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Randomized Control Trials

Inflammatory markers as diagnostic and precision nutrition tools for metabolic dysfunction-associated steatotic liver disease: Results from the Fatty Liver in Obesity trial

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SUMMARY

Background & aims: Metabolic dysfunction-associated steatotic liver disease (MASLD) is a growing public health concern. The disease is silent, and its diagnosis is often delayed. Inflammatory markers constitute an interesting tool to act as subrogate, non-invasive markers. This study aimed to evaluate the changes of inflammatory markers throughout a two-year dietary intervention in subjects presenting MASLD, to determine which of the markers are suitable to predict the disease, and act as a customizing tool for MASLD's dietary treatment.

Methods: Ninety-eight subjects with MASLD and forty-five controls from the Fatty Liver in Obesity (FLiO) Study were analyzed. MASLD was diagnosed and graded by ultrasound. The MASLD subjects were randomly assigned to two different dietary strategies, the American Heart Association (AHA diet) or a dietary strategy based on the Mediterranean pattern, which was specially designed for the study (FLiO diet), and then followed for two years. Hepatic status was additionally assessed through Magnetic Resonance Imaging (MRI), elastography, and determination of transaminases.

Results & discussion: Inflammatory markers improved throughout the intervention in the MASLD subjects and managed to reach similar levels to controls, especially at 6 and 12 months. Additionally, leptin, adiponectin, M30, and LECT2 managed to significantly diagnose the disease at all time marks of the intervention, making them candidates for subrogate non-invasive markers of the disease. Moreover, baseline chemerin, leptin, LECT2, and M65 were used to build a predictive score to achieve greater weight loss, and therefore, which strategy could be more useful for MASLD 's treatment. The predictive score was significantly able assign a specific diet to 55% of the study participants, meaning that the remaining 45% could achieve the same amount of weight loss following either diet equally.

Conclusion: Inflammatory markers constitute a potential non-invasive tool to be used in MASLD screening and could also constitute an interesting tool for MASLD's treatment customization, being able to predict the effectiveness of a dietary strategy based on the initial inflammatory state of each subject.

Trial registration: www.clinicaltrials.gov (NCT03183193).

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1. Introduction

Metabolic dysfunction-associated steatotic liver disease (MASLD) is defined as the existence of hepatic steatosis that presents itself together with one cardiometabolic risk factor and the absence of excessive alcohol consumption or other discernible cause [1,2]. MASLD is currently considered the most common cause of steatotic liver, which at the same time is the most common cause of liver disease [2,3]. Among the pathophysiological mechanisms involved in its development, fatty acid accumulation in the liver parenchyma increased oxidative stress, and the activation of the inflammatory cascade stand out [4].

The disease spectrum encompasses both simple steatosis and Metabolic dysfunction-associated steatohepatitis (MASH), the inflammatory and most dangerous phenotype of the disease [1]. MASH dramatically increases the risk of developing liver fibrosis, cirrhosis, hepatocarcinoma, and end-stage liver disease [5,6]. Studies have suggested that inflammation precedes liver fibrosis and is an important pathophysiological mechanism inducing it [7]. Therefore, inflammation is a key component regulating not only the development of the disease but also its progress [7–9].

Currently, MASLD is considered a silent disease because it is usually not diagnosed until advanced stages of the disease [10,11]. Biopsy continues to be the diagnosis gold standard, but this technique is invasive and not used in regular clinical practice. Other imaging methodologies, such as echography and Magnetic Resonance Imaging (MRI) have also been proposed to diagnose the disease, however, these techniques are expensive [12]. The development and finding of non-invasive markers, which could be applied in clinical practice screenings continues to be a challenge [12,13].

Moreover, the primary treatment for this disease according to the American Association for the Study of Liver Diseases (AASLD) consists of lifestyle and dietary interventions that lead to weight loss. In this sense, weight loss percentages of 3–5% of an individual's initial body weight are said to ameliorate steatosis, while percentages ranging from 7 to 10% could even improve liver fibrosis [14,15].

Different dietary strategies have been proposed to achieve the needed weight loss and, therefore, treat MASLD. Among the most known strategies are the Mediterranean diet, the American Heart Association (AHA), and high-protein diets, among others [16]. Contrarily, unhealthy dietary habits [17,18] and high intake of processed foods, rich in sugars and saturated fats encourages the secretion of inflammatory factors, which contribute to the dysfunction in energy, glucose, and lipid homeostasis [19,20]. Nevertheless, evidence regarding the effects of specific diets, especially from longitudinal studies, is scarce. More information is needed to draw conclusions on the effect of diet on the inflammatory state and liver status.

Given the previously exposed facts, this study aimed to evaluate the changes of inflammatory markers throughout a two-year dietary intervention in subjects presenting MASLD, as well as to determine which markers are suitable to predict the disease and act as a customizing tool for MASLD ‘s dietary treatment.

2. Material and methods

2.1. Study design and participants

This study includes data from the FLiO (Fatty Liver in Obesity) study (NCT03183193), a randomized controlled clinical trial where 98 adults with MASLD, diagnosed by ultrasonography, and who were obese or overweight (BMI between 27.5 kg/m2 and 40 kg/m2), were randomized and assigned to one of two different dietary strategies (Fig. 1). Exclusion criteria included elevated alcohol consumption (>21 units of alcohol per week for men and >14 units for women), presence of known liver disease, endocrine disorders (hyperthyroidism or uncontrolled hypothyroidism), weight loss >3 Kg in the past 3 months, the use of weight modifiers, severe psychiatric disorders, active autoimmune diseases, acute infections, pharmacological treatment with drugs that could cause hepatic steatosis or alteration in hepatic tests (immunosuppressants, cytotoxic agents, corticosteroids), the inability to follow the diet (food allergies/intolerances) and difficulty in following the scheduled visits. None of the MASLD or control subjects took pioglitazone, vitamin E, or other drugs that could affect their steatosis degree. However, 4 subjects from the AHA group and 3 subjects from the FLiO group took metformin. None of the control individuals took metformin. The changed in medications were negligible in this study as previously reported [21].

The power and sample size for this study was calculated based on a previous nutritional intervention done by the Centre for Nutrition Research of the University of Navarra (CIN) [22], taking weight loss as the primary outcome given the current AASLD guideline recommendations for MASLD patients. The sample size was calculated to detect a difference in the reduction of weight of 1.5 Kg between the dietary interventions (AHA vs. FLiO) [21], with a statistical power of 80% ($\beta = 0.8$) and a 95% Confidence Interval ($\alpha = 0.05$). This approach estimated a total sample size of 36 subjects per group. According to the researcher's experience, a 35–40% dropout rate was considered, therefore estimating a sample size of 50 participants per group. Two individuals from the AHA group were excluded for presenting significant alterations in their initial biochemical evaluations. In consequence, the FLiO group started with 50 individuals while the AHA group started with 48 (Fig. 1). The randomization of the subjects was done by a physician who was not involved in the study, and allocation was done using a computer program that generated random numbers. More information regarding the study protocol can be found elsewhere [21,23].

Additionally, a control group was recruited for a continuation of the project while the MASLD subjects completed the 24-month dietary interventions. This group consisted of 45 individuals who presented the same age range and sex distribution as the subjects with MASLD but were specifically selected for not presenting the disease according to the ultrasonography and had normal weight (BMI <25 kg/m2). Controls were recruited by the same team at the Centre for Nutrition Research of the University of Navarra and were subjected to the same tests and determinations than the MASLD subjects at the Clínica Universidad de Navarra.

