.9	85.4±13.1*	86.8±14.2*	89.4±14.8*	<0.0001
BMI (kg/m^2)	33.4±3.7	30.1±3.8*	30.7±4.3*	31.5±4.8*	<0.0001
Waist circ (cm)	109.1±8.8	99.7±9.7*	96.9±19.2*	105.0±11.9*	0.001
Total body fat (kg)	38.4±8.6	31.1±9.0*	32.2±9.4*	34.6±10.1*	<0.0001
Visceral fat (kg)	2.2±0.9	1.5±0.7*	1.7±1.0*	1.9±1.0*	<0.0001
Neck circ (cm)	39.6±3.7	38.0±3.5*	38.2±3.5*	39.4±4.0	0.172
NHtR	23.4±1.8	22.6±1.8*	22.8±1.8*	23.4±2.1	0.216
NWtR	0.41±0.04	0.45±0.05*	0.44±0.04*	0.44±0.05*	<0.0001
Biochemical variables					
Glucose (mg/dl)	103.2±17.1	93.8±12.6*	94.2±17.6*	96.2±19.4*	<0.0001
Insulin (mU/L)	17.3±8.2	11.2±7.2*	12.5±7.2*	12.0±0.9*	<0.0001
HOMA-IR	4.5±2.4	2.6±2.0*	3.1±2.5*	3.0±2.0*	<0.0001
Totalcholesteroll (mg/dl)	191.1±36.5	180.9±41.9*	180.0±34.1*	188.6±41.7	0.299
Triglycerides (mg/dl)	129.8±61.1	94.5±50.6*	105.8±46.9*	125.9±79.0	0.240
HDL-c (mg/dl)	51.8±13.0	53.8±12.8*	54.8±13.2*	53.5±13.6	0.237
LDL-c (mg/dl)	113.2±32.2	107.7±36.0	104.2±29.4*	109.9±32.5	0.353
Hepatic variables					
ALT (IU/L)	33.2±17.1	22.2±8.8*	25.0±12.0*	26.9±15.1*	0.001
AST (IU/L)	25.3±10.1	21.7±7.3*	22.9±8.7	24.4±7.7	0.577
GGT (IU/L)	38.6±28.6	27.3±34.3*	28.4±19.6*	29.4±31.3	0.025
Hepatic fat (hist) (%)	10.5±6.3	5.8±4.0*	6.7±5.7*	7.5±6.1*	<0.0001
Hepatic fat (dix) (%)	7.8±8.2	3.2±3.2*	5.3±4.8*	5.7±4.5	0.043
Hepatic volumen (cm^3)	1757.6±399.9	1591.2±318.5*	1620.2±380.3*	1660.1±493.4*	<0.0001

*statToferences basal vs 6, 12 and 24 months. NHtR neck circumference to height ratio; NWtR neck circumference to weight ratio

Table 1: Descriptive variables (anthropometric and body composition, biochemical and imaging technique variables) of study participants at baseline and after 6, 12 and 24 months of nutritional intervention.

To assess the relationship between NC and neck ratios with hepatic steatosis (by ultrasonography) a correlation analysis was performed at all the study time points. NC and NHtR were significantly associated with the steatosis degree at baseline (r=0.29; r=0.32), 6 (r=0.22; r=0.39), 12 (r=0.25; r=0.46), and 24 months (r=0.39; r=0.62), respectively, while NWtR was only associated with the steatosis degree at 12 (r=0.25) and 24 months (r=0.26).

The potential predicting of anthropometric variables (NC, NHtR, NWtR) for hepatic fat content (Table 2) and steatosis degree (Table 3) was assessed by means of a Receiver Operating Curve (ROC) analysis. This analysis was performed at all the study time points. The combination panel made up of NC or NHtR or NWtR, ALT levels and HOMA-IR showed a steady good predictive value for hepatic fat content (Table 2) and steatosis degree (Table 3) at all the study time points. The predictive ability of these combination panels improved during the nutritional intervention, showing the highest predictive ability

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for both liver fat content (ROC: 0.85-0.90) and steatosis degree (ROC: 0.95-0.97) at the end of the intervention (Tables 2 and 3). When models were adjusted by the weight loss percentage the predictive scores were improved in both cases the hepatic fat content (Figure 2) and steatosis degree Figure 3). These results were validated by calculating the optimism-corrected value using Tibshirani's enhanced bootstrap method described by Harrell.

