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(RESEARCH ARTICLE)

CIRCULATING NEUREGULIN-4 AND FIBROBLAST GROWTH FACTOR-21 IMBALANCE IN IRAQI ADULTS WITH METABOLIC DYSFUNCTION-ASSOCIATED STEATOTIC LIVER DISEASE

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ABSTRACT

Background: Metabolic dysfunction-associated steatotic liver disease (MASLD) involves adipose-tissue dysfunction, hepatic metabolic stress, insulin resistance, and oxidative injury. Neuregulin-4 (NRG4) is an adipose-enriched factor with anti-lipogenic actions, whereas fibroblast growth factor-21 (FGF21) is a predominantly hepatic stress-responsive hormone.

Objective: To compare circulating NRG4 and FGF21 among Iraqi adults with MASLD, obesity without steatosis, and healthy normal-weight controls; examine their associations with metabolic, hepatic, and oxidative-stress variables; and explore the diagnostic performance of an NRG4/FGF21 index.

Methods: This comparative cross-sectional study included 162 adults divided equally among the three study groups. Hepatic steatosis was assessed by FibroScan using the Controlled Attenuation Parameter (CAP). Serum NRG4 and FGF21 were measured by sandwich ELISA, and HOMA-IR, FIB-4, MDA, and TAC were assessed. For the exploratory diagnostic analysis comparing MASLD with obesity without steatosis, the relevant participants were divided into derivation (70%) and internal-validation (30%) subsets.

Results: Across the full sample, NRG4 was lower in MASLD (5.12 ± 1.02 ng/mL) than in obesity without steatosis (6.81 ± 0.92 ng/mL) and healthy controls (8.52 ± 0.85 ng/mL), whereas FGF21 was higher in MASLD (345.2 ± 81.4 pg/mL) than in obesity without steatosis (238.5 ± 65.3 pg/mL) and healthy controls (152.8 ± 74.2 pg/mL); both overall $p < 0.001$. The NRG4/FGF21 index yielded an AUC of 0.892 (95% CI 0.826-0.951) in the derivation subset and 0.879 (95% CI 0.801-0.942) in the internal-validation subset.

Conclusion: A low-NRG4/high-FGF21 pattern was associated with MASLD beyond the presence of obesity alone. The index showed promising internal discrimination, but prospective external validation and improved assessment of visceral adiposity and liver fat are required before clinical application.

Keywords: Metabolic Dysfunction-Associated Steatotic Liver Disease; Neuregulin-4; Fibroblast Growth Factor-21; Insulin Resistance; Oxidative Stress; Biomarkers.

1 INTRODUCTION

Metabolic dysfunction-associated steatotic liver disease (MASLD) is the current consensus term for hepatic steatosis occurring in the presence of at least one cardiometabolic risk factor and without a dominant alternative cause of steatosis. The terminology was adopted to replace the exclusion-based label nonalcoholic fatty liver disease and to emphasize the metabolic drivers of the condition [1]. Contemporary European and American guidance recognizes MASLD as a common, multisystem disorder that ranges from isolated steatosis to metabolic dysfunction-associated steatohepatitis, progressive fibrosis, cirrhosis, and hepatocellular carcinoma [2,3].

The global burden is substantial and continues to increase in parallel with obesity and type 2 diabetes. A large systematic review estimated a worldwide adult prevalence of approximately 32%, with marked geographic and methodological heterogeneity [4]. The clinical importance of MASLD extends beyond liver-related outcomes because affected individuals have increased risks of diabetes, cardiovascular disease, chronic kidney disease, and extrahepatic malignancies [5,6]. Fibrosis stage is the strongest histological predictor of liver-related outcomes, which explains the current emphasis on sequential noninvasive risk stratification using FIB-4 followed by elastography or other second-line tests [2,7,8].

MASLD is driven by an interaction among adipose-tissue insulin resistance, excessive delivery of free fatty acids to the liver, increased de novo lipogenesis, impaired lipid export, mitochondrial dysfunction, endoplasmic-reticulum stress, inflammation, and oxidative injury [9,10]. The multiple-hit model proposes that these abnormalities act simultaneously and vary between individuals rather than occurring as a fixed sequence [10]. Insulin resistance is central to this network: it increases adipose lipolysis and hepatic substrate availability while hyperinsulinemia promotes sterol-regulatory-element-binding-protein-1c-mediated lipogenesis. Lipotoxic species and reactive oxygen species then amplify hepatocellular stress and inflammatory signaling [9-11].

Adipose tissue and liver communicate through secreted proteins that may integrate these pathophysiological processes. Neuregulin-4 (NRG4) is a member of the epidermal growth factor family that is enriched in brown and beige adipose tissue. Experimental work showed that NRG4 signals through ErbB4 in hepatocytes, suppresses de novo lipogenesis, improves systemic glucose homeostasis, and protects against diet-induced steatosis [12,13]. NRG4 enhances fuel oxidation and a beneficial adipokine profile in preclinical models [14]. Together, these data support the notion of diminished NRG4 as an adipose-derived protective signal that is lost in metabolic dysfunction associated with obesity.

In general, human data are supportive, although not unequivocally so, of this interpretation. Dai et al.identified decreased serum NRG4 in adults with ultrasound diagnosed fatty liver and suggested it as an independent correlate of disease [15]. Lower levels were later reported in children with obesity and non-alcoholic fatty liver [16], in Iranian adults with NAFLD [17], and in an Egyptian case control study [18]. A 2025 systematic review and meta-analysis reported a synthesis of findings indicating a decline in circulating NRG4 levels in NAFLD although relevant heterogeneity in methodologies, population and diagnostic criteria was found [19]. This differences call for the inclusion of hepatosteatosis- and obesity-matched controls to assess whether the biomarker changes are specific to hepatic steatosis or related to general adiposity. On the other hand, a biopsy-based investigation by de Munck et al. failed to found an obvious association of serum NRG4 with histological disease severity [20]. This discrepancy indicates a need for obesity-matched controls and the examination of other complementary hepatic signals simultaneously.

Fibroblast growth factor-21 (FGF21) is an endocrine FGF family member which is mainly secreted from the liver under fasting conditions as well as upon nutrient deprivation, mitochondrial dysfunction, and endoplasmic-reticulum stress. FGF21 alters glucose and lipid metabolism in white adipose tissue and other tissues through beta-Klotho-receptor complexes [21,22]. While therapeutic FGF21 mimetics reduce hepatic fat, inflammation and fibrosis, endogenous FGF21 is typically raised in obesity, insulin resistance and fatty liver disease. This apparent paradox has been interpreted as a compensatory response or relative FGF21 resistance [22,23].