Control subjects were evaluated at baseline. Conversely, the MASLD subjects were followed for two years, with visits at baseline, 6 months, 12 months, and 24 months of the intervention. In these visits, metabolic, hepatic, biochemical, and dietary variables were measured.

The study protocol was approved by the Research Ethics Committee of the University of Navarra (ref. 54/2015). All the procedures were done in accordance with the declaration of Helsinki and the CONSORT 2010 guidelines. All the participants gave written informed consent prior to their inclusion in the study. No harms were reported in the study.

2.2. Dietary interventions

The first strategy was based on the American Heart Association (AHA) guidelines, while the second strategy, named FLiO diet, was an adapted Mediterranean-based diet. Both diets had the same 30% caloric restriction extracted out of the energy requirements, calculated with the Institute of Medicine equation [24].

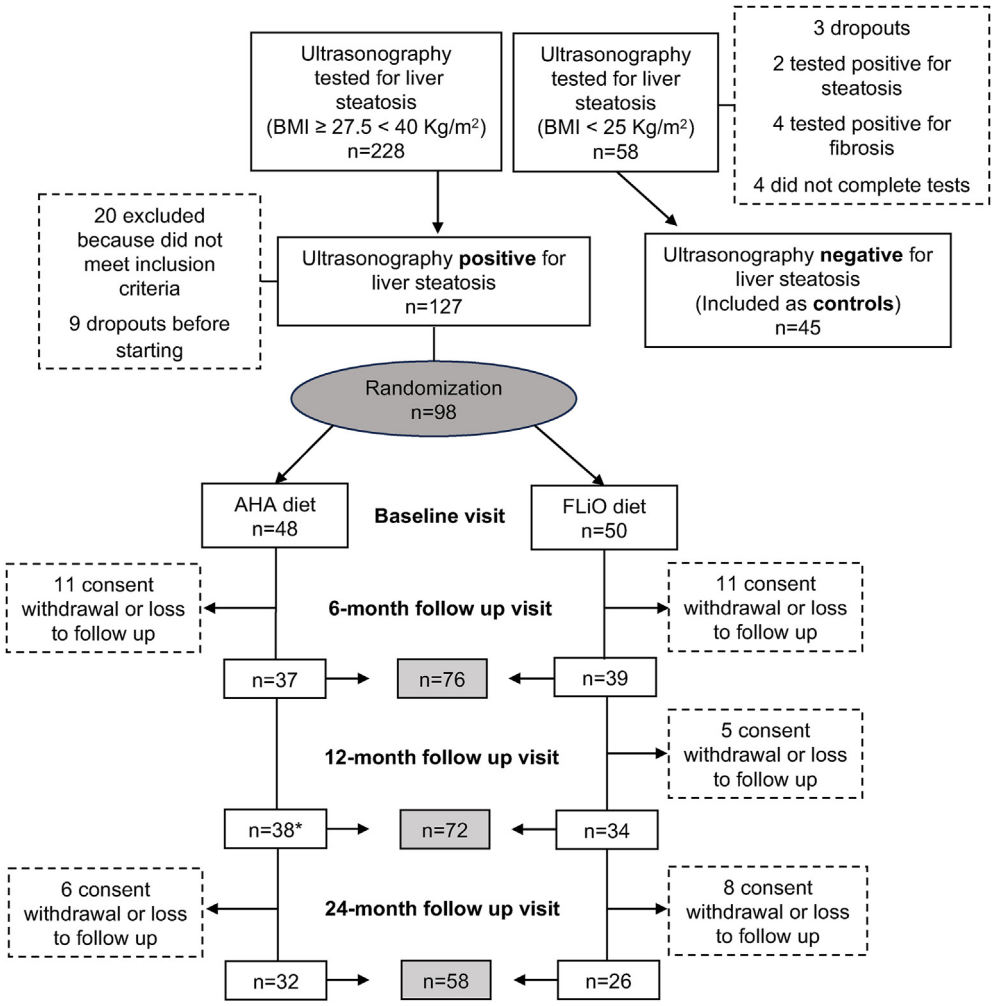

Fig. 1. Study Flowchart showing the screening, selection, and follow up process for the volunteers. MASLD volunteers were randomized into two different dietary strategies, the AHA diet, and the FLiO diet. Subjects were measured at baseline, 6 months, 12 months, and 24 months of the intervention. Anthropometric and body composition, hepatic status, inflammatory status, and general biochemical measurements from all volunteers were taken at each visit.

Nevertheless, the diets had different macronutrient distribution and number of meals per day.

The AHA diet prescribed 3–5 meals per day and had a macronutrient distribution respecting the total energy intake of 55% carbohydrates, 15% protein, and 30% of lipids. Conversely, The FLiO diet promoted high adherence to the Mediterranean Diet and consumption of natural antioxidants. It prescribed 7 meals per day with adequate size and composition suitable for each moment. Moreover, the FLiO diet had a macronutrient distribution respecting the total energy intake of 45%–40% in the form of carbohydrates (from low-glycemic sources), 25% proteins (preferably from vegetable sources), and 30–35% of lipids (promoting the consumption of extra virgin olive oil and omega 3 fatty acids in detriment of saturated fats, trans fat, and cholesterol).

Both strategies were personalized and based on an exchange system, which allowed more freedom to the participants and enabled them to choose their preferred food items as long as they followed their respective strategies and study conditions. This was done to encourage volunteer's adherence to their intervention. Regardless of their assigned strategy, each volunteer was given 7-day menu models, an explanation of the nutritional intervention assigned, overall healthy living recommendations for MASLD subjects, a list of foods to avoid, and a series of recommendations in case they had to eat outside of their homes. Moreover, each participant was followed by the same dietitian throughout the two-year intervention.

The dietary intervention had a duration of two years, with the following visits at 6, 12, and 24 months [21]. Compliance to the interventions and dietary intake was assessed through validated Food Frequency Questionnaires (FFQ) [25] at every visit of the study. The questionnaires were completed by each participant with the assistance of the study's dietitian. Mediterranean Diet Adherence was evaluated through a 17-point screening questionnaire [26,27], while physical activity was assessed with the Spanish Minnesota Leisure Time questionnaire [27,28].

2.3. Anthropometric and body composition measurements

Anthropometric measurements were collected by trained dietitians of the Center for Nutrition Research (Metabolic Unit) of the University of Navarra, as previously described [21]. Body Mass Index (BMI) was calculated as the body weight of the subject measured in Kilograms divided by the squared height measured in meters (Kg/m2). Body composition, including Visceral Adipose Tissue (VAT), was determined by dual-energy X-ray absorptiometry (DXA) following the instructions of the manufacturer (Lunar iDXA, enCORE 14.5, Madison, WI).

2.4. Biochemical and inflammatory markers determinations

Blood samples were collected under 8–10 h of fasting conditions, as previously described [22]. The samples were processed to obtain biochemical determinations in the biochemistry laboratory of the University of Navarra Clinic (CUN). Blood triglycerides (TG), total cholesterol, high-density lipoprotein cholesterol (HDL-c), glucose, and insulin were quantified on an autoanalyzer with specific commercial kits and following the instructions of the company (Cobas 8000, Roche Diagnostics, Switzerland). Low-density lipoprotein cholesterol (LDL-c) was calculated using the Friedewald formula [29]. The Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) was calculated as (fasting insulin (μU/mL) x fasting glucose (mmol/L))/22.5.

