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Research article

The association of Neuromedin U levels and non-alcoholic fatty liver disease: A comparative analysis

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ABSTRACT

This comprehensive study delves into the potential link between Neuromedin U (NmU) serum levels and the development of non-alcoholic fatty liver disease (NAFLD), a condition of increasing global prevalence and significant public health concern. The research provides a nuanced understanding of the disease's etiology by examining a cohort of 112 participants, including individuals with and without NAFLD. The study meticulously considers a spectrum of variables such as demographic factors, body composition metrics, and blood parameters. Advanced diagnostic tools like Fibroscan® are employed to ascertain NAFLD presence, ensuring accurate and reliable results.

The investigation reveals a noteworthy correlation between NAFLD and several risk factors, notably obesity, increased waist and neck circumferences, hypertriglyceridemia, and insulin resistance. These findings underscore the multifactorial nature of NAFLD and its intricate connection with metabolic syndromes. Intriguingly, the study observes lower NmU levels in individuals diagnosed with NAFLD. However, the role of NmU as an independent risk factor for NAFLD remains inconclusive, warranting further investigation. Although triglyceride level was observed to be an independent risk factor for NAFLD, this relationship was not associated with NmU.

This research contributes significantly to the existing knowledge on NAFLD, highlighting the disease's complexity and the interplay of various risk factors. It also opens up new avenues for future research, particularly in exploring the role of NmU within the metabolic pathways associated with NAFLD. The insights gained from this study could guide the development of novel diagnostic and therapeutic strategies for NAFLD, addressing a crucial need in contemporary healthcare.

In conclusion, the findings of this study not only enhance the understanding of NAFLD's pathophysiology but also emphasize the importance of comprehensive risk factor analysis in the management and prevention of this growing health concern.

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1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is the world's most common chronic liver disease, defined as the presence of fatty liver by imaging methods or histologically after differential diagnosis of secondary causes that cause fat accumulation in the liver. The prevalence of NAFLD worldwide is approximately 25%, and it has become a significant health problem that is highly prevalent among patients who are obese, have metabolic syndrome, and have type 2 diabetes mellitus [1,2]. Recent meta-analytical findings indicate a concerning upward trend in the global prevalence of Non-Alcoholic Fatty Liver Disease (NAFLD). Currently estimated at around 30%, this rising prevalence underscores the urgent need for increased awareness and proactive healthcare measures [3]. Known risk factors for NAFLD include genetic predisposition, environmental factors, age, gender, race, eating habits, metabolic syndrome, obesity, dyslipidemia, diabetes mellitus, hypertension, and some medication [4–7].

The intricate and controversial matter of renaming NAFLD to convey its metabolic foundations more precisely. The renaming of NAFLD to Metabolic (Dysfunction) Associated Fatty Liver Disease (MAFLD) and further to Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD) emphasizes the emphasis on metabolic dysfunction in the new terminology. Nevertheless, the act of renaming has ignited a contentious discussion among scientists regarding its potential ramifications on disease awareness, research, and patient treatment [8].

Neuromedin U (NmU) is a biologically active peptide that plays crucial roles in physiological processes. The NMU system, including NMU-specific receptors, plays a pivotal role in the contraction of smooth muscles and is implicated in eating behavior, energy expenditure, stress reactions, circadian rhythmicity, and inflammation [9–16].

Over the last ten years, several findings have shown that the NMU system plays various physiological functions concerning obesity and obesity-related illnesses in the central nervous system (CNS) and peripheral tissues [17].

Neuromedin U (NmU) is a neuropeptide with diverse roles in physiology, including its emerging significance in metabolic disorders like NAFLD. Research has indicated that NmU may be pivotal in regulating lipid metabolism and hepatic lipid accumulation [17,18]. Hepatosteatosis, characterized by excessive fat accumulation in the liver, is a hallmark of non-alcoholic fatty liver disease (NAFLD). Recent studies have shown that NmU and its receptors are expressed in the liver, and their activation may impact lipid homeostasis. NmU has been associated with regulating appetite and energy expenditure, which can indirectly influence hepatic fat storage [12,16, 17,19,20].

Furthermore, NmU's potential role in inflammatory and fibrotic pathways may contribute to the progression of NAFLD to more severe liver conditions. While the exact mechanisms by which NmU influences NAFLD are not elucidated yet, this neuropeptide has emerged as a promising avenue for understanding and potentially modulating the development and progression of NAFLD [17,18]. Further research in this field holds the potential to provide valuable insights into the pathogenesis of hepatosteatosis. It may open doors to novel therapeutic strategies for this increasingly prevalent metabolic disorder.