Animal studies linked obesity to reduced circulating levels of FGF21 and early clinical investigation showed circulating FGF21 was increased in obesity and NAFLD [24], positively correlated to hepatic triglyceride content [25], and raised in patients with steatotic liver disease [26]. Plasma FGF21 was also associated with the histological severity of steatohepatitis in obese adults with type 2 diabetes [27]. A 2025 meta-analysis confirmed an increase in circulating FGF21 in NAFLD, particularly in more advanced disease, albeit stressing between-study heterogeneity [28]. The therapeutic importance of this pathway is underlined by the clinical trials of FGF21 analogues and recent meta-analyses demonstrating improvements in liver fat and histological outcomes [29,30]. Oxidative stress acts as a mechanistic link between the two endocrine signals. Decreased NRG4 might promote liver fat accumulation, whereas elevated FGF21 may reflect hepatocellular metabolic stress. Products of lipid peroxidation such as malondialdehyde (MDA) increase when reactive oxygen species surpass antioxidant defenses while total antioxidant capacity (TAC) reflects an integrated measure of the circulating antioxidant pool. Impaired antioxidant pathways, mitochondrial dysfunction and lipid

peroxidation have been highlighted in recent reviews as key aspects in MASLD progression [31,32]. The joint assessment of NRG4 and FGF21 along with MDA, TAC, and HOMA-IR may thus yield a more consistent biochemical phenotype than that obtained by analyzing the single hormone.

To the best of our knowledge , there are no data concerning NRG4 and FGF21 in MASLD Iraqi adults. Moreover, the majority of biomarker investigations pit individuals with fatty liver against lean controls that do not allow for separation of the effect of hepatic steatosis from that of obesity. For this reason, the present study also included an obesity comparator with no CAP-defined steatosis. We aimed to assess NRG4 and FGF21 in subjects with MASLD, obesity but no steatosis, and in healthy controls. Secondary objectives: To assess their associations with insulin resistance, liver enzymes, FIB-4 and oxidative-stress markers and to investigate the potential performance of an NRG4/FGF21 index to discriminate MASLD from obesity without steatosis.

2 MATERIALS AND METHODS

2.1 Study design and setting

This study was designed and applieded as a cross-sectional biomarker study in Baghdad, Iraq. 162 Iraqi adults were included and classified into 3 equal groups: 54 participants with CAP-defined MASLD, 54 participants with obesity without hepatic steatosis, and 54 apparently healthy with normal-weight controls. The laboratory tests and biomarker analysis were done at Al-Bilad Laboratory, Baghdad, Iraq. The reporting followed the STROBE guide for observational studies and the relevant STARD aspects for diagnostic exploratory analyses [34,35].

2.2 Ethical approval

The study was approved by the appropriate institutional review board. Informed consent: All participants gave written informed consent. The investigation was performed in accordance with the principles expressed in the Declaration of Helsinki.

2.3 Eligibility framework and group definitions

The inclusion criteria were that the adults were located in Iraq and aged between 20-60 years. MASLD was defined as CAP-based hepatic steatosis (CAP ≥248 dB/m) in the context of overweight/obesity or a cardiometabolic risk factor and in the absence of a dominant alternative cause of steatosis, according to the current consensus construct [1,2]. The group of obesity without steatosis consisted of individuals with BMI ≥30 kg/m2 and CAP <248 dB/m. Healthy controls had a BMI ranging 18.5-24.9 kg/m2, CAP <248 dB/m, routine liver tests were normal, and they had no known metabolic disease. Exclusion criteria included significant alcohol consumption, viral hepatitis, autoimmune or genetic liver disorders, liver failure, pregnancy, active cancer, chronic inflammatory disease, corticosteroid therapy, and use of medications that are significantly linked to hepatic steatosis.

2.4 Sample-size determination

A total sample size of ~144 was required a priori for one-way analysis of variance with three groups, a moderate effect size (f = 0.30), two-sided alpha = 0.05, and 90% power. The analytical sample was defined as 162 participants (54 per group) to allow balanced comparisons and limited multivariable adjustment.

2.5 Clinical and anthropometric assessment

Age, sex, history of disease, drug exposure, and smoking status were reported in a standardized questionnaire. Weight was recorded to the nearest 0.1 kg with subjects dressed in light clothing, and height to the nearest 0.1 cm. BMI was computed as the weight divided by the height squared. Waist circumference was measured at the midpoint between the lower margin of the last palpable rib and the top of the iliac crest at the end of a normal expiration. Blood pressure Using an appropriately sized cuff, the blood pressure was taken at least five minutes after the subject had been seated.

2.6 Quantitative assessment of hepatic steatosis and fibrosis

All patients were evaluated for the liver with a FibroScan 530 Compact system (Echosens, Paris, France) and the M or XL probe was used depending on the skin-to-liver capsule distance. Hepatic steatosis was measured with the Controlled Attenuation Parameter (CAP) in [dB/m]. Prespecified investigational categories were S1 (248-267 dB/m), S2 (268-279 dB/m), and S3 (≥280 dB/m) [36,37]. These categories were interpreted with caution as published CAP cut-offs may differ by body size, probe, and reference method. Liver stiffness measurement (LSM) in kPa was concomitantly obtained for noninvasive fibrosis-risk stratification. None of the patients had an LSM value ≥12 kPa and thus none was excluded according to the predefined LSM criterion. Among the MASLD patients, CAP grades were S1 in 22 (40.7%), S2 in 20 (37.0%) and S3 in 12 (22.2%).

2.7 Blood sampling and routine biochemical measurements

After a 10–12 hour overnight fast, venous blood was collected between 08:00 and 10:00. Serum and plasma aliquots were separated promptly and stored at −80 °C until analysis. Fasting glucose, total cholesterol, triglycerides, HDL-C, ALT, AST, and GGT were measured using standard enzymatic methods on an automated chemistry analyzer. LDL-C was calculated using the Friedewald equation when triglycerides were below 400 mg/dL. Platelet counts were obtained from an automated hematology analyzer. HbA1c was measured by an NGSP-aligned method.