The inflammatory status of the subjects was assessed through the analysis of specific markers in previously collected blood samples that were conveniently stored at −80 °C until the analyses. Cytokeratin-18 (CK-18) antigens, M30 and M65, which are considered subrogate hepatic fibrosis markers [30] were quantified with an enzyme-linked immunosorbent assay (ELISA) commercial kit (PEVIVA, Bromma, Sweden) according to the manufacturer's instructions. C-reactive Protein (CRP), Retinol Binding Protein 4 (RBP4), leptin, adiponectin, and chemerin were also analyzed and quantified using specific ELISA kits (Demeditec; Kiel-Wellsee, Germany) in a Triturus autoanalyzer (Grifols, Barcelona, Spain) at the Laboratory of Biochemistry of the Center for the Nutrition Research at the University of Navarra (CIN, Pamplona, Spain). Leukocyte cell-derived chemotaxin-2 (LECT2) was analyzed and quantified using the same Triturus autoanalyzer with specific kits for this chemotaxin (Biovendor LLC, North Carolina, USA). Ferritin levels were externally analyzed by a certified laboratory (Eurofins Megalab S.A, Madrid, Spain) using a chemiluminescent microparticle immunoassay (CMIA) technology (Abbott Architect Ferritin Assay). M30 and M65, which are considered hepatic fibrosis markers were quantified with an enzyme-linked immunosorbent assay (ELISA) method using commercial kits (PEVIVA, Bromma, Sweden) according to the manufacturer's instructions.

2.5. Liver status assessment

The entire hepatic assessment was performed by qualified professionals of the CUN under fasting conditions. The diagnosis of MASLD was determined by ultrasonography (Siemens ACUSON S2000 and S3000), classifying the subjects into groups: 0 (controls) and ≥1 (MASLD). The steatosis degrees diagnosed through ultrasonography are classified as: 0 (liver fat <5%); 1 (mild steatosis, liver fat = 5%–33%); 2 (liver fat = 34%–66%); 3 (liver fat >66%) [31,32]. MRI (Siemens Aera 1.5T) determined liver fat content by the Dixon technique. Acoustic Radiation Force Elastography (ARFI) and Transient Elastography (TE) were performed to assess liver stiffness as a subrogate marker for liver fibrosis, as described elsewhere [21]. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyl transferase (GGT) were quantified on an autoanalyzer with specific commercial kits and following the instructions of the company (Cobas 8000, Roche Diagnostics, Switzerland). The Fatty Liver Index (FLI) was computed using a previously described formula, that considers serum triglycerides, BMI, waist circumference, and GGT concentrations to determine the probability of suffering MASLD [33].

2.6. Statistical analyses and decision model

All statistical analyses were performed in R Studio (Version 2023.09.0 + 463). Statistically significant effects and differences were considered with p-values <0.05. Variable distribution was evaluated using Shapiro-Wilk tests, and parametrical or non-parametrical tests were used if the variables presented normal distribution or not, respectively. Linear Mixed Models from thelmer package (CRAN) were used to evaluate group-dependent longitudinal changes (differences in changes between groups) of the anthropometric, hepatic, biochemical, and inflammatory parameters. Baseline measurements were compared to 6-, 12-, and 24-month measurements of each variable. Contrast analyses were performed with the emmeans package (CRAN) to evaluate the changes with time within the group. Wilcoxon tests were used to compare differences with the control group.

Univariate and multivariate logistic regressions were performed to evaluate the predictive capacity of inflammatory markers to diagnose MASLD. For this, the presence of MASLD according to ultrasonography results was used as the dependent variable. Univariate regressions with all the inflammatory markers were performed at every time point of the study. Afterward, those that presented statistical significance at the four-time points were selected and used in the multivariate logistic regression, which was additionally adjusted by sex, age, and physical activity. Receiver Operating Characteristic Curve Analyses (ROC) and the areas under the ROC curve (AUROC) with their confidence intervals were calculated to evaluate the power of prediction and the diagnostic performance of said regression models. Collinearity and the Hosmer-Lemeshow goodness-of-fit test were assessed after each logistic regression to evaluate their efficacy.

To evaluate the capacity and use of inflammatory markers in precision nutrition and in the efficacy of the dietary treatments, a decision model was created using baseline measurements of the markers. To create the decision model, we considered weight loss percentage as the dependent outcome variable, since this is the main desirable outcome to ameliorate MASLD proposed by AASLD guidelines. Weight loss percentage was calculated as the percentage lost between baseline and 6 months measurements, since at this time the participants of the study lost the greatest amount of initial their weight. The reason why BMI loss percentage was not used as the dependent variable is because, as seen in Table 1, results are the same as weight loss percentage, since the variable part of BMI throughout the study time is precisely weight and not height.

For the first step to create the decision model, the study population was divided according to their dietary strategy, either AHA or FLiO diet. Stepwise linear regressions based on the Akaike Information Criterion (AIC) were then calculated for both the AHA and FLiO diets, using weight loss percentage as the dependent variable, and baseline inflammatory markers as independent ones. This method is used to select those variables who contribute in a more significant way to the study outcome. Collinearity of each model was evaluated.

The second step for building the model consisted in extracting a selection of variables from each stepwise model. The markers selected for the sub-scores presented a positive or negative association with weight loss in one of the groups, but an inverse or no association in the other group. These inverse associations are crucial to achieving the interaction term between baseline inflammation sub-scores and diet, which allows the deduction of the individualized treatment effects.

For the third step, the β-coefficients for each inflammatory marker obtained from the stepwise regression were used to construct the inflammation sub-scores for each diet (AHA and FLiO). Lastly, an overall inflammation score was created by subtracting one of the scores from the other, as seen in Table 5.

Finally, for the four and final step to construct the decision model, a Linear Mixed Model was performed with thelmerpackage to predict weight loss according to the total inflammation score. This model included the interaction term between diet and

Table 1
Changes in anthropometric, biochemical, and hepatic markers between AHA and FLiO strategies throughout the 2-year intervention.