	Hepatic fat content (MRIDixonn)				Hepatic fat content (MRI-histo)			
Combination panels	**Study time-point**	**lroc**	**Sensitivity**	**Specificity**	**Study time-point**	**lroc**	**Sensitivity**	**Specificity**
NC + ALT + HOMA-IR	Baseline	0.79	63.6	74.5	Baseline	0.79	85	70.5
	6-month follow-up	0.79	28.5	98.4	6-month follow-up	0.83	42.1	94.2
	12-month follow-up	0.75	38.8	95.9	12-month follow-up	0.79	47.3	95.9
	24-month follow-up	0.85	56.2	95.0	24-month follow-up	0.89	68.1	91.4
NHtR + ALT + HOMA-IR	Baseline	0.81	70.4	78.4	Baseline	0.81	83.3	61.7
	6-month follow-up	0.82	28.5	98.3	6-month follow-up	0.87	52.6	94.2
	12-month follow-up	0.78	61.1	95.9	12-month follow-up	0.79	47.3	95.9
	24-month follow-up	0.88	56.2	95.0	24-month follow-up	0.90	68.1	88.5
NWtR + ALT + HOMA-IR	Baseline	0.79	95.9	80.3	Baseline	0.80	83.3	58.8
	6-month follow-up	0.79	28.5	100	6-month follow-up	0.81	47.3	96.1
	12-month follow-up	0.77	38.8	95.9	12-month follow-up	0.81	42.1	95.9
	24-month follow-up	0.84	50.0	92.5	24-month follow-up	0.88	59.1	88.5

NHtR: neck circumference to height ratio; NWtR: neck circumference to weight ratio

Table 2: Receiver operating characteristic curve (ROC) analysis considering the hepatic fat content as the binary dependent variable and neck and neck ratios combined with ALT and HOMA, Dixon as independent variables at baseline and all the study time-points (6, 12 and 24 months of follow-up).

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	Steatosis degree by ultrasonography			
Combination panels	**Study time-point**	**lroc**	**Sensitivity**	**Specificity**
NC + ALT + HOMA-IR	Baseline	0.78	59.4	83.0
	6-month follow-up	0.70	83.3	29.1
	12-month follow-up	0.74	79.0	57.1
	24-month follow-up	0.95	84.3	84.6
NHtR + ALT + HOMA-IR	Baseline	0.78	59.4	83.0
	6-month follow-up	0.73	85.7	50.0
	12-month follow-up	0.77	76.7	53.5
	24-month follow-up	0.97	93.7	92.3
NWtR + ALT + HOMA-IR	Baseline	0.76	45.9	86.4
	6-month follow-up	0.71	80.9	29.1
	12-month follow-up	0.75	76.7	57.1
	24-month follow-up	0.95	81.2	84.6

NC: neck circumference; NHtR: neck circumference to height ratio; NWtR: neck circumference to weight ratio

Table 3: Receiver operating characteristic curve (ROC) analysis considering the steatosis degree as the dependent variable (considering "0"= steatosis grade 1 and "1"= steatosis grades 2 and 3) and neck and neck ratios (NHtR and NWtR) combined with ALT and HOMA-IR as independent variables at baseline and all the study time-points (6, 12 and 24 months of follow-up).