2.8 NRG4 and FGF21 assays

Serum NRG4 was quantified using a sandwich ELISA kit (Human Neuregulin-4 ELISA Kit, Catalog No. CSB-EL016080HU; Cusabio; detection range, 0.781-50 ng/mL). Serum FGF21 was quantified using a sandwich ELISA kit (Quantikine Human FGF-21 Immunoassay, Catalog No. DF2100; R&D Systems; detection range, 31.3-2000 pg/mL). Standards, controls, and participant samples were assayed in duplicate. The inter-assay and intra-assay coefficients of variation were <8% and <5%, respectively, for both assays. Laboratory personnel were blinded to clinical group. Results were reported in ng/mL for NRG4 and pg/mL for FGF21. The prespecified NRG4/FGF21 index was calculated as (NRG4 in ng/mL × 1000)/FGF21 in pg/mL, yielding a dimensionless measure of adipose protective signaling relative to hepatic stress signaling.

2.9 Oxidative-stress measurements

MDA was measured using a validated thiobarbituric-acid-reactive-substances spectrophotometric method and reported in μmol/L. TAC was measured using a standardized colorimetric assay and reported in mmol/L. Internal quality-control materials at low and high concentrations were analyzed with each batch, and samples from all three groups were distributed across analytical plates to reduce batch-related imbalance.

2.10 Derived indices

Insulin resistance was estimated using the homeostasis model assessment: HOMA-IR = fasting insulin (μIU/mL) × fasting glucose (mg/dL)/405 [38]. Fibrosis risk was estimated using FIB-4 = age (years) × AST (U/L)/[platelet count (10^9/L) × square root of ALT (U/L)] [39]. FIB-4 was interpreted as a risk-stratification measure rather than a diagnostic substitute for elastography or histology [2,7].

2.11 Statistical analysis

Continuous variables were assessed for distributional form using the Shapiro-Wilk test and were summarized as mean ± SD when approximately normally distributed. Overall group comparisons used Welch ANOVA with Games-Howell post-hoc tests because equal variances were not assumed. Sex distributions were compared using the chi-square test. Pairwise p values were adjusted using the Holm method, and effect magnitude was summarized using eta squared or Cohen d, as appropriate. Spearman correlations among participants in the two obesity groups were treated as exploratory pooled associations.

Robust multiple linear regression with heteroscedasticity-consistent HC3 standard errors was used to assess standardized HOMA-IR among participants with obesity. Age, sex, BMI, MASLD status, NRG4, and FGF21 were entered simultaneously, and a sensitivity model additionally included MDA. For the comparison of MASLD with obesity without steatosis, each biomarker was examined in a separate binary logistic-regression model adjusted for age, sex, and BMI and expressed per 1-SD change. Descriptive analyses across CAP grades were considered exploratory because of the modest subgroup sizes.

For exploratory diagnostic analysis, participants in the MASLD and obesity-without-steatosis groups were randomly divided, with group stratification, into a derivation subset (70%) and an internal-validation subset (30%). Receiver operating characteristic curves were generated from the continuous values of NRG4, FGF21, and the NRG4/FGF21 index. Optimal cutoffs were selected in the derivation subset using the Youden index and then applied unchanged to the internal-validation subset to estimate sensitivity and specificity. Validation AUCs were calculated independently from the continuous marker values, and 95% confidence intervals were estimated using 2,500 stratified bootstrap resamples. Formal pairwise comparison of AUCs using DeLong's test was not performed because the diagnostic analysis was prespecified as exploratory. Therefore, the AUCs were interpreted descriptively, and no claim of statistical superiority between biomarkers was made. All tests were two-sided, with $p < 0.05$ considered statistically significant. Analyses were performed using SPSS version 26.0 and MedCalc.

3 RESULTS

3.1 Participant distribution

The analytic dataset included 162 participants, with 54 participants in each study group. Figure 1 shows participant grouping; Table 1 summarizes group characteristics. All included participants completed the study procedures. Age and sex distributions were comparable across groups, whereas BMI and waist circumference were higher in both obesity groups than in healthy controls. The two obesity groups showed substantial overlap in BMI; however, mean BMI and waist circumference were modestly higher in the MASLD group than in the obesity-without-steatosis group. Within the MASLD group, CAP grades were S1 in 22 participants (40.7%), S2 in 20 (37.0%), and S3 in 12.

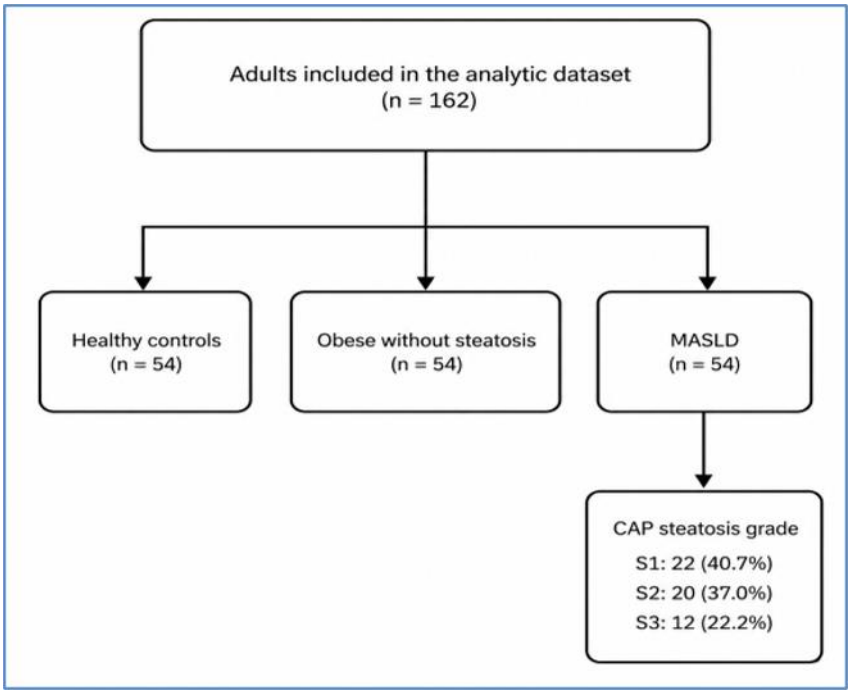

Figure 1: Participant flow and analytic grouping.

Table 1: Demographic and metabolic characteristics of the study groups.