	CONTROLS	MASLD SUBJECTS						AHA vs. FLiO		
		AHA (n = 48)			FLiO (n = 50)					
	Baseline (n = 45)	Δ 6 months (n = 37)	Δ 12 months (n = 38)	Δ 24 months (n = 32)	Δ 6 months (n = 39)	Δ 12 months (n = 34)	Δ 24 months (n = 26)	Pvalue[a]	Pvalue[b]	Pvalue[c]
Body weight (kg)	65.61 (12.01)	−9.16 (5.03)***	−6.37 (6.32)***	−4.70 (6.85)***	−9.83 (6.71)***	−9.34 (7.11)***	−7.31 (5.82)***	NS	0.098	NS
Weight loss (%)	NA	9.67 (5.01)***	6.74 (6.51)***	4.83 (7.28)***	10.08 (6.45)***	9.61 (6.97)***	7.55 (5.80)***	NS	NS	NS
BMI (kg/m2)	23.27 (2.46)	−3.22 (1.64)***	−2.18 (2.09)***	−1.53 (2.31)***	−3.42 (2.33)***	−3.24 (2.45)***	−2.49 (2.00)***	NS	0.080	NS
BMI loss (%)	NA	9.67 (5.01)***	6.74 (6.51)***	4.83 (7.28)***	10.08 (6.45)***	9.61 (6.97)***	7.55 (5.80)***	NS	NS	NS
Waist circunference (cm)	78.78 (9.10)	−9.23 (6.65)***	−7.56 (7.71)***	−2.83 (8.28)	−9.58 (6.38)***	−10.61 (6.66)***	−6.67 (6.35)***	NS	0.053	**0.021**
VAT (kg)	0.5 (0.4)	−0.81 (0.59)***	−0.57 (0.72)***	−0.39 (0.65)**	−0.62 (0.52)***	−0.62 (0.49)***	−0.38 (0.57)**	NS	NS	NS
Lean Body Mass (kg)	44.01 (11.63)	−1.78 (1.52)***	−1.52 (1.91)***	−1.85 (2.11)***	−1.98 (1.54)***	−2.11 (1.62)***	−2.27 (1.93)***	NS	NS	NS
Body Fat (%)	19.30 (5.70)	−4.51 (3.37)***	−2.97 (3.60)***	−1.55 (3.24)	−4.27 (4.10)***	−3.81 (4.35)***	−1.85 (4.13)	NS	NS	NS
Glucose (mg/dL)	90.73 (6.49)	−11.14 (12.51)***	−10.08 (14.61)***	−9.19 (18.29)**	−7.79 (10.01)***	−9.94 (11.04)***	−8.08 (11.09)**	NS	NS	NS
Insulin (mU/L)	4.16 (1.79)	−6.41 (7.55)***	−4.88 (6.77)***	−4.81 (7.17)**	−5.92 (7.94)***	−4.09 (5.83)**	−5.64 (5.46)***	NS	NS	NS
HbA1c (%)	5.14 (0.38)	−0.22 (0.40)*	−0.12 (0.45)	−0.10 (0.43)	−0.18 (0.44)**	−0.21 (0.53)*	−0.08 (0.43)	NS	NS	NS
Cholesterol (mg/dL)	218 (40.20)	−8.19 (28.50)	−9.13 (29.26)	2.53 (43.75)	−12 (36.71)	−17.06 (27.24)	−5.15 (25.66)	NS	NS	NS
LDL-c (mg/dL)	133.51 (4.81)	−5.12 (27.29)	−7.71 (28.53)	3.12 (35.60)	−5.87 (31.50)	−13.28 (22.24)	−3.48 (22.57)	NS	NS	NS
HDL-c (mg/dl)	67.76 (17.65)	2.14 (7.04)	1.29 (8.98)	−0.37 (14.98)	1.79 (9.46)	4.04 (8.34)	3.88 (8.73)	NS	NS	NS
Triglycerides (mg/dL)	83.57 (38.16)	−27.41 (52.82)*	−13.63 (45.69)	−0.81 (71.80)	−42.95 (65.22)***	−42.82 (62.04)***	−20.08 (59.01)	NS	**0.039**	NS
HOMA-IR	0.94 (0.43)	−1.97 (2.33)***	−1.50 (2.05)***	−1.55 (2.18)***	−1.68 (2.10)***	−1.22 (1.69)**	−1.55 (1.54)***	NS	NS	NS
ALT (IU/L)	21.49 (14.34)	−12.16 (13.22)***	−9.53 (17.64)***	−2.72 (15.76)	−9.76 (15.66)***	−8.74 (16.13)***	−9.35 (15.60)***	NS	NS	NS
AST (IU/L)	23.63 (10.92)[#]	−5.51 (10.67)**	−1.53 (13.85)	0.31 (6.53)	−1.76 (9.49)	−3.65 (7.33)	−1.08 (6.84)	NS	NS	NS
GGT (IU/L)	27.26 (38.48)	−15.47 (27.40)**	−13.32 (24.91)*	−4.72 (34.14)	−7.45 (35.71)	−9.26 (14.16)	−10.46 (18.57)	NS	NS	NS
FLI Index	17.00 (18.10)	−24.14 (17.75)***	−17.66 (17.31)***	−11.03 (21.18)**	−31.93 (20.68)***	−28.95 (21.77)***	−21.76 (18.92)***	0.060	**0.019**	0.054
Steatosis degree	0 (0)	−0.68 (0.67)***	−0.55 (0.69)***	−0.56 (0.62)***	−0.72 (0.73)***	−0.85 (0.80)***	−0.85 (0.73)***	NS	NS	NS
Hepatic volume (mL)	1312.34 (225.26)	−159.82 (201.08)**	−138.12 (208.46)**	−72.56 (289.86)	−172.42 (277.74)***	−168.28 (325.1)***	−97.35 (173.86)**	NS	NS	NS
Hepatic fat (%)	3.62 (1.93)	−3.96 (5.04)***	−2.50 (4.53)***	−0.12 (4.09)	−3.59 (3.03)***	−1.22 (3.34)	−1.09 (2.84)	NS	NS	NS
ARFI stiffness (m/s)	1.25 (0.32)	0.31 (0.13)	0.22 (0.13)	0.05 (0.14)	−0.14 (0.13)	0.16 (0.13)	0.14 (0.14)	**0.017**	NS	NS
TE stiffness (kPa)	4.26 (0.8)[#]	−0.25 (0.47)	−0.12 (0.48)	−0.57 (0.49)	−0.23 (0.46)	−0.42 (0.48)	−0.90 (0.54)	NS	NS	NS

Data from controls represents mean (sd) at baseline. NA, Not Applicablle. #No significant differences with the MASLD groups at baseline. Data from MASLD subjects is represented as mean change between baseline and 6, 12, and 24 months, and standard deviation (sd). Significant differences among the comparisons within AHA and FLiO diets: *P < 0.05; **P < 0.005; ***P < 0.001. Comparisons of the changes between AHA and FLiO groups: [a]Baseline vs. 6 months; [b]Baseline vs. 12 months; [c]Baseline vs. 24 months. NS states non-significant results for those p-values of less than 0.10. Abbreviations: ALT, Alanine aminotransferase; ARFI, Acoustic Radiation Force elastography; AST, Aspartate aminotransferase; BMI, Body Mass Index; FLI, Fatty Liver Index; GGT, Gamma-glutamyl transferase; HbA1c; Glycated Hemoglobin; HDL, High Density Lipoprotein; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; LDL, Low Density Lipoprotein; TE, Transient elastography; VAT, Visceral Adipose Tissue.

inflammation score and was additionally adjusted by sex, age, and physical activity. As previously stated, the interaction term is deliberately sought to select a specific diet for greater weight loss results.

3. Results

3.1. Changes between and within groups throughout a 2-year dietary intervention

No statistically significant differences were found regarding the age of the control and MASLD subjects. At baseline, the mean age of the FLiO group was 49.2 (±8.9) years, the mean age AHA group was 51.1 (±9.8) years, and the mean age in the controls was 48.8 (±5.5) years. The sex distribution for the AHA, FLiO, and control groups was 20 women/28 men, 23 women/27 men, and 29 women/16 men, respectively.

Additionally, no differences were found at baseline between AHA and FLiO groups in metabolic, biochemical, or hepatic variables. The AHA and FLIO groups started the intervention with a mean BMI (Kg/m^2) of 33.7 (±4) Kg/m2, and 33.3 (±4) Kg/m^2, respectively.

Moreover, at baseline, the steatosis degree determined by ultrasonography of the AHA group was 1.5 (±0.7); while the FLiO group was 1.5 (±0.6). MRI was also used to determine more accurate liver fat percentage. The mean liver fat percentage at baseline of the AHA and FLiO groups were 7.4%, (±5) and 7.0% (±5), respectively. The differences between the controls and MASLD subjects were statistically significant (Table 1).