This study aims to compare the NAFLD risk factors, particularly NmU levels (the first human study in the literature), in volunteers with and without a diagnosis of NAFLD proven by Fibroscan.

2. Methods

Ethics statement: The local ethics committee approval (2011-KAEK-25 2019/07-01) was obtained, and the study was carried out in accordance with the declaration of Helsinki. Written informed consent was obtained from all participants.

A hundred and twelve volunteers between the ages of 18–65, who do not use drugs regularly, have any known chronic diseases (diabetes mellitus, chronic liver disease, chronic kidney disease, malignancy, etc.), and whose alcohol consumption is below 20 g/day, and no steatogenic drugs according to Patel et al. [21] used participants were included in the study.

The volunteers ' sociodemographic characteristics and detailed anamnesis were obtained, and physical examinations were performed. Blood pressure, waist circumference, and neck circumference were measured. Body fat mass (BFM), body fat ratio (BFR), Visceral fat ratio (VFR), Body muscle mass (BMM), body muscle ratio (BMR), and Bone mass (BM) were measured with Tanita®.

Venous blood samples were taken after 8 h of fasting for testing complete blood count (CBC), Neuromedin-U (NmU), fasting blood glucose (FBS), insulin, lipid profile [Total cholesterol, low-density lipoprotein (LDL), triglyceride, high-density lipoprotein (HDL)], aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), thyroid stimulating hormone (TSH) and plasma cortisol level.

Participants' visceral adiposity indexes were calculated using formulas created by Amato et al. [22], with the procedure specified separately for men and women using the participants' waist circumference, body mass index, triglyceride, and HDL values.

The participants' fatty liver and fibrosis levels were measured by sending low-frequency and amplitude vibrations over the probe with the Fibroscan® (transient elastography) device. Fatty liver was determined quantitatively by the controlled attenuation parameter (CAP™) feature, which was measured based on the principle of weakening of the signals created by the radiofrequency waves propagating backward. In our study, the participants were divided into groups using 257 dB/m as the threshold value for CAP, and Yilmaz et al. [23] reported that they could distinguish significant steatosis with 89% sensitivity and 83% specificity (AUROC = 0.93). According to Foucher et al. [24], the threshold values of 7.2 kPa or higher indicate moderate fibrosis, 12.5 kPa or higher indicate severe fibrosis, and 17.6 kPa indicate cirrhosis. The obtained data were compared between the groups.

3. Statistical analysis

The study examined the compliance of continuous variables to a normal distribution using the Shapiro-Wilk Test. Variables were

reported with median (minimum: maximum) and mean (± standard deviation) values. In the comparisons between the groups, the Kruskal-Wallis test was used if there was no compliance with the normal distribution, and the ANOVA test was used if there was no compliance with the normal distribution. Dunn Bonferroni approached the Kruskal-Wallis test in subgroup analysis in case of finding general significance after tests; After the ANOVA test, the Tukey test was used. Correlation Analysis was performed to examine the relationships between CAP Median values and continuous variables, and Pearson (Rho) or Spearman Correlation Coefficients (Rs) were calculated according to the data distribution. SPSS (IBM Corp. Released 2012. IBM SPSS Statistics for Mac, Version 26.0. Armonk, NY: IBM Corp.) program was used for statistical analysis, and $p < 0.05$ was considered statistically significant.

4. Results

Volunteers participating in the study were divided into two groups: those with (n = 49) and those without fatty liver (n = 63), according to CAP median measurements. There was no significant difference in gender distribution (Table 1). The mean age of all participants was 35.71 ± 10.49 years, and the mean age of those with NAFLD was observed to be higher in the intergroup comparison (Table 2). While a higher rate of fatty liver was found in those who were married and/or had children, the monthly incomes of the groups were similar (Table 1, Table 2). Education level demonstrated a significant association with NAFLD status (p = 0.034). Notably, individuals with higher education levels, particularly those with university-level education and above, were more prevalent in the non-NAFLD group compared to the NAFLD group. The smoking rate was found to be significantly higher in those with NAFLD, and the groups were similar in terms of alcohol use (<20 g/day) (Table 1).

When compared according to the obesity classification determined by the World Health Organization, it was noted that higher rates of NAFLD were observed in those with higher BMI. Regarding liver stiffness measurements, significant differences were noted (P = 0.009). A significant association was observed regarding liver stiffness measurement (LSM) with NAFLD status (p = 0.009). The NAFLD group exhibited a higher prevalence of moderate and severe fibrosis compared to the non-NAFLD group, where mild fibrosis was predominant (Table 1).