Variable	Healthy controls (n = 54)	Obese without steatosis (n = 54)	MASLD (n = 54)	Overall p	η^2
Age (years)	39.5 ± 7.6	40.1 ± 7.4	41.5 ± 8.6	0.438	0.011
Male sex, n (%)	22 (40.7%)	25 (46.3%)	26 (48.1%)	0.689	—
BMI (kg/m^2)	23.0 ± 1.3	31.9 ± 2.1	33.1 ± 2.3	< 0.001	0.852
Waist circumference (cm)	80.8 ± 6.2	99.0 ± 5.8	103.8 ± 6.2	< 0.001	0.741
CAP (dB/m)	189.5 ± 24.1	195.2 ± 22.8	291.4 ± 44.6	< 0.001	0.812
LSM (kPa)	4.2 ± 0.7	4.5 ± 0.9	6.1 ± 1.6	< 0.001	0.354
Fasting glucose (mg/dL)	88.6 ± 7.2	96.8 ± 7.6	108.5 ± 7.9	< 0.001	0.548
Fasting insulin (μIU/mL)	6.9 ± 2.4	12.6 ± 3.0	17.7 ± 2.8	< 0.001	0.731
HOMA-IR	1.52 ± 0.58	3.04 ± 0.86	4.75 ± 0.98	< 0.001	0.732
HbA1c (%)	5.18 ± 0.22	5.49 ± 0.20	5.79 ± 0.22	< 0.001	0.579

Total cholesterol (mg/dL)	177.2 ± 20.4	199.1 ± 18.6	211.8 ± 16.5	< 0.001	0.385
Triglycerides (mg/dL)	102.1 ± 20.5	151.6 ± 27.9	193.5 ± 21.5	< 0.001	0.726
HDL-C (mg/dL)	52.9 ± 5.9	43.8 ± 5.7	39.8 ± 5.3	< 0.001	0.494
LDL-C (mg/dL)	103.8 ± 21.3	125.0 ± 20.1	133.2 ± 17.8	< 0.001	0.291

Values are mean ± SD unless otherwise indicated. Overall p values are from Welch ANOVA, except sex (chi-square test). η^2 denotes eta squared. CAP: Controlled Attenuation Parameter; LSM: Liver Stiffness Measurement.

3.2 Liver, endocrine, and oxidative-stress profiles

ALT, AST, and GGT increased progressively from healthy controls to obesity without steatosis and MASLD. Platelet counts were lower and FIB-4 was higher in MASLD, although mean FIB-4 values remained within a range generally associated with low fibrosis probability in younger adults. MDA rose markedly across groups, while TAC declined, indicating a graded redox imbalance.

NRG4 was highest in healthy controls, intermediate in participants with obesity without steatosis, and lowest in MASLD. FGF21 showed the opposite pattern. The NRG4/FGF21 index therefore produced a pronounced numerical separation between MASLD and the other groups. All three endocrine measures differed across groups with large effect sizes (Table 2; Figure 2).

Table 2: Liver-related, endocrine, and oxidative-stress measurements.

Variable	Healthy controls (n = 54)	Obese without steatosis (n = 54)	MASLD (n = 54)	Overall p	η^2
ALT (U/L)	18.9 ± 6.3	29.4 ± 7.2	47.9 ± 7.5	< 0.001	0.752
AST (U/L)	20.0 ± 5.0	23.2 ± 5.1	33.6 ± 4.5	< 0.001	0.598
GGT (U/L)	18.8 ± 8.9	29.5 ± 9.8	51.2 ± 8.2	< 0.001	0.700
Platelets (10^9/L)	279.5 ± 18.5	274.5 ± 20.5	251.0 ± 20.4	< 0.001	0.291
FIB-4	0.67 ± 0.21	0.63 ± 0.18	0.82 ± 0.21	< 0.001	0.131
NRG4 (ng/mL)	8.52 ± 0.85	6.81 ± 0.92	5.12 ± 1.02	< 0.001	0.685
FGF21 (pg/mL)	152.8 ± 74.2	238.5 ± 65.3	345.2 ± 81.4	< 0.001	0.518
NRG4/FGF21 index	80.2 ± 51.8	32.4 ± 16.5	16.5 ± 6.9	< 0.001	0.431
MDA (μmol/L)	2.03 ± 0.42	3.14 ± 0.59	4.68 ± 0.47	< 0.001	0.832
TAC (mmol/L)	1.47 ± 0.12	1.21 ± 0.10	0.96 ± 0.11	< 0.001	0.791

The NRG4/FGF21 index was calculated as (NRG4 × 1000)/FGF21. Higher η^2 values indicate a larger proportion of variance attributable to group membership.

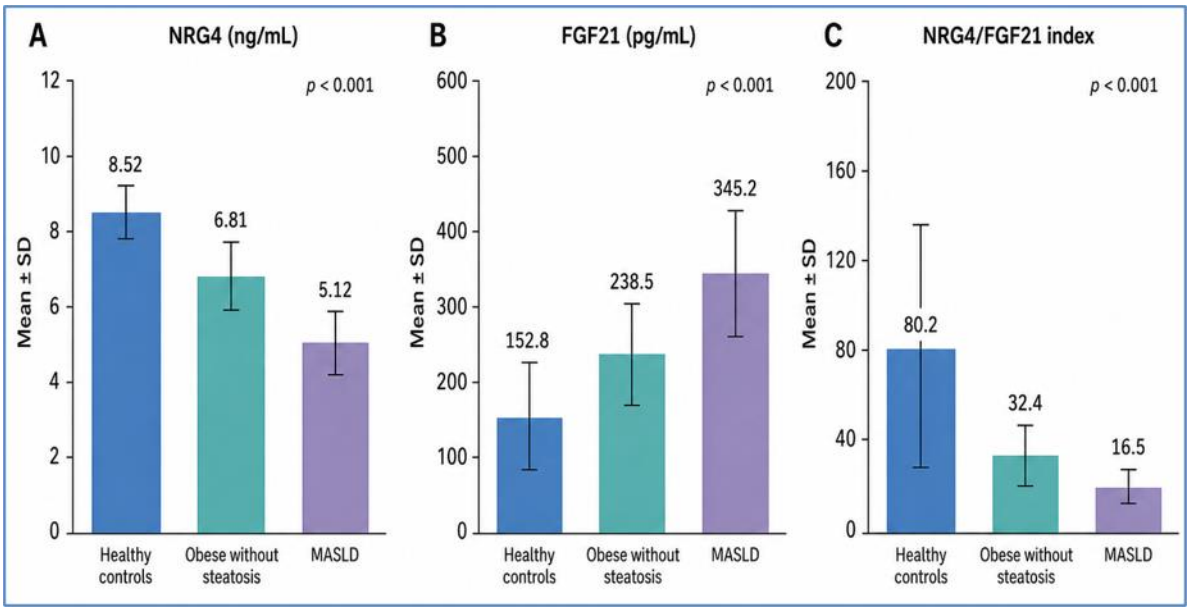

Figure 2: Mean (± SD) circulating NRG4, FGF21, and NRG4/FGF21 index values across the three study groups.