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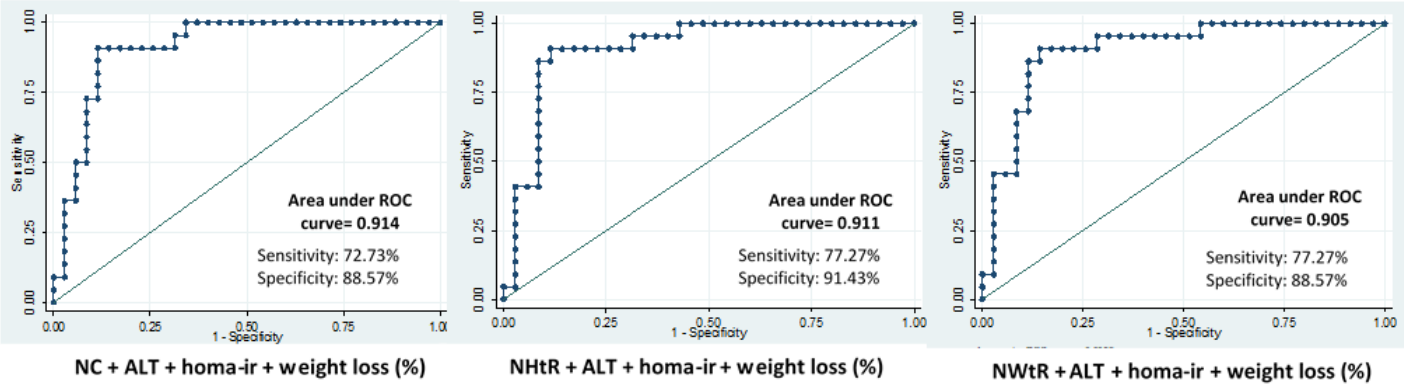

Figure 3: Receiver operating characteristic curve (ROC) analysis considering the hepatic fat content (MRI-histo) as the binary dependent variable and neck and neck ratios combined with ALT, HOMA-IR and total weight loss (%) as independent variables after 24 months of follow-up