Both dietary interventions were successful in ameliorating metabolic, biochemical, and hepatic parameters in all visits (6, 12, and 24 months) compared to the baseline (Table 1). These changes were notably superior at 6 months, where weight loss percentage, which is the main desired treatment outcome according to AASLD guidelines, reached its peak with a mean of 9.67% and 10.08% in AHA and FLiO groups, respectively. Furthermore, the changes in hepatic parameters were not only statistically significant but also clinically relevant, given the fact that several subjects reached a degree of steatosis equal to zero, especially at the 6-month visit, pointing out that they lowered their liver fat percentage to less than 5%. These results can also be corroborated with those observed with MRI [21].

There were no differences in lifestyle or dietary variables between the AHA and FLiO groups at baseline. Nevertheless, differences between groups regarding dietary intake were found, especially at 6 and 12 months of the intervention. Concretely, FLiO subjects had a higher intake of proteins and Mediterranean Diet adherence at 6 and 12 months of the intervention, and a higher intake of polyunsaturated fatty acid at 6, 12, and 24 months of the intervention. On the other hand, the AHA group subjects consumed more carbohydrates and their diets had higher glycemic load at 6 and 12 months of the intervention. No differences were found regarding the physical activity of the subjects.

More information regarding the baseline and longitudinal dietary, hepatic, and general characteristics of MASLD and control subjects can be found elsewhere [21,34].

Results were similar in both groups for most variables at all measures. Nevertheless, the FLiO group's change in several variables, such as waist circumference, triglycerides, and FLI index, was significantly greater than the AHA group (Table 1).

As shown in Table 2, inflammatory markers clearly differed between the controls and both MASLD groups, but no differences were found between the AHA and FLiO group at baseline. Nevertheless, most of the inflammatory markers improved significantly throughout the study, especially at 6- and 12-month marks. Among the markers that changed at 6 months were ferritin, chemerin, LECT2, RBP4, leptin, M30 and M65. Adiponectin also improved but its change was superior in the FLiO group. At 24 months, RBP4 was the only inflammatory marker that significantly changed in both groups. Decreased ferritin and adiponectin were statistically significant only in the FLiO group, while statistically significant increased chemerin was statistically significant only in the AHA group.

The markers that reached control-like levels at 6 months were chemerin, RBP4, and leptin in both groups. Ferritin reached control-like levels only in the FLiO group and M65 reached control levels in the AHA group as well, but the FLiO group's M65 levels were even lower than those observed in the controls. The markers that reached control-like levels at 12 months were ferritin, chemerin, RBP4, and M65 in both groups, while leptin also reached control-like levels but only in the FLiO group. At 24 months, the markers that reached normal levels were ferritin, RBP4, and M65 in the FLiO group.

Additionally, the levels of adiponectin, and then both chemerin and adiponectin demonstrated a significant interaction among groups at the 6-month, and 24-month mark, respectively. Regarding the behavior of these two markers, chemerin seems to have increased significantly more in the AHA diet subjects compared to FLiO. Conversely, adiponectin seems to have increased significantly more in the FLiO subjects compared to the AHA ones.

3.2. Evaluating the use of inflammatory markers as non-invasive diagnostic tools for MASLD

As seen in Table 2 most inflammatory markers improved along the metabolic outcomes with dietary intervention. Given the literature background on the role of inflammation in the pathophysiology of MASLD, this study aimed to find markers that could serve as non-invasive tools for the diagnosis of the disease. To prove this, the first step taken was to perform univariate logistic regressions at every time mark between the presence of the disease diagnosed with echography (dependent variable) and the inflammatory markers as independent ones (Supplementary Table 1). Among the markers that had a common significant effect at every time mark of the study were adiponectin, leptin, M30, and LECT2. Given these results, those four markers were selected for multivariate logistic regressions adjusted by sex, age, and physical activity (Table 3).

As shown in Table 3, all regressions were statistically significant with pseudo-R2 varying from 0.94 to 0.86 (corrected pseudo-R2 varying from 0.82 to 0.70). These results indicate that the four selected markers could be used as non-invasive diagnostic tools at every time mark in this study.

Additionally, ROC curves were performed after these regressions to evaluate the diagnostic capacity of said models, obtaining AUROC values that ranged between 0.983 and 0.948. These curves can be observed in Fig. 2.

3.3. Specific dietary treatment decision model for subjects with MASLD based on their initial inflammatory state

To evaluate the role of inflammation in precision medicine and, more specifically, precision nutrition, a decision model evaluating baseline inflammatory markers was created to predict which dietary strategy could be more beneficial to everyone. The main objective was to assess which of the two diets (AHA or FLiO) is the most suitable to achieve the greater amount of weight loss, which is the main desired treatment objective according to AASLD guidelines for MASLD treatment. The weight loss percentage outcome was calculated using the weight loss percentage from each subject

Table 2
Evolution of inflammatory markers between AHA and FLiO strategies throughout the 2-year intervention and comparison with controls.

	CONTROLS (n = 45)	MASLD SUBJECTS								AHA vs. FLIO		
		AHA				FLiO						
		Baseline (n = 48)	6 months (n = 37)	12 months (n = 38)	24 months (n = 32)	Baseline (n = 50)	6 months (n = 39)	12 months (n = 34)	24 months (n = 26)	Pvalue[a]	Pvalue[b]	Pvalue[c]
CRP (mg/dl)	0.09 (0.16)	0.65 (1.85)	0.33 (0.41)	0.26 (0.34)	0.34 (0.54)	0.4 (0.59)	0.18 (0.16)	0.27 (0.81)	0.19 (0.2)	NS	NS	NS
Ferritin (ng/ml)	76.01 (63.17)	151.51 (140.84)	133.67 (122.15)*	84.32 (81.24)***#	135.38 (105.42)	139.94 (116.68)	114.54 (100.29)#	69.04 (62.77)***#	98.1 (89.34)**#	NS	NS	NS
Chemerin (ng/ml)	186.51 (79.71)	214.77 (35.01)	196.03 (36.19)#	188.74 (46.83)**#	239.5 (55.74)*	209.38 (38.57)	191.28 (33.11)#	180.32 (30.23)**#	207.22 (37.28)	NS	NS	**0.005**
RBP4 (mg/l)	26.69 (7.03)	32.85 (7.82)	27.08 (17.66)*#	26.24 (5.79)**#	25.76 (6)**	37.74 (11.07)	30.46 (15.81)***#	24.64 (6.16)***#	24.08 (7.27)***#	NS	NS	NS
Leptin (ng/ml)	14.14 (11.52)	37.14 (27.02)	22.3 (18.97)***#	23.15 (16.76)***	27.06 (17.75)	38.76 (31.14)	23.29 (20.3)***#	20.26 (14.8)***#	28.97 (16.54)	NS	NS	NS
Adiponectin (ug/ml)	14.02 (5.72)	6.66 (2.16)	7.95 (3.04)***	8.32 (3.52)***	8.36 (3.2)	6.64 (2.25)	9.47 (3.72)***	8.82 (3.31)***	10.62 (3.43)***	**0.035**	0.529	**0.009**
LECT2 (ng/ml)	23.83 (9.04)	40.86 (10.2)	36.04 (9.08)**	38.04 (10.93)	43.49 (10.03)	42.26 (10.07)	38.44 (8.88)*	37.65 (9.39)*	39.85 (8.08)	NS	NS	NS
M30 (U/L)	40.77 (23.36)	93.1 (56.19)	69.27 (35.36)	132.3 (69.73)*	95.55 (81.08)	95.86 (85.83)	72.46 (48.39)	140.51 (106.3)***	66.24 (46.87)	NS	NS	NS
M65 (U/L)	114.70 (67.09)	135.09 (72.51)	93.67 (54.4)*#	123.92 (83.23)#	128.34 (69.28)	159.92 (116.02)	83.45 (46.77)***	126.23 (89.5)#	111.49 (48.53)#	NS	NS	NS

Data represents mean (sd). Significant differences among the comparisons within AHA and FLiO diets: *P < 0.05; **P < 0.005; ***P < 0.001. Comparisons of the changes between AHA and FLiO groups: [a]Baseline vs. 6 months; [b]Baseline vs. 12 months; [c]Baseline vs. 24 months. #No significant differences with the control group. NS states non-significant results for those p-values of less than 0.10. Abbreviations: CRP, C-reactive protein; LECT2, Leukocyte cell-derived chemotaxin-2; RBP4, Retinol Binding Protein 4. M30 and M65 are cytokeratin 18 (CK-18) antigens.