While no significant difference was observed between the heights of the groups, it was found that overweight patients had a higher rate of fatty liver. The waist circumference, neck circumference, systolic/diastolic blood pressure, and pulses of the participants with NAFLD were higher (Table 2).

In the comparison of complete blood count data, WBC and hemoglobin levels were significantly higher in patients with fatty livers. In contrast, platelet counts were found to be similar. While no significant difference was observed between creatinine and GGT levels, it was noted that AST and ALT levels were higher in patients with NAFLD. A significant decrease in HDL levels was noted in comparing lipid profiles, while total cholesterol, LDL, and triglyceride levels were more elevated in patients with NAFLD. TSH and cortisol levels were similar in participants whose insulin, blood glucose levels, and HOMA scores were significantly higher in participants with NAFLD. In comparing bioimpedance measurement data, BFM, BFR, VFR, and BMM measurements were higher in those with NAFLD. In contrast, BMR and BM measurements were significantly lower (Table 2).

Logistic regression analysis was performed to determine the risk factors affecting the development of NAFLD. The significant variables including age, gender, marital status, education level, BMI, waist circumference, neck circumference, SBP, DBP, heart rate, visceral adiposity index, WBC, hemoglobin, FBG, AST, ALT, total cholesterol, LDL, HDL, triglyceride, Insulin, HOMA-IR, NmU, Cortisol, BFM, BFR, VFR, BMM, BMR and BM in Table-1 and Table-2 are included for the analysis.

Table 1
Demographics.

	Non-NAFLD (n = 63)	NAFLD (n = 49)	P[a]
Sex (Male/Female)	24/39	26/23	0.114
Education, n (%)			**0.034**
Illiterate	0 (0)	1 (2)	
Primary School	3 (4.7)	9 (18.3)	
Middle School	2 (3.1)	5 (10.2)	
High School	10 (15.8)	8 (16.3)	
University and above	48 (76.1)	26 (53.0)	
Married, n (%)	25 (39.6)	41 (83.6)	**<0.001**
Alcohol consumed, n (%)	12 (19.0)	9 (18.3)	0.927
Smoker, n (%)	18 (28.5)	24 (48.9)	**0.027**
BMI (kg/m^2), n (%)			**<0.001**
<18.5	23 (36.5)	0 (0)	
18.5–24.9	21 (33.3)	1 (2)	
25–29.9	11 (17.4)	11 (22.4)	
30–34.9	6 (9.5)	10 (20.4)	
35–39.9	2 (3.1)	15 (30.6)	
>40	0 (0)	12 (24.4)	
Liver stiffness measurement, n (%)			**0.009**
Mild Fibrosis	63 (100)	44 (89.7)	
Moderate Fibrosis	0 (0)	4 (8.1)	
Severe Fibrosis	0 (0)	1 (2)	

[a] Chi-square test.

Table 2
The comparison of data between participants with and without fatty liver.