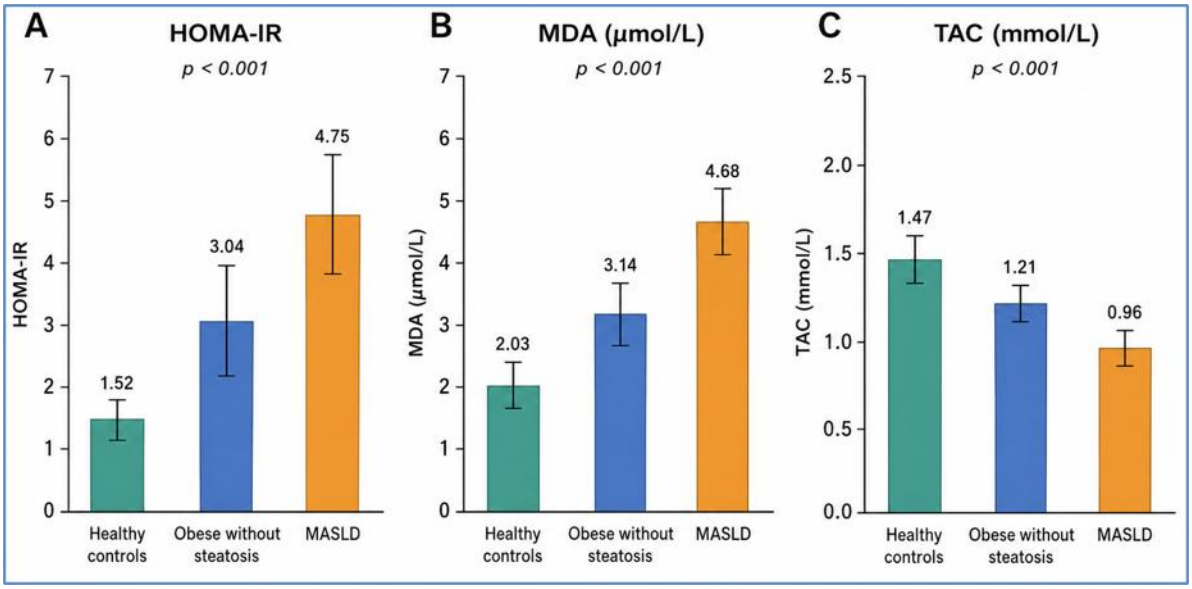

Figure 3: Mean (± SD) HOMA-IR, MDA, and TAC values across the three study groups.

3.3 Pairwise comparisons

Pairwise comparisons and standardized effect sizes are presented in Table 3.

Table 3: Holm-adjusted pairwise comparisons and standardized effect sizes.

Variable	HC vs ONS d	Adjusted p	HC vs MASLD d	Adjusted p	ONS vs MASLD d	Adjusted p
BMI	−5.34	<0.001	−5.60	<0.001	−0.57	0.005
HOMA-IR	−2.07	<0.001	−4.06	<0.001	−1.90	<0.001
ALT	−1.55	<0.001	−4.20	<0.001	−2.53	<0.001
NRG4	1.86	<0.001	3.53	<0.001	1.71	<0.001
FGF21	−1.27	<0.001	−2.37	<0.001	−1.29	<0.001
NRG4/FGF21 index	1.24	<0.001	1.72	<0.001	1.26	<0.001

MDA	−2.17	<0.001	−5.94	<0.001	−2.89	<0.001
TAC	2.40	<0.001	4.50	<0.001	2.37	<0.001
FIB-4	0.17	0.399	−0.68	0.002	−0.89	<0.001

HC: healthy controls; ONS: obesity without hepatic steatosis; MASLD: metabolic dysfunction-associated steatotic liver disease. Adjusted p-values were calculated using the Holm method.

3.4 Correlation analysis

In the pooled exploratory analysis of participants in the two obesity groups, lower NRG4 and a lower NRG4/FGF21 index were associated with greater insulin resistance, higher liver enzymes, higher MDA, lower TAC, and higher FIB-4, whereas FGF21 showed associations in the opposite direction. The strongest pooled correlations involved the NRG4/FGF21 index and HOMA-IR (rho = -0.778), MDA (rho = -0.772), TAC (rho = 0.757), GGT (rho = -0.716), and ALT (rho = -0.698). Because these correlations combined two clinically distinct groups, they were considered exploratory and may partly reflect between-group separation rather than independent within-group monotonic relationships. Accordingly, the pooled coefficients should not be overinterpreted as evidence of graded biological associations at the individual level.

Table 4: Exploratory pooled Spearman correlations among participants in the two obesity groups (n = 108).

Clinical variable	NRG4 ρ	p value	FGF21 ρ	p value	NRG4/FGF21 ρ	p value
BMI	−0.279	0.005	0.180	0.073	−0.264	0.008
Waist circumference	−0.301	0.002	0.301	0.002	−0.330	<0.001
HOMA-IR	−0.707	<0.001	0.682	<0.001	−0.778	<0.001
Triglycerides	−0.642	<0.001	0.520	<0.001	−0.643	<0.001
ALT	−0.677	<0.001	0.578	<0.001	−0.698	<0.001
GGT	−0.654	<0.001	0.599	<0.001	−0.716	<0.001
MDA	−0.730	<0.001	0.655	<0.001	−0.772	<0.001
TAC	0.695	<0.001	−0.642	<0.001	0.757	<0.001
FIB-4	−0.315	0.001	0.307	0.002	−0.358	<0.001

ρ: Spearman's rank correlation coefficient. Correlations were calculated using pooled data from participants with obesity without hepatic steatosis and those with MASLD.