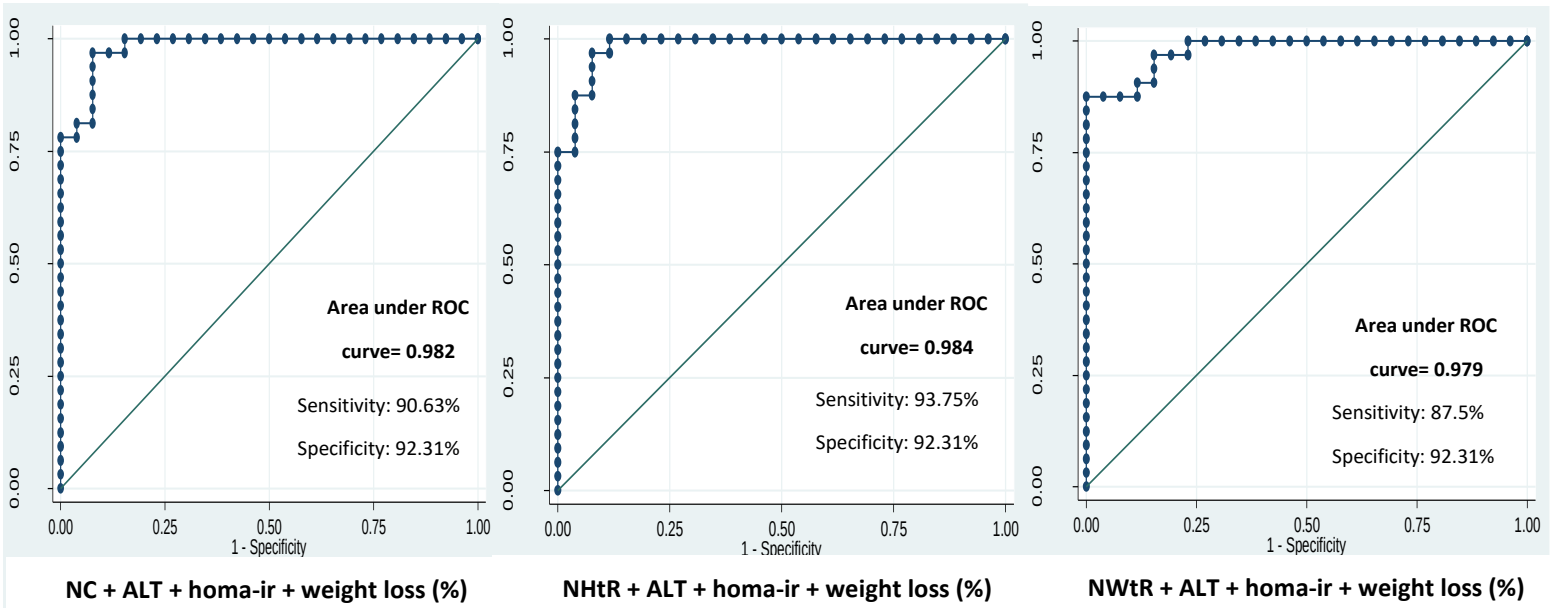

Figure 4: Receiver operating characteristic curve (ROC) analysis considering the steatosis degree as the binary dependent variable (0= grade 1; 1= grade 2+3) and neck and neck ratios combined with ALT, HOMA-IR and total weight loss (%) as independent variables after 24 months of follow-up.

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4. Discussion

This study is a randomized controlled trial that involved 98 patients with ultrasound-proven steatosis. All participants followed two different energy-restricted diets: the AHA and FLIO diets, both with a 30% of energy restriction. The intervention lasted 24 months with assessment visits at baseline, 6, 12 and 24 months. The most important finding obtained was those anthropometric and biochemical variables such as NC, NHtR or NWtR combined with ALT levels and HOMA-IR resulted in a combination panel able to predict the hepatic fat content and the steatosis degree at all the study time points. Moreover, this predictive ability was improved when the weight loss achieved during the nutritional intervention was also considered in the models. The utility of NC and neck ratios as viable and low-cost alternatives for the assessment of fat accumulation in the hepatic tissue has been also analyzed by other authors [17–19]. In a cross-sectional study where 2,761 subjects underwent a medical check-up including a physical examination, fasting blood samples and abdominal ultrasonography, authors analyzed potential relationships between NC and liver outcomes. NC in NAFLD patients was significantly wider than in patients with other metabolic conditions or healthy control [19]. More recent cross-sectional studies have also corroborated that NC is significantly associated with NAFLD and metabolic syndrome [11,20,21]. Interestingly a cross-sectional survey conducted among Chinese postmenopausal women with normal BMI showed that relatively large NC levels were associated with increased risk of NAFLD [22] and previously another cross-sectional survey on the prevalence of metabolic diseases and risk factors in East China in 2014 considered a total of 2,668 normal weight participants. Data showed that NC was an independent indicator for NAFLD in normal-weighted men [10].

NC has been suggested as an important and simple measurement reflecting the deposition of subcutaneous fat in the neck or fat surrounding the respiratory tract that can help to determine the degree of obesity, particularly upper body adiposity. The upper-body subcutaneous adipose tissue, estimated by NC, is a unique fat depot that confers additional metabolic risks beyond generalized and abdominal adiposity [18,20,21–24]. Likewise, NC, as well as neck ratios are strongly associated with insulin levels, HOMA-IR, lipid alterations and diabetes [17,21,25–27]. In the Framingham Heart Study, participants with large NC had various cardiometabolic risk factors when compared to those with small NC even after adjustment for visceral adipose tissue and BMI.

The Korean Genome and Epidemiologic Study observed that NC was associated with type 2 diabetes incidence. Participants in the highest NC quartile showed the highest diabetes incidence in comparison with participants from the other quartiles [28]. In the present work, the HOMA-IR was an important variable in the predictive model for liver fat and liver steatosis at all the study time points. The combination of HOMA-IR with ALT levels and NC or neck ratios improved the predictive ability than that observed with each variable separately. Insulin resistance is a known risk factor for NAFLD and the addition of the HOMA-IR to the model confirmed its important role in liver fat accumulation. NC is positively correlated to the body mass index, waist circumference and glycemia [29,30].