	Non-NAFLD (N = 63)		NAFLD (N = 49)		P
	Mean ± SD	Median (Min-Max)	Mean ± SD	Median (Min-Max)	
Age	30.62 ± 11.11	27 (18–56)	33.8 ± 7.43	32 (28–46)	<0.001[a]
SBP	115.38 ± 13.3	110 (100–150)	138 ± 8.37	140 (130–150)	<0.001[a]
DBP	70.77 ± 9.32	70 (60–90)	79 ± 8.22	75 (70–90)	<0.001[b]
Pulse	76.92 ± 11.64	70 (65–100)	90 ± 6.12	90 (80–95)	<0.001[a]
BMI	21.55 ± 5.77	20.1 (14.5–37.5)	39.44 ± 7.71	40.6 (30.1–50.8)	<0.001[a]
WC	81 ± 16.62	77 (57–123)	129 ± 11.4	130 (110–140)	<0.001[b]
NC	32.77 ± 4.62	32 (27–43)	43 ± 4.24	42 (37–48)	<0.001[b]
WBC	6607.69 ± 1595.77	6900 (3250–8900)	8062 ± 1082.97	7840 (6900–9300)	0.002[b]
Hemoglobin	13.82 ± 1.43	13.7 (11.8–16)	14.94 ± 1.49	15.1 (12.8–16.4)	0.017[b]
Platelet	222k±37k	222k(158k-283k)	239k±29k	251k (188k-262k)	0.304[b]
FBG	86.77 ± 10.54	82 (76–112)	85.4 ± 9.18	84 (77–101)	0.003[a]
Creatinine	0.64 ± 0.13	0.6 (0.5–0.9)	0.7 ± 0.12	0.7 (0.5–0.8)	0.215[b]
AST	16 ± 4.76	16 (9–27)	18.8 ± 7.05	16 (10–27)	0.010[b]
ALT	15.46 ± 8.93	13 (8–35)	31 ± 23.8	15 (11–58)	<0.001[a]
GGT	15.45 ± 11.39	12 (8–47)	36.6 ± 32.27	36 (9–89)	0.072[a]
Total Cholesterol	155.77 ± 30.46	152 (119–210)	219.8 ± 49.68	217 (152–290)	0.002[b]
HDL	54.15 ± 12.05	52 (34–72)	41.6 ± 9.61	46 (25–49)	<0.001[a]
LDL	86.54 ± 26.49	90 (57–140)	141 ± 31.56	143 (90–170)	0.003[b]
Triglycerides	73.46 ± 19.44	70 (44–104)	206.8 ± 242.56	113 (68–638)	<0.001[a]
TSH	2.12 ± 1.09	1.9 (0.2–3.8)	1.84 ± 0.55	1.7 (1.2–2.5)	0.206[b]
Cortisol	14.31 ± 4.99	13.7 (7.9–24)	12.74 ± 7.16	13.8 (3.2–22)	0.189[b]
Neuromedin U	9.13 ± 12.11	4.09 (0.14–37.38)	4.08 ± 3.26	4.34 (0.51–8.05)	0.001
Insulin	11.93 ± 10.14	9.9 (2–40.5)	20.16 ± 17.04	10.3 (8.2–47.9)	<0.001[a]
HOMA-IR	2.82 ± 2.81	1.9 (0.7–11.2)	4.48 ± 4.41	1.9 (1.6–11.9)	<0.001[a]
VAI	2.51 ± 1.02	2.49 (0.84–3.85)	8.08 ± 8.08	3.84 (2.75–22.08)	<0.001[a]
BFM	12.65 ± 11.98	13.7 (1–46.4)	50.1 ± 21.82	50.3 (22.7–83.2)	<0.001[a]
BFR	17.21 ± 10.83	18.1 (3–34.3)	41.32 ± 14.06	39.6 (23.8–62.4)	<0.001[b]
VFR	2.92 ± 3.71	1 (1–14)	17.8 ± 9.2	15 (8–31)	<0.001[b]
BMM	48.47 ± 14.53	44.6 (29.2–84.5)	64.22 ± 10.86	69 (46.6–73)	<0.001[b]
BMR	78.78 ± 10.51	77.8 (62.5–93.9)	55.58 ± 13.67	57.4 (34.9–72.4)	<0.001[b]
BM	2.56 ± 0.72	2.4 (1.6–4.3)	3.56 ± 0.23	3.6 (3.2–3.8)	<0.001[b]

BMI: body mass index. BP: blood pressure. CAP: controlled attenuation parameter. LDL: low-density lipoprotein. HDL: high-density lipoprotein. FBS: fasting blood sugar. AST: aspartate transaminase. ALT: alanine transaminase. GGT: gamma-glutamyl transferase. TSH: thyroid stimulating hormone. VAI: visceral adiposity index. BFM: body fat mass. BFR: body fat ratio. VFR (visceral fat ratio). BMM: body muscle mass. BMR: Body muscle ratio. BM: Bone mass. Normal distributed data were presented as mean ± SD. Data without normal distribution were presented as median and minimum–maximum values. a: Mann-Whitney *U* test. b: independent samples *t*-test.

Each variable was first examined by univariate logistic regression analysis, and variables meeting the $p < 0.05$ condition were analyzed by multivariate logistic regression analysis. As a result of univariate logistic regression analysis, all variables that meet the condition are included in the multivariate logistic regression analysis. The forward selection approach has been adopted as the variable selection method. Analysis results of the final step are presented in Table 3. The study encompassed a cohort of 112 participants, and the final model exhibited a noteworthy chi-square value of 36.951 ($p < 0.001$), indicating the model's overall effectiveness in elucidating the variability in NAFLD. The model's goodness-of-fit was assessed using the Hosmer-Lemeshow test, yielding a non-significant p-value of 0.982. This result indicates that the model adequately aligns with the observed data, bolstering its reliability in predicting the likelihood of NAFLD based on the included variables.