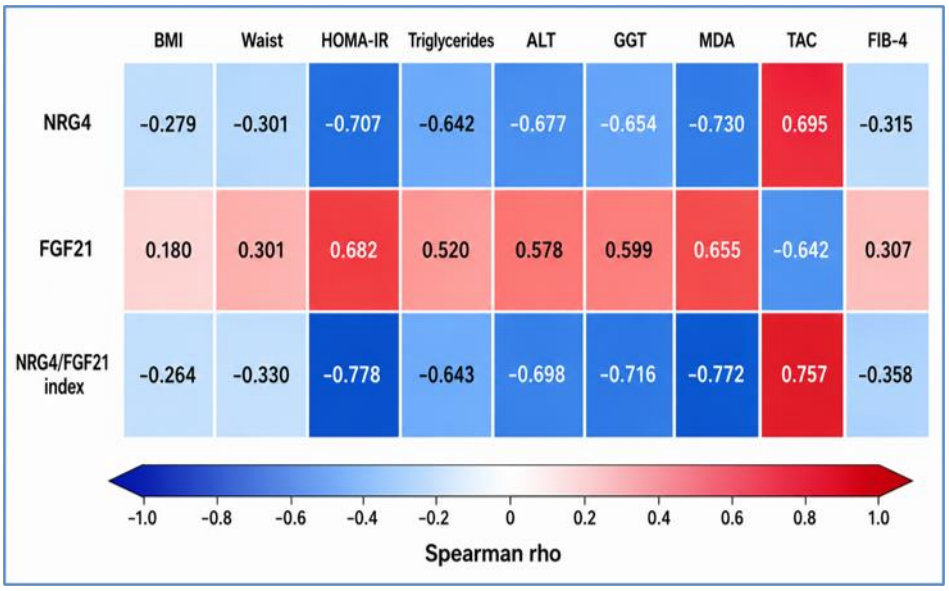

Figure 4: Heatmap of exploratory pooled biomarker correlations among participants in the two obesity groups (n = 108).

3.5 Multivariable associations with insulin resistance

The primary robust linear-regression model explained 69.6% of the variance in standardized HOMA-IR (adjusted R^2 = 0.676; model p < 0.001). After adjustment for age, sex, BMI, and MASLD status, NRG4 remained inversely associated with HOMA-IR, whereas FGF21 remained positively associated. BMI and MASLD status were also independent positive predictors. Age and sex were not significant in this model.

Table 5: Robust multivariable linear regression for standardized HOMA-IR among participants with obesity.

Predictor	Standardized beta	95% CI	p value
Age (per SD)	0.012	–0.094 to 0.119	0.821
Male sex	0.149	–0.084 to 0.382	0.209
BMI (per SD)	0.144	0.012 to 0.276	0.033
MASLD group	0.550	0.240 to 0.859	< 0.001
NRG4 (per SD)	–0.261	–0.414 to –0.108	< 0.001
FGF21 (per SD)	0.372	0.228 to 0.517	< 0.001

Model covariates were entered simultaneously. HC3 robust standard errors were used. R^2 = 0.696; adjusted R^2 = 0.676.

In the sensitivity model adding MDA, model R^2 increased to 0.752. MDA was independently associated with HOMA-IR (standardized beta = 0.516, p < 0.001), while the coefficients for NRG4 and FGF21 were attenuated. This pattern is compatible with partial overlap among endocrine imbalance, oxidative stress, and insulin resistance.

3.6 Adjusted odds and diagnostic discrimination

In separate logistic-regression models adjusted for age, sex, and BMI, lower NRG4, higher FGF21, and a lower NRG4/FGF21 index were each associated with MASLD rather than obesity without steatosis. The index showed the largest standardized association magnitude, although each biomarker was evaluated in a separate model.

Table 6: Adjusted associations of biomarkers with MASLD versus obesity without steatosis.

Biomarker (per 1 SD)	Adjusted OR	95% CI	p value
NRG4	0.091	0.034 to 0.243	< 0.001
FGF21	4.945	2.458 to 9.950	< 0.001
NRG4/FGF21 index	0.042	0.011 to 0.154	< 0.001

Each biomarker was analyzed in a separate logistic model adjusted for age, sex, and BMI.

Table 7: Receiver operating characteristic analysis for MASLD versus obesity without steatosis in derivation and internal-validation subsets.

Marker	Cohort	AUC	95% CI	Cutoff	Sensitivity	Specificity
NRG4	Derivation	0.887	0.815-0.944	≤ 6.32	94%	72%
NRG4	Internal validation	0.876	0.792-0.938	≤ 6.32	92%	70%
FGF21	Derivation	0.819	0.735-0.896	≥ 309.5	66%	86%
FGF21	Internal validation	0.808	0.718-0.884	≥ 309.5	64%	84%
NRG4/FGF21 index	Derivation	0.892	0.826-0.951	≤ 21.3	86%	82%
NRG4/FGF21 index	Internal validation	0.879	0.801-0.942	≤ 21.3	84%	80%

AUCs in both subsets were calculated from continuous marker values. Cutoffs were selected in the derivation subset and applied unchanged to the internal-validation subset. The results are exploratory and require external validation.

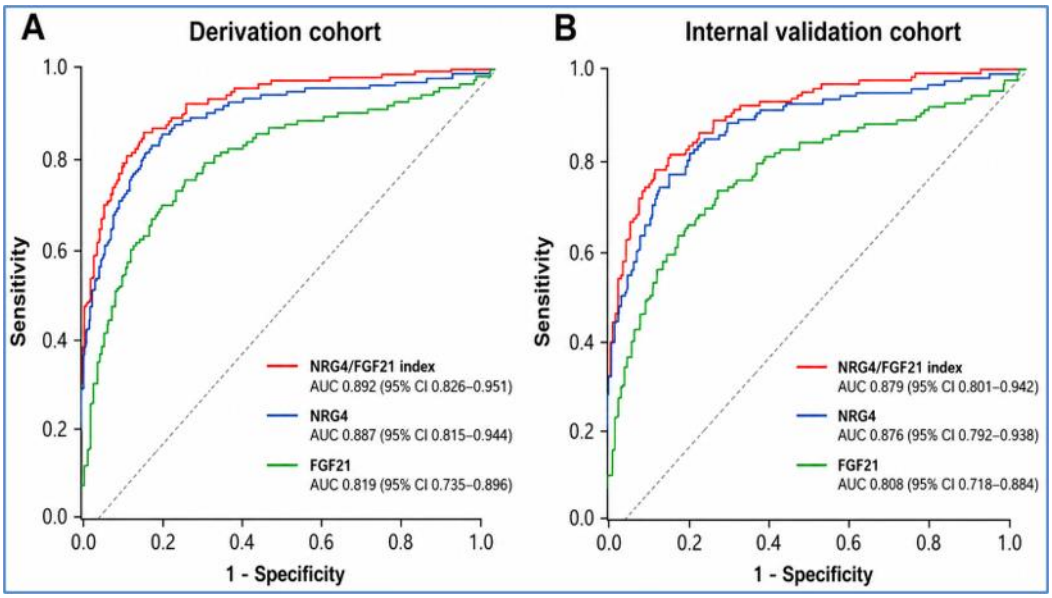

Figure 5: Receiver operating characteristic curves for NRG4, FGF21, and the NRG4/FGF21 index.