Table 3
Multivariable Logistic regressions between inflammatory markers (leptin, adiponectin, M30, and LECT2) and presence of MASLD (as dependent variable).

	Baseline			6 months			12 months			24 months		
Pseudo-R2	0.96			0.93			0.95			0.93		
Corrected R2	0.88			0.81			0.85			0.83		
p-value	<0.001			<0.001			<0.001			<0.001		
AUC (95% CI)	0.995 (0.988−1.00)			0.952 (0.912−0.992)			0.989 (0.975−1.00)			0.975 (0.950−1.00)		
	OR	OR 95% CI	Pvalue	OR	OR 95% CI	Pvalue	OR	OR 95% CI	Pvalue	OR	OR 95% CI	Pvalue
Adiponectin	0.41	(0.16−0.69)	0.014	0.72	(0.58−0.86)	<0.001	7.59^{-1}	(5.45^{-5}-0.46)	0.050	0.76	(0.54−0.97)	0.056
Leptin	1.37	(1.11−2.10)	0.035	1.03	(0.98−1.10)	0.226	1.10	(0.98−1.28)	0.113	1.11	(1.01−1.25)	0.041
M30	1.05	(1.01−1.14)	0.040	1.05	(1.02−1.09)	<0.001	1.07	(1.04−1.13)	<0.001	1.03	(1.01−1.06)	0.034
LECT2	1.27	(1.10−1.73)	0.022	1.18	(1.09−1.30)	<0.001	1.16	(1.04−1.39)	0.024	1.23	(1.10−1.43)	<0.001
Sex	7.47^{-4}	(1.70^{-8}-0.14)	0.060	0.29	(0.04−1.63)	0.174	1.12	(5.13^{-5}-0.46)	0.042	0.03	(5.30^{-4}-0.71)	0.059
Age	1.21	(1.02−1.62)	0.074	1.11	(1.01−1.23)	0.027	1.06	(0.91−1.27)	0.448	1.05	(0.93−1.22)	0.373
Physical activity	8.54	($1.22\text{-}3.16^{-12}$)	0.102	0.80	(0.38−1.68)	0.567	1.38	(0.47−4.98)	0.568	1.12	(0.39-3-35)	0.827

Data represents Odds Ratio (OR), Pseudo R-squared and corrected pseudo-R-squared, and p-values obtained through the multivariate logistic regressions. The regressions are adjusted by sex, age, and physical activity. Abbreviations: AUROC, Area Under the Curve; CI, Confidence Interval (95%); LECT2, Leukocyte cell-derived chemotaxin-2; OR, Odds Ratio. M30 is a cytokeratin 18 (CK-18) antigen.

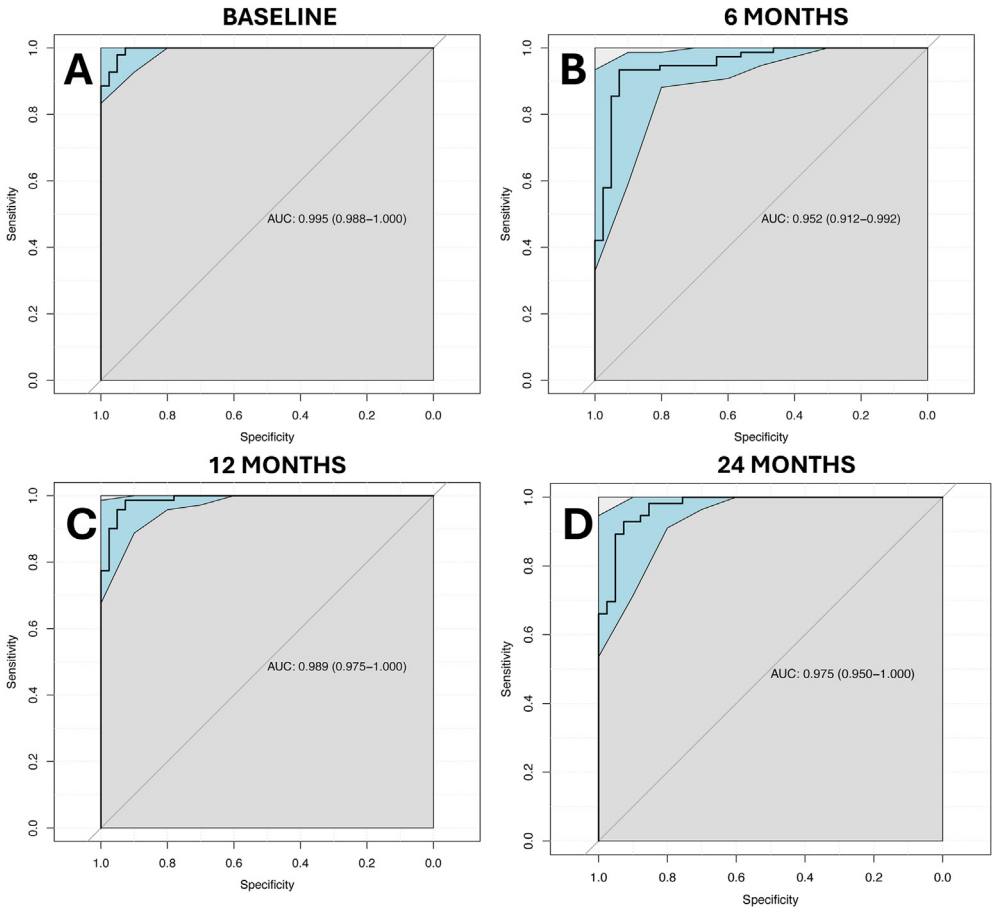

Fig. 2. Receiver Operating Characteristic (ROC) Curves for the presence of Metabolic dysfunction-associated steatotic liver disease (MASLD) diagnosed with ultrasonography as dependent variable, and leptin, adiponectin, Leukocyte cell-derived chemotaxin-2 (LECT2), and M30 as independent variables at **A.** Baseline, **B.** 6 months, **C.** 12 months, and **D.** 24 months. Analyses were adjusted for sex, age, and physical activity. Area Under the Curve (AUC) represented with 95% confidence intervals.

between the baseline and 6-month measurements since the last one represents the greater change in this variable among all the time marks.

The algorithms mentioned in the methods section were applied to construct the inflammation sub-scores. By using stepwise regressions, a selection of variables was extracted and one model for each diet was constructed (Table 4). The inflammation sub-scores were then assembled using the β-coefficients from the multiple regressions in the previous steps. The sub-score formulas, which include chemerin, leptin, M65, and LECT2, are shown in Table 5. The total inflammation score was created by subtracting both sub-scores (Table 5).