The analysis uncovered that waist circumference surfaced as a substantial predictor of NAFLD (Wald = 7.41, $p = 0.006$), with an odds ratio (OR) of 1.138 (95% CI: 1.04–1.25). This denotes that for each unit increase in waist circumference, the vulnerability to NAFLD escalates by a factor of 1.138 while keeping other variables constant. Similarly, neck circumference exhibited a robust

Table 3
Independent risk factors affecting steatosis.

n = 112				95% CI for OR	
	Wald	p-value	Exp-B	Lower	Upper
Waist Circumference	7.41	**0.006**	1.138	1.04	1.25
Neck Circumference	9.01	**0.003**	2.091	1.29	3.38
High-Density Lipoprotein	3.58	**0.058**	1.087	0.99	1.18
Triglyceride	4.71	**0.030**	1.027	1.00	1.05
Bone Mass	6.46	**0.011**	0.061	0.07	0.52
	Final Model $\chi^2 = 36.951$; **p<0.001**				
	Hosmer-Lemeshow Test p = 0.982				

OR: Odds Ratio, CI: Confidence Interval.

association with NAFLD (Wald = 9.01, p = 0.003), manifesting an OR of 2.091 (95% CI: 1.29–3.38). The outcomes imply that individuals with larger neck circumferences are over twice as likely to experience NAFLD as those with smaller neck circumferences. HDL levels showcased a trend toward significance (Wald = 3.58, p = 0.058), featuring an OR of 1.087 (95% CI: 0.99–1.18). Although the p-value did not achieve conventional significance, the trend suggests that lower HDL levels might contribute to NAFLD risk. Triglyceride levels are notably associated with NAFLD (Wald = 4.71, p = 0.030), revealing an OR of 1.027 (95% CI: 1.00–1.05). This indicates that a one-unit increase in triglyceride levels corresponds to a 2.7% escalation in the odds of developing NAFLD.

Interestingly, bone mass substantially impacted NAFLD risk (Wald = 6.46, p = 0.011), with an OR of 0.061 (95% CI: 0.07–0.52). This suggests that for each unit increase in bone mass, the odds of NAFLD significantly diminish. Thus, NAFLD appears to be a severe risk factor for loss of bone mass.

In summary, this study identified waist circumference, neck circumference, triglyceride levels, and bone mass as independent risk factors significantly influencing the development of NAFLD. These findings provide a nuanced understanding of the intricate nature of risk factors associated with NAFLD and contribute to ongoing efforts to unravel the complexities of this prevalent metabolic disorder.

The relationship between NmU and clinical and metabolic parameters was evaluated using Spearman's rank correlation coefficient (rho) in Table 4. Key findings include significant negative correlations between NmU and CAP-Median (rho = −0.275, p = 0.003), E-Median (rho = −0.203, p = 0.032), BMI (rho = −0.354, p < 0.001), waist circumference (rho = −0.339, p < 0.001), and neck circumference (rho = −0.335, p < 0.001). Furthermore, insulin levels (rho = −0.211, p = 0.026) and HOMA (rho = −0.228, p = 0.016) were negatively correlated with NmU. No significant correlations were found with visceral adiposity index, HDL, triglycerides, total cholesterol, LDL, or cortisol. These results suggest a noteworthy association of Neuromedin U with obesity-related measures and insulin resistance markers.

5. Discussion

In our study, it becomes evident that identifying and understanding factors contributing to the risk of NAFLD holds paramount importance. Our investigation reveals a compelling association between various salient parameters and the propensity for NAFLD. Notably, individuals afflicted with NAFLD exhibit distinctive characteristics, including increased waist and neck circumferences, elevated triglyceride levels, and diminished bone mass (BM), independently promoting the susceptibility to NAFLD. However, the focal point of our discussion lies in the noteworthy correlation we unveil – that of NmU levels, which present a compelling relationship with the risk of NAFLD. This revelation prompts a persuasive rationale for further, more in-depth investigations into the intricate role of NmU in NAFLD pathogenesis, thereby setting the stage for a more nuanced and comprehensive exploration in future research endeavors.

It has been shown that the prevalence rate of NAFLD is higher in people aged 40 years and older than in other age groups [6]. In accordance with the literature, fatty liver disease increased statistically significantly with age in our study. In our study, there was no significant difference between NAFLD and gender. In contrast, the rate of fatty liver disease was lower in people with higher education levels. Lin et al. [7] also reported that the rate of fatty liver disease was lower in people with higher education levels. Still, unlike our study, fatty liver disease was higher in women than in men. In our results, the fatty liver rate was higher in those who were married and/or had children. This result may be explained by decreased physical activity in these groups.

The relationship between smoking and the development of NAFLD is of growing importance in medical research, and recent studies have yielded valuable insights into this connection. As our study did, it is intriguing to observe that the incidence of fatty liver disease was notably higher in smokers. However, when scrutinizing the association between smoking and NAFLD, it is crucial to account for various confounding factors. The current study has elucidated that while an increased prevalence of fatty liver among smokers is evident, logistic regression models may not consistently designate smoking as an independent risk factor. The association of smoking with advanced fibrosis in patients with NAFLD has been demonstrated in a large multicenter cohort study [25]. Souza et al. [26]

Table 4
The evaluation of the relationship between NmU and various clinical and metabolic parameters.