3.7 Biomarkers across CAP-defined steatosis grades

Within the MASLD group, descriptive analysis across CAP-defined steatosis categories showed progressively lower mean NRG4 and progressively higher mean FGF21 from S1 to S3 (Figure 6). The lowest NRG4 and highest FGF21 values were observed in the S3 subgroup. Because the subgroup sizes were modest (S1, n = 22; S2, n = 20; S3, n = 12) and no grade-specific multivariable or diagnostic model was developed, this pattern was considered exploratory and should not be interpreted as evidence of a dose-response relationship. Confirmation is required in larger, adequately powered cohorts using more precise liver-fat quantification methods, such as MRI-PDFF, which was not available in the present study.

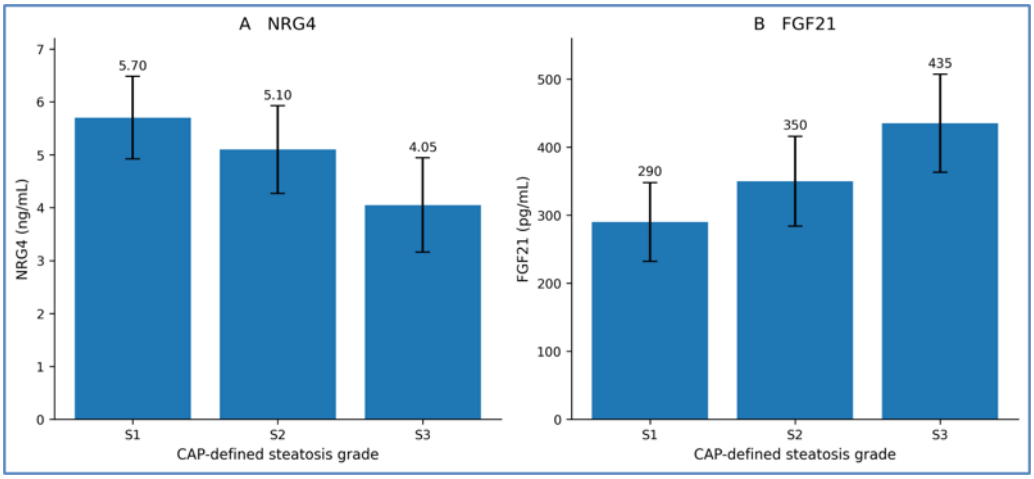

Figure 6: Mean (± SD) NRG4 and FGF21 values across CAP-defined steatosis categories within the MASLD group.

4 DISCUSSION

This comparative cross-sectional study identified a coordinated endocrine pattern in which circulating NRG4 decreased and FGF21 increased across healthy controls, participants with obesity without steatosis, and participants with MASLD. Both obesity groups met the BMI criterion for obesity, although BMI and waist circumference were modestly higher in MASLD. The NRG4/FGF21 index integrated the opposing directions of the two biomarkers and showed an AUC of 0.892 in the derivation subset and 0.879 in the internal-validation subset. Its discrimination was numerically similar to that of NRG4 alone and greater than that of FGF21, but the analysis was exploratory and did not establish statistical superiority. The endocrine changes occurred alongside higher HOMA-IR, dyslipidemia, liver-enzyme activity, and lipid peroxidation and lower antioxidant capacity.

The reduction in NRG4 is consistent with its proposed role as an adipose-derived hepatoprotective signal. Wang et al. demonstrated that NRG4-ErbB4 signaling suppresses hepatic lipogenesis and protects mice from diet-induced metabolic disease [12]. Subsequent experimental studies linked NRG4 to increased fuel oxidation, improved adipokine profiles, and reduced steatohepatitis-related injury [13,14]. In the present analysis, NRG4 was inversely associated with HOMA-IR, ALT, GGT, MDA, and FIB-4 and positively associated with TAC. These correspond to biologically plausible relationships with NRG4 signaling being maintained to attenuate liver lipid influx and oxidative stress. The clinical direction is consistent with the case-control study of Dai et al. where serum NRG4 was decreased in NAFLD and independently linked to the disease [15]. Similar decreases have been found in children with obesity-related fatty liver [16], in the Iranian case-control study of Tutunchi et al. [17] and in the Egyptian study of Gado et al. [18]. The recent meta-analysis by Tapak et al. likewise corroborated reduced circulating NRG4 in NAFLD but there was significant heterogeneity [19]. The progressive decline from healthy controls to obese subjects without steatosis and then to MASLD in the present study implies that NRG4 may be a marker of both adipose dysfunction and the superimposed insult of hepatic steatosis. Not all published results agree. De Munck et al. reported no strong association between circulating NRG4 and the severity of NAFLD as assessed by biopsy [20]. Differences could be related to plasma or serum matrices, ELISA standardization, sample handling, matching for obesity, exposure to medication, the spectrum of histology, and whether the difference was between the presence and stages of disease. Brown-adipose activity, age, sex, ambient temperature and host genetics may also affect NRG4 while circulating levels of NRG4 were suggested to correlate with brown-adipose activity nociceptin/orphanin FQ. Therefore, a single concentration should not be interpreted as reflecting hepatic tissue signalling directly. This discordance highlights the importance of standardised assays and independent confirmation.

The FGF21 elevation in MASLD is in line with ample clinical literature. Dushay et al. has been shown to increase in the obesity and NAFLD [24], while Li et al. associated both circulating and hepatic FGF21 expression with the intrahepatic triglyceride content [25]. Yilmaz et al. also reported elevated serum FGF21 in steatotic liver disease [26]. In a biopsy-proven population of individuals with overweight/obesity and T2DM, Barb et al. observed that plasma FGF21 levels were linked to more severe steatohepatitis [27]. The 2025 review and meta-analysis by Filimidou et al. stated that "circulating FGF21 is mostly elevated in NAFLD, with the greatest discrimination between fibrosis stages and through the different disease stages " [28]. This positive correlation between FGF21 and HOMA-IR should not be taken as an indication that FGF21 induces insulin resistance. The metabolic and cellular stress induces the endogenous FGF21, which can function as the compensatory hormone. In obesity, increased production may be associated with decreased downstream sensitivity, changes in beta-Klotho expression, or an insufficient response to metabolic demands, a phenomenon commonly referred to as FGF21 resistance [22,23]. Results from randomized trials demonstrating therapeutic benefits of long-acting FGF21 analogues further corroborate the separation of elevated endogenous levels and pharmacological pathway activation [29,30].