Table 4
Linear regression models constructed based on Stepwise Regressions, with weight loss percentage as the dependent variable and the inflammatory markers selected for the inflammation scores in each group.

Models	Variables	Estimate[a]	SE	T-value	R^2	Adjusted R^2	P-value
AHA	Chemerin	0.041	±0.02	1.994	0.29	0.20	0.025
	Leptin	−0.103	±0.03	−2.877			
	LECT2	0.151	±0.07	2.052			
	M65	0.016	±0.01	1.556			
FLiO	LECT2	−0.211	±0.09	−2.294	0.12	0.10	0.027

[a] Represents changes in outcomes for the increasing number of units of percentage of weight loss separating by dietary groups. Abbreviations: SE, Standard error; LECT2, Leukocyte cell-derived chemotaxin-2. M65 is a cytokeratin 18 (CK-18) antigen.

To fully evaluate the effect of initial inflammation on weight loss outcomes, a mixed-effect regression model (Table 6) that included the interaction term between the inflammation score and dietary strategy was used. The interaction between these terms is necessary to evaluate if the markers can predict which diet would be more effective regarding weight loss, and therefore, act as a precision nutrition tool. The model was additionally adjusted by sex and age. As seen in Table 6 and Fig. 3, the interaction term between the inflammation score and dietary strategies was statistically significant, making the assignation to a specific diet possible.

Z tests were then applied to calculate the statistical differences between the prediction of the percentage of weight loss with each dietary strategy. From these results, we calculated the percentage of subjects susceptible to be assigned the most suitable dietary strategy. The model was able to significantly assign a specific diet to 55% of the study participants, which means that the remaining 45%,

Table 5
Diet specific inflammation sub-score formulas and total inflammation score formulas.

Score	Calcultating formula
AHA diet sub-score	(0.04155*Chemerin) + (−0.10373*Leptin) + (0.15183*LECT2) + (0.01621*M65)
FLiO diet sub-score	(-0.21166*LECT2)
Total inflammation score	AHA diet sub-score - FLiO diet sub-score

Abbreviations: LECT2, Leukocyte cell-derived chemotaxin-2. M65 is a cytokeratin 18 (CK-18) antigen.

Table 6
Linear mixed model regression for total inflammation score to predict weight loss (decrease in body weight%).

Total inflammation score			
Independent variables	β coefficient	SE	p-value
Score	0.938	0.249	0.015
Diet	594.93	178	0.095
Age	5.44	2.95	0.064
Sex	14.3	60.6	0.813
Diet*Score	−0.896	0.306	0.003

Abbreviations: SE, Standard error. The model includes an interaction between dietary strategy and inflammation score, as well as adjusting variables such as sex, age, and physical activity as fixed effects.

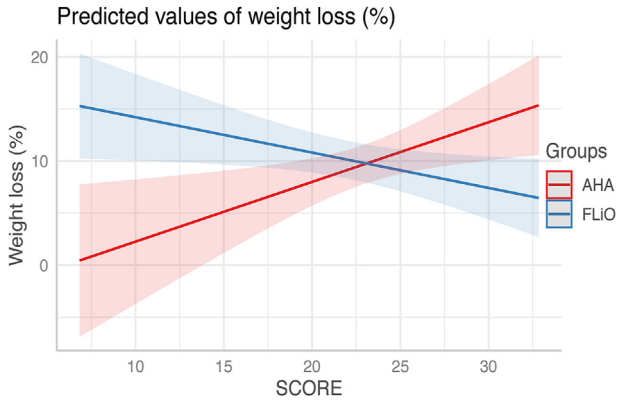

Fig. 3. Linear Mixed Regression Model to predict weight loss (decrease in body weight %) based on initial inflammatory status. The model includes an interaction between dietary strategy and inflammation score, as well as adjusting variables such as sex, age, and physical activity as fixed effects. The graph show the interaction between the inflammation score and each diet (AHA and FLiO) in the linear mixed model regression. The line in red represents the AHA diet, while the blue line represents the FLiO diet. 95% Confidence intervals are depicted in the graph as well.

which correspond to those individuals with a baseline inflammation score between 21 and 26, could achieve the same amount of weight loss either following the AHA or FLiO diets equally. Among the subjects that could be assigned to a specific dietary strategy, those with a score between 6 and 20 at baseline (58.5%) would lose significantly more weight if prescribed the FLiO diet (blue line in Fig. 3), while those with a baseline score between 26 and 33 (41.5%) would lose more weight if prescribed AHA diet (red line in Fig. 3).

4. Discussion

As previously mentioned, both dietary strategies (AHA and FLiO diet) were successful and had similar effects in ameliorating hepatic and metabolic parameters. There were practically no differences among groups when analyzing metabolic, biochemical, hepatic, and inflammatory between AHA and FLiO diets (Tables 1 and 2). Both groups significantly reduced their weight and managed to achieve weight loss percentages up to 10% of their initial weight, which is the main desired outcome when treating MASLD according to European and American guidelines [14,15], especially at 6 months. Along with the weight reduction, hepatic parameters such as hepatic fat percentage, transaminase levels, and FLI index, as well as biochemical parameters including insulin resistance and lipidic profile markers improved in both diets.

The results in both diets were better at the 6-month visit, which is plausible considering that at this time the individuals had overall better adherence to their diets and lifestyle habits, as shown in previously published data from this study [21,23]. At 24 months there was a decrease in body weight reduction along with considerably higher dropout rates, which again went along with overall worst adherence to the dietary strategies and caloric restriction. Similar results have been found in long-term lifestyle interventions [35,36]. This demonstrates the struggle many patients with metabolic diseases face when dealing with long-term lifestyle modifications [35,36]. These results also highlight the need for interdisciplinary interventions that include periodical reinforcements, not only from physicians and dietitians, but also from psychology, cognitive, and behavioral therapy professionals that address treatment from an integrative point of view and promote better adherence to dietary strategies.

Although results were quite similar among both diets, the FLiO group did manage to achieve slightly better results, for example, at waist circumference, triglycerides, and FLI index, especially at 12 and 24 months. Although only reaching a tendency towards statistical significance, FLiO also presented a slight advantage compared to AHA because of its ability to decrease BMI and body weight. These results highlight the importance of dietary treatment as a pivotal part of the care of the disease. Additionally, FLiO's results may suggest the ability to maintain this specific dietary strategy for prolonged periods of time compared to AHA. A correct adherence to the diet is a key component in the treatment for MASLD, since maintaining the weight loss is usually the utmost complication patients have [37,38].

Moreover, similar results were observed in the analyses of the inflammatory markers, where FLiO was able to obtain slightly better results. Several of the inflammatory markers, such as CRP, ferritin, chemerin, and RBP4, presented better results at 12 months rather than at 6 months, contrary to most of the metabolic, hepatic, and biochemical parameters. A plausible explanation for this could be that, since inflammation is one of the first mechanisms involved in the genesis of obesity and metabolic syndrome, it could also take longer to change and improve compared to other parameters [7,8,20]. Additionally, it is worth mentioning that obesity-related inflammation is a complex process involving many pathways, which could be difficult to modulate [39]. Nevertheless, other inflammatory markers, such as leptin, adiponectin, LECT2, M30, and M65, peaked its improvement at 6 months, consistent with most of the metabolic and hepatic parameters. Several studies have previously presented similar results, suggesting lifestyle interventions are quite effective in improving systemic inflammatory state, especially those including energy-restricted dietary strategies within the metabolic syndrome treatments [18,20,39].