	Neuromedin U	
	Spearman's Rho	p
CAP median, dB/m	**−0.275**	**0,003**
Elastography median, kPa	**−0.203**	**0,032**
BMI	**−0.354**	**0,000**
Waist Circumference	**−0.339**	**0,000**
Neck Circumference	**−0.335**	**0,000**
Insulin	**−0.211**	**0,026**
HOMA-IR	**−0.228**	**0,016**
Visceral Adiposity Index	−0,075	0,434
Total Cholesterol	−0,027	0,779
Triglyceride	−0,099	0,298
LDL	−0,029	0,758
HDL	0,101	0,288
Cortisol	0,038	0,692

CAP: controlled attenuation parameter, BMI: body mass index, HDL: high-density lipoprotein, LDL: low-density lipoprotein, Bold values represents statistical significance.

reported that smoking may lead to partial progression of NAFLD through its effect on insulin resistance and may lead to increased fibrosis. Jang et al. [27] conducted an in-depth study on the relationship between smoking habits and NAFLD in 9603 people. It showed that the risk of NAFLD was 1.38 and 1.12 times higher in smokers and quitters, respectively, compared to non-smokers. The researchers also reported that the risk of adiposity was 1.39 times higher in smokers with 10–20 packs/year and 1.51 times higher in smokers with>20 packs/year. While the exact mechanisms underlying this association remain the subject of ongoing research, it is increasingly evident that smoking may play a role in the development and exacerbation of NAFLD. These findings emphasize the importance of smoking cessation as a potential preventive measure for NAFLD. Such results highlight the intricate web of factors involved in fatty liver development, underlining the need for comprehensive investigations to disentangle the multifaceted pathogenesis of this disease.

Obesity is considered the most prominent and independent risk factor for NAFLD. Among obese patients with a BMI >30 kg/m^2, the prevalence of NAFLD is over 91% [28–30]. Ma et al. [31] reported that the risk for NAFLD increased 1.7, 1.9, and 9.1-fold in overweight, grade 1 obese, and grade 2 obese patients, respectively, compared with the normal BMI group. Increased waist circumference, an indicator of abdominal fat accumulation, is a risk factor for fatty liver in many studies [30,32]. Neck circumference, a significant predictor of metabolic syndrome and NAFLD, is a valid substitute for measuring subcutaneous fat storage in the upper body [33–35]. Our study showed that the rate of fatty liver was significantly increased in patients with high BMI and increased waist and neck circumference. We also observed that neck and waist circumferences were independent risk factors for NAFLD.

Insulin resistance (IR) is considered a significant risk factor in NAFLD. Oxidative stress and inflammation accelerate disease progression [36]. IR, one of the essential cellular abnormalities in the development of type 2 DM and NAFLD, is recognized as an integral part of NAFLD pathogenesis and disease progression [37]. Studies with large groups of patients with fatty liver, insulin resistance, and hyperinsulinemia have been emphasized as the laboratory findings most closely associated with NAFLD [38–40]. In our study, blood glucose and IR were significantly elevated in patients with NAFLD, consistent with the literature.

In parallel with our study, many studies have shown that patients with hyperlipidemia have a higher prevalence of NAFLD than those with normal blood lipid levels [41]. Patients with severe hypertriglyceridemia and mixed hyperlipidemia have been reported to have a 5- or 6-fold increased prevalence of fatty liver compared to ordinary people [42]. In the regression analysis model in our study, we observed that serum triglyceride level was an independent risk factor that increased the risk of NAFLD 1.027-fold. Although serum ALT level is an indicator of liver disease, it can be found at high levels in many liver diseases other than NAFLD. Increased ALT levels in patients with NAFLD indicate the severity of necroinflammation rather than steatosis or fibrosis [43]. In our study, although serum AST and ALT levels were within normal limits, they were higher in the NAFLD patient group.