The redox results are consistent with the interpretation of endocrine results. MDA was elevated by about twofold from controls to MASLD while TAC was markedly reduced. Mitochondrial electron leakage, peroxisomal and microsomal oxidation, NADPH oxidase activity, and inflammatory cell activation contribute to oxidative stress. Products of lipid peroxidation can alter membranes, proteins, and even DNA, and can also trigger stellate-cell activation. Recent reviews highlight that alterations in glutathione-dependent pathways, superoxide dismutase, catalase, and other antioxidants coincide with MASLD progression [31,32]. The fact that the NRG4 and FGF21 coefficients are attenuated after including MDA into the regression model implies that these routes have biologic shared variance as opposed to be independently functioning pathways. The NRG4/FGF21 ratio was intended to reflect the relative amounts of a protective adipose tissue secreted signal and a stress liver secreted signal. It achieved an AUC of 0.892 in the derivation subset and 0.879 in the internal-validation subset. However, the derivation AUC was only slightly different from that for NRG4 alone and there was no formal superiority. The comparable AUC in the internal-validation subsample provides evidence for the replicability of the signal within this dataset; however, the small validation sample and lack of a separate external cohort constrain the precision and generalizability of these estimates. There is no established clinical NRG4/FGF21 ratio or cutoff value yet.

One important design feature was the inclusion of a comparator group of obese individuals without steatosis by CAP analysis as many previous studies included only lean controls. However, the two obese groups were not comparable: the mean BMI and waist circumference were slightly higher in MASLD. Although BMI was included as a covariate in the multivariable regression models, adjustment for BMI alone may not fully capture visceral adiposity and body-fat distribution, which are important determinants of both MASLD and the circulating biomarkers examined in this study. The observed differences in HOMA-IR, triglycerides, liver enzymes, MDA, NRG4, and FGF21 therefore support a metabolic-liver phenotype associated with steatosis but do not exclude residual confounding by visceral adiposity. Diet, physical activity, sleep, medication exposure, and other unmeasured factors may also have contributed.

FIB-4 was higher in MASLD than in the other groups, but its absolute mean remained low. This finding is plausible in a relatively young sample dominated by CAP-defined steatosis without high LSM values. FIB-4 is useful for noninvasive fibrosis-risk stratification but has reduced precision in younger adults and should be interpreted alongside elastography

when risk remains uncertain [2,7,8]. Associations with FIB-4 in the present study therefore indicate relationships with routine fibrosis risk rather than histologically proven fibrosis.

Descriptive analyses by CAP-defined steatosis categories also revealed a decrease in mean NRG4 and an increase in mean FGF21 from S1 to S3. This is reminiscent of enhanced hepatic stress signaling and decreased adipose-derived protective signaling with increasing liver fat, although the small subgroups do not allow definitive statements. Dai et al. [15] previously failed to detect a meaningful NRG4 gradient among steatosis grades determined by standard ultrasonography. CAP is a useful quantitative index of hepatic attenuation, whereas MRI-PDFF provides more accurate noninvasive quantification of liver fat [36,37]. The FGF21 pattern observed here is in agreement with described correlations of circulating FGF21 with hepatic triglyceride content [25] and with histological severity of disease [27].

Strengths of the investigation include balanced groups, an included obesity comparator, concurrent evaluation of adipokine, hepatokine, metabolic, hepatic and oxidative measures, multiplicity adjusted comparisons, robust regression, and exploratory split-sample internal validation with bootstrap confidence intervals. The major limitations are the cross-sectional design not allowing causal interpretation; recruitment from a single geographic location; lack of liver biopsy or MRI-PDFF; residual confounding owing to visceral adiposity and lifestyle factors; potential ELISA platform variability; absence of inflammatory cytokines such as TNF-alpha and IL-6; and no PNPLA3 genotyping. The pooled correlations may, contribute at least in part to separating the two obesity groups, whereas the internal-validation subset, was too small to be used as independent external validation. In the future, studies should prospectively enroll representative multicenter Iraqi populations, preregister primary hypotheses, harmonize fasting sample collection and ELISA protocols, and measure visceral adiposity and liver fat by validated imaging techniques such as MRI-PDFF, if possible. A prespecified model should be developed in an adequately sized derivation cohort and tested by blinded external validation in an independent setting. Longitudinal measurements during weight loss or pharmacotherapy could determine whether changes in the index track reductions in liver fat and improvements in insulin sensitivity. Mechanistic studies should also examine adipose NRG4 expression, hepatic beta-Klotho signaling, and relevant genetic or environmental determinants.

Study limitations

There are multiple limitations of this study worth noting. First, the cross-sectional nature of this research does not allow us to establish causation or longitudinal correlations between NRG4, FGF21, insulin resistance, oxidative stress, and NAFLD. The participants were recruited at one research center. Hence, these findings cannot be extrapolated to the whole population of Iraq. Although FibroScan provided us with non-invasive measurements of hepatic fat and stiffness, we did not carry out liver biopsy or MRI-PDFF, and therefore could not validate the stage of inflammation and fibrosis and the exact percentage of liver fat. Internal validation sample size was small and the proposed NRG4/FGF21 cut-off requires further testing in larger independent multicenter cohorts. There could have been unknown confounders including visceral fat, diet, physical activity, sleep, socioeconomic characteristics, and medications. Levels of circulating NRG4 and FGF21 could vary due to assay-related factors and processing, as well as due to biological factors including fat mass distribution and liver sensitivity to receptors. We did not measure inflammatory biomarkers, adipose NRG4 expression, hepatic β-Klotho signaling and genetic variants such as PNPLA3. Thus, NRG4/FGF21 index can be considered as an exploratory biomarker only.

5 CONCLUSION

The findings describe a coherent low-NRG4/high-FGF21 phenotype associated with MASLD in adults with obesity. Reduced NRG4 was associated with greater insulin resistance, liver-enzyme activity, lipid peroxidation, and lower antioxidant capacity, whereas FGF21 showed the opposite pattern. The NRG4/FGF21 index demonstrated promising internal discrimination between MASLD and obesity without steatosis, but its performance was numerically similar to that of NRG4 alone. Prospective external validation, standardized assays, and more precise assessment of visceral adiposity and liver fat are required before clinical application.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

Disclosure of conflict of interest

The authors declare no conflicts of interest.

Data availability

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

Author contributions

All authors contributed to study conception, data analysis, manuscript preparation, and final approval.

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