Lifestyle modification is established as the first-line treatment of NAFLD by scientific societies for the study of liver diseases. A healthy dietary intervention is essential to induce progressive weight loss, reduce liver fat accumulation and improve insulin resistance as well as associated metabolic comorbidities. The nutritional intervention program applied in the present work was based on an energy restriction of 30% of the total energy requirements and a high adherence to the Mediterranean diet. Data showed a relevant weight loss, especially after the first 6 months of intervention, which induced at the same time important liver fat reductions, transaminases modification and improvements in glucose profile. Except for NWtR, NC and NHtR were not importantly modified during the intervention, however, both measures were associated with the steatosis degree at all the study time points suggesting that NC and NHtR seem to be good indicators of hepatic fat accumulation. When associations were analyzed considering changes observed in neck

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measurements and liver fat content by MRI, we noticed that evolution of hepatic fat content during the intervention was associated with the changes registered in NC, NHtR and NWtR which corroborates that neck measurements could be sensitive markers of the hepatic status.

Receiver operating characteristic curve analysis showed that the combination of neck measurements with ALT and HOMA-IR was a good predictive panel of the steatosis degree and liver fat content, and interestingly, predictions were improved after the 24-month nutritional intervention program. When weight loss was added to the models, these were importantly improved showing an area under the ROC curve between 0.90 to 0.91 for the liver fat content and 0.97 to 0.98 for the steatosis degree. Neck circumference and neck ratios in addition to being important indicators of hepatic status could be also considered good markers for the monitoring of NAFLD subjects that are included in a nutritional intervention program. All the studies found in the scientific literature have a cross-sectional design; however, the present study is the first longitudinal study assessing the impact of a nutritional intervention program on NAFLD subjects.

The main underlying mechanisms suggested are that upper body obesity causes metabolic abnormalities, including increased circulating free fatty acids (FFAs). The excess FFAs may contribute to the development of fatty liver disease by contributing to triglyceride formation and storage in the liver (24). It has been described that the 59% of hepatic fat is derived from circulating FFAs, with lesser contributions from *de novo* lipogenesis (26%) and diet (15%). In addition, the excess FFAs may induce insulin resistance, which is thought to be related to the first "hit" in the multistep pathogenesis of NAFLD, and by increasing oxidative stress, thereby triggering the inflammatory response and progressive liver damage (18). More studies indicate that NC is closely correlated with glucolipid dysregulation, hyperinsulinemia, homeostasis model assessment of insulin resistance and other CVD risk factors (19). Therefore, the combination of NC or neck ratios with the HOMA-IR and ALT levels achieved a higher predictive ability for hepatic status than variables independently.

5. Conclusions

The present study shows that NC and neck ratios might be valuable predictors of fatty liver disease, being the NHtR the best of them, especially when combined with other important NAFLD risk factors like insulin resistance and ALT levels. More longitudinal studies should be performed to assess the validity and sensitivity of these simple and easy variables as surrogate markers of NAFLD since their application could improve the prevention and management of this prevalent disease.

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STATEMENTS AND DECLARATIONS

Author Contributions: Conceptualization, M.E., A.B.-B., I.A. and M.A.Z.; methodology, M.E., A.B.-B., B.A.M., I.A. and M.A.Z.: validation, M.E., A.B.-B., I.A. and M.A.Z.; formal analysis, M.E., A.B.-B., I.A. and M.A.Z.; investigation, M.E., A.B.-B., J.I.H., I.A. and M.A.Z.; resources, M.E., A.B.-B., J.I.H., I.A. and M.A.Z.; data curation, M.E., A.B.-B., B.A.M., I.A. and M.A.Z.; writing—original draft preparation, M.E., A.B.-B., I.A. and M.A.Z.; writing—review and editing, M.E., A.B.-B., N.P.-D.-d.-C., J.I.M, I.A. and M.A.Z.; visualization, M.E., A.B.-B., I.A. and M.A.Z.; supervision, M.E., A.B.-B., J.I.M., I.A. and M.A.Z.; project administration, J.A.T., J.A.M., I.A. and M.A.Z.; funding acquisition, J.A.T., J.A.M. and M.A.Z. All authors have read and agreed to the published version of the manuscript.

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