Bone mineral density (BMD) is known to be decreased in patients with advanced, severe liver disease. Our study observed that bone mass (BM), determined by impedance measurements, was lower in the group with NAFLD. Evidence has been found to suggest an increased risk of osteopenic fractures in patients with NAFLD [44]. Additionally, reduced BMD has been found in patients with metabolic syndrome and NAFLD as a part of it [45–48]. Many studies show a decrease in BM and an increased risk of osteoporosis in NAFLD patients without advanced liver disease [49–51]. A decrease in BMD was found in obese children with NAFLD compared to those without NAFLD, which was thought to be related to insulin resistance. In a study where low BMD was observed in obese children with NAFLD, a positive correlation was observed between BMD and fat mass. In contrast, a negative correlation was found between BMD and insulin resistance and leptin [49–51]. In another study, postmenopausal women with NAFLD had lower lumbar spine BMD than those without NAFLD [52]. Although all these studies have found a relationship between NAFLD and decreased BM, their relationship has yet to be fully elucidated. It is thought that many factors, such as some cytokines secreted from the inflamed liver, vitamin D deficiency, and decreased physical activity, may explain the decreased BMD in NAFLD [53]. Several independent studies have found that the circulating tumor necrosis factor-alpha (TNF-α) level increases in patients with NAFLD. TNF-α has essential roles in the pathophysiology of hepatic inflammation and bone loss [54–56]. There is evidence showing that TNF-α leads to inhibition of activation of osteoblasts and stimulation of osteocalcigenesis [57].

Osteocalcin is an amino acid secreted from osteoblasts that play an essential role in bone formation and calcium homeostasis [58]. Decreased osteocalcin concentrations, a sensitive marker for bone formation, are associated with postmenopausal osteoporosis [59,60]. Yilmaz et al. [61] reported decreased serum osteocalcin levels in NAFLD patients diagnosed by biopsy and that this finding was significantly related to the ballooning of hepatocytes, insulin resistance, and metabolic syndrome.

Similar findings were found in studies conducted with fetuin-A, osteoprotegerin, and osteopontin in patients with NAFLD [53]. In our study, we also found a decrease in BM in patients with NAFLD. When all the information and our findings are evaluated together, the reduction in BM is not a risk factor for NAFLD. On the contrary, there is a decreased BM and an increased risk of osteopenic fracture in patients with NAFLD.

NmU, a neuropeptide secreted mainly from the brain, adipose tissue, and gastrointestinal tract, plays a role in important events such as appetite regulation and energy homeostasis and also has many functions, such as bone remodeling, smooth muscle contraction, and gastric secretion regulation [62]. Some evidence has been found to suggest that NmU has significant effects on hepatosteatosis and eating behaviors. In transgenic rat models, it has been shown that NmU null rats have hyperphagia and decreased energy consumption, increased adiposity, decreased insulin sensitivity, and increased bone mass [10,63]. In experimental studies, intracerebrovascular administration of NmU in rats caused a decrease in food intake and nutrition-related behaviors, decreased weight loss, and increased locomotor activity and heat production [19,20,64,65]. It has been shown that rats have increased insulin sensitivity following NmU injection [66]. In another clinical study, it has been demonstrated that NmU suppresses glucose-induced insulin secretion through mitochondrial dysfunction and endoplasmic reticulum [67]. In our study, we observed that the NmU level was significantly lower in patients with NAFLD, but NmU was not identified as an independent risk factor.

Although the relationship between obesity, fatty liver, and NmU has been shown in the animal studies mentioned above, no study

has evaluated the relationship in humans. Our study is the first to assess the relationship between NAFLD and NmU in humans. Our findings reveal that the relationship between NmU and NAFLD is similar to animal studies. The fact that NmU levels are significantly low in patients with NAFLD and do not stand out as an independent risk factor suggests that NmU plays a role in the pathogenesis of NAFLD by affecting metabolic pathways. Studies with more patients are needed to determine which metabolic pathways it affects in NAFLD.

This study explores the complex relationship between NmU and several clinical and metabolic factors, revealing the crucial role of NmU in regulating metabolism. The study employs Spearman's rank correlation coefficient to identify significant connections that provide insights into the role of NmU in obesity-related pathophysiology and insulin resistance. These correlations are crucial for comprehending the intricate mechanisms of metabolic diseases.

Our study discovered a substantial negative correlation between NmU and adiposity metrics such as BMI, waist circumference, and neck circumference (rho values ranging from -0.335 to -0.354, $p < 0.001$). Recent studies further elucidate the role of Neuromedin U (NmU) in metabolic disorders and its potential implications in regulating body weight and energy balance. Teranishi and Hanada [17] provided a comprehensive overview of the physiological roles of NmU, particularly in feeding behavior, energy expenditure, and insulin secretion, highlighting its direct role in obesity pathophysiology. Malendowicz and Ruciński [68] focused on the detailed role of NmU and NMS in controlling energy homeostasis, mentioning the need for highly specific NMUR1 and NMUR2 receptor antagonists for a deeper understanding of NMU/NMS mechanisms. These recent insights provide a more nuanced understanding of NmU's role in metabolic regulation, with implications for future research and therapeutic approaches in treating obesity and related metabolic conditions. These discoveries are significant, given the rapidly growing worldwide obesity crisis and the pressing requirement for new therapeutic objectives.