Furthermore, the significant interaction observed in chemerin levels at 24 months and adiponectin levels at 6 and 24 months is worth mentioning. Chemerin increased at 24 months in the AHA group. On the other hand, adiponectin increased more in the FLiO group compared to AHA at 6 months and increased only in the FLIO group at 24 months of the intervention. Since chemerin is considered a proinflammatory marker [40,41], while adiponectin is considered an anti-inflammatory one [42,43], these results also suggest that the FLiO diet might be more beneficial than the AHA diet regarding its effect on inflammation.

The fact that, firstly, several markers reached levels like those observed in controls only in the FLiO group, and secondly, that only in this group did several markers decrease significantly, might indicate a desirable effect of this specific diet. It is worth noticing that, as this strategy was based on a Mediterranean dietary pattern, it provided high consumption of vegetables, legumes, and fruits, which are rich in bioactive compounds such as polyphenols and other antioxidants. Antioxidants are known for their anti-inflammatory properties, which could be a feasible explanation for these results.

When evaluating the diagnostic capacity of inflammatory markers, it was interesting to find that the four selected markers

were able to predict MASLD at every time mark in this study with very high AUC levels in all, suggesting an accurate diagnostic capacity of the markers (Table 3). In this case, leptin, adiponectin, M30, and LECT2 demonstrated their capacity to act as a surrogate non-invasive marker for MASLD's diagnosis, which, as previously mentioned, is a silent disease whose diagnosis is usually delayed until it reaches advanced stages.

The markers included in this regression have also been proven to be associated with MASLD by other authors previously [30,44,45]. In this case, M30 was more effective than M65 in identifying the disease. Regarding these results, as previously mentioned when discussing M65 levels, some authors have published similar results, while others suggest that M65 is more suitable for predicting MASLD [30,46]. It is important to consider that both markers are contemplated as liver fibrosis surrogate markers, which can not only diagnose the disease but also predict its stage [30,46].

Leptin and adiponectin were also included in the regression model. Leptin is known to be increased in obese subjects and individuals with related comorbidities, such as insulin resistance and MASLD [47,48]. Former studies have found significant associations of leptin with insulin secretion and triglyceride accumulation in the hepatocytes. Moreover, other researchers have also suggested that serum leptin can detect and identify MASLD [49,50]. Adiponectin is a widely studied marker in obesity and MASLD [42,49,50]. This adipokine is known to be decreased in obese individuals and subjects with MASLD. Multiple studies have suggested adiponectin's anti-inflammatory properties through the activation of 5′ adenosine monophosphate-activated protein kinase (AMPK) pathways [42]. Additionally, adiponectin has also been proven to promote fat oxidation and regulate insulin sensitivity and glucose metabolism [42,45].

LECT2 is a chemotaxin, also proposed as an hepatokine by some researchers [44]. This marker is involved in the regulation of glucose metabolism because it prevents insulin signaling and induces inflammatory cytokines secretion [51,52]. Similar results to the ones found in this study have been published by other authors, where LECT2 levels seem to be enhanced in subjects with obesity and MASLD, and the hepatokine levels also tend to decrease after weight and hepatic fat loss [51]. LECT2 has been suggested as a MASH and hepatic fibrosis marker as well [44].

Moreover, this study was able to demonstrate the capacity of inflammatory markers to act as diet customizing tools. This is a particularly interesting result, given the recent interest in personalized medicine and nutrition. Considering the formulas used to construct the inflammation sub-scores and total inflammation score shown in Table 5, to have a lower total inflammation score (from 6 to 20 marks) and, therefore, be assigned to FLiO, one individual presenting MASLD must have lower initial concentrations of chemerin, LECT2, and M65, and higher or very high leptin concentrations. On the other hand, individuals with high score marks that are assigned to the AHA diet (from 26 to 33 points) should have high initial concentrations of chemerin, LECT2, and M65, and/or low concentrations of leptin. In this sense, inflammatory markers could be considered a precision medicine and precision nutrition tool, useful for customizing MASLD dietary treatments.

Leptin and LECT2 were found in both the diagnostic regression models and the dietary decision model, suggesting that these markers could be highly involved in the pathophysiology of MASLD as well as its prognosis. On the other hand, adiponectin and M30 could be considered good subrogate serum markers for the diagnosis of the disease, while M65 and chemerin could function as markers for the customization of the dietary treatment.

It is worth noticing that, while this study's results are interesting, this study also presents some limitations. One limitation is the lack of mechanistic information regarding the effect of inflammatory markers on the subject's metabolism. This trial was also done on a sample of Spanish population, therefore its conclusions may not apply to other populations because of ethnic or genetic differences. Moreover, another limitation is the fact that the sample size was calculated based on weight loss as a primary outcome and not the inflammatory state of the subjects. This means that type II errors cannot be completely ruled out, especially at the 24-month visit where higher dropout rates occurred. Additionally, the fact that although the MASLD groups improved most of the metabolic outcomes but did not reach normal weight could also be considered a limitation, as the fact that the control individuals were not followed for the two years of the intervention as were the MASLD subjects. Finally, it is worth mentioning that the subjects of this study were screened for MASLD using ultrasonography, which may have underestimated liver fat percentage, especially in control individuals. Nevertheless, all subjects were highly characterized with other imaging techniques, such as MRI, and many other hepatic determinations.

Nevertheless, this study also presents several strengths. In this sense, it is worth mentioning the long duration of this trial as well as the adequate adhesion of the participants to their assigned diets. The study participants are also highly characterized not only regarding hepatic parameters but also concerning their body composition and biochemical information. The fact that several inflammatory markers were assessed and studied throughout the 2-year intervention is quite advantageous, considering that this study assessed the effect of two dietary strategies on the inflammatory state. Additionally, the dietary strategies proposed are healthy, sustainable, and feasible for long-term following.

Additional information, especially from dietary intervention trials on MASLD subjects, is needed to further explore the behavior of inflammatory markers through time, as well as the capacity to discriminate the disease and act as a customizing tool for precision nutrition.

5. Conclusion

This study's results align with previous literature, highlighting the key role inflammation has in metabolic diseases and, in this case, MASLD. Inflammatory markers constitute a potential, non-invasive tool to be used in MASLD screening. Additionally, inflammatory markers could also constitute an interesting tool for MASLD's treatment customization, being able to predict the effectiveness of a dietary strategy based on the initial inflammatory state of each subject.

Author contributions

Paola Mogna-Peláez: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing—original draft preparation, Writing—review and Editing, Visualization. **José I Riezu-Boj:** Conceptualization, Writing - review & editing, Visualization, Supervision. **Fermin I. Milagro:** Methodology, Investigation, Writing—review and Editing. **Ana Luz Tobaruela**: Investigation, Writing—review and Editing. **José I. Herrero:** Methodology, Investigation, Writing—review and Editing. **Mariana Elorz:** Methodology, Investigation, Writing—review and Editing. **Alberto Benito-Boillos:** Methodology, Investigation, Writing—review and Editing. **Josep A. Tur:** Funding acquisition, Resources, Writing—review and Editing **J. Alfredo Martínez:** Funding acquisition, Resources, Writing—review and Editing. **Itziar Abete:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing—original draft preparation, Writing—review and Editing, Visualization,

Supervision, Project administration and Funding acquisition, **M. Angeles Zulet:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing—original draft preparation, Writing—review and Editing, Visualization, Supervision, Project administration and Funding acquisition.

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Conflict of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnu.2024.05.042.

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