The negative associations between NmU, CAP-Median, and E-Median indicate that NmU may have a protective impact against hepatic steatosis and fibrosis. The recent studies on hepatic steatosis and fibrosis offer valuable insights, though they do not directly address the role of Neuromedin U (NmU). Bae et al. [69] underscored the intricate relationship between hepatic steatosis, liver fibrosis, and insulin resistance, emphasizing the need to identify better and manage metabolic-associated fatty liver disease. Mak et al. [70] delved into the complex interplay between chronic hepatitis B and NAFLD, highlighting how hepatic steatosis can influence fibrosis progression and hepatitis B surface antigen seroclearance. Additionally, Seto et al. [71] provided evidence of a link between hepatic steatosis and increased fibrosis burden in chronic hepatitis B, further illustrating the impact of steatosis on liver health. These studies contribute to our understanding of hepatic steatosis and fibrosis, albeit without directly connecting to NmU, and underline the importance of addressing metabolic factors in liver disease management.

The negative correlations established between NmU and insulin levels and HOMA (rho $= -0.211$ and -0.228, respectively) highlight the impact of NmU on insulin sensitivity and glucose metabolism. The recent literature does not address the relationship between NmU and insulin resistance. Kaczmarek et al. [72] showed that NmU receptors are expressed in pancreatic islets and that NmU dose-dependently decreased insulin output by isolated pancreatic islets, suggesting a role in regulating insulin secretion. Zhang et al. [67] found that NmU induced mitochondrial dysfunction and endoplasmic reticulum stress in pancreatic β-cells, leading to suppression of glucose-stimulated insulin secretion, which might influence insulin resistance. Doggrell et al. [66] noted that NmU $-/-$ mice become obese with higher insulin levels, suggesting a potential link between NmU and insulin sensitivity. These studies indicate a complex interplay between NmU, insulin secretion, and potentially insulin resistance, although the direct relationship remains to be clearly established.

The metabolic impact of NmU is characterized by its specificity, as it largely affects adiposity and glycemic control without significant associations with lipid profile components and cortisol levels. Hanada et al. [10] found that mice lacking NmU developed obesity, hyperleptinemia, hyperinsulinemia, late-onset hyperglycemia, and hyperlipidemia. Their study highlighted NmU has an essential role in regulating feeding behavior and energy metabolism independent of the leptin signaling pathway, impacting adiposity and glycemic control but not explicitly mentioning effects on lipid profiles or cortisol levels. Dalbøge et al. [73] observed that a lipidated NMU analog reduced gastric emptying and food intake in mice and improved glycemic control, again underscoring NmU has a role in energy homeostasis and glycemic regulation, with no mention of its effects on lipid profiles or cortisol. Neuner et al. [74] developed a Neuromedin U–human serum albumin conjugate as a long-acting candidate for treating obesity and diabetes. This study points to NmU has the potential to manage adiposity and glycemic control, again without specific reference to effects on lipid profiles or cortisol levels. Peier et al. [75] found that peripheral administration of NmU in rodents reduced food intake and body weight, increased core body temperature and metabolic rate, and improved glucose excursion. This study supports NmU has a role in energy and glucose homeostasis without directly associating it with lipid profiles or cortisol levels. These studies collectively highlight the specific metabolic impact of NmU on adiposity and glycemic control, independent of significant associations with lipid profiles and cortisol levels.

Identifying NmU's notable connections with crucial metabolic factors underscores its potential as both a biomarker and a target for therapeutic interventions. Further research is needed to investigate the timing and cause-and-effect linkages related to NmU.

To summarize, this study enhances our comprehension of NmU's function in metabolic well-being, highlighting its potential in obesity and insulin resistance. The results, which suggest that NmU plays a complex role in regulating metabolism, provide us with opportunities for additional investigation in this fascinating area of endocrine research.

6. Conclusion

Obesity, increased waist and neck circumference, hypertriglyceridemia, and insulin resistance are important risk factors for NAFLD. Low BMD may increase the risk of osteopenic fracture in patients with NAFLD. A decrease in NmU levels may be an essential

risk factor for NAFLD; more extensive studies are needed to elucidate this relationship.

CRediT authorship contribution statement

Murat Keskin: Writing – original draft, Investigation, Data curation, Conceptualization. **Sercan Avul:** Writing – original draft, Investigation, Data curation, Conceptualization. **Aylin Beyaz:** Investigation, Data curation. **Nizameddin Koca:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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