Muğla Sıtkı Koçman Üniversitesi Tıp Dergisi 2026;13(1):30-37
Medical Journal of Mugla Sitki Kocman University 2026;13(1):30-37
Doi: 10.47572/muskutd.1706423

Research Article

Comparing The Levels of Serum Netrin-1, Nesfatin-1 and Adropin in Subclinical and Overt Hypothyroid Patients

Subklinik ve Aşikar Hipotiroid Hastalarda Serum Netrin-1, Nesfatin-1 ve Adropin Düzeylerinin Karşılaştırılması

Emine GEMCİ[1*], Gülhan AKBABA[2], Neşe ÇINAR[2], Tuba EDGÜNLÜ[3], Kürsad TOSUN[4]

[1]Bursa City Hospital, Department of Internal Medicine, Division of Geriatrics, Bursa, Turkey

emine_koca@hotmail.com.tr

[2]Muğla Sıtkı Koçman University Faculty of Medicine, Department of Internal Medicine, Division of Endocrinology and Metabolism, Muğla, Turkey

[3]Muğla Sıtkı Koçman University Faculty of Medicine, Department of Medical Biology, Muğla, Turkey

[4]Siena College, Loudonville, NY, 12211, USA

*Sorumlu Yazar / Corresponding Author

Received: 26.05.2025
Accepted: 26.02.2026

Abstract

Netrin-1, nesfatin-1, and adropin are regulatory peptides that play significant roles in energy homeostasis, inflammation, cardiovascular function, and appetite regulation. The present study was designed to assess serum concentrations of netrin-1, nesfatin-1 and adropin in patients with overt hypothyroidism (OH) and subclinical hypothyroidism (SH), and to examine their relationships with thyroid function tests and metabolic parameters. This single-center, cross-sectional study was performed prospectively. A total of 83 people: 27 OH patients, 29 SH patients and 27 control group were included in our study. Body mass index (BMI), WC (waist circumference), free triiodothyronine (fT3), free thyroxine (fT4), thyroid stimulating hormone (TSH), anti-thyroid peroxidase (anti-TPO), anti thyroglobulin (anti-TG), fasting blood glucose (FBG), insülin, homeostatic model assessment of insulin resistance (HOMA-IR), triglyceride (TG), total cholesterol (TOTAL-C), low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C), high density lipoprotein cholesterol (HDL-C), C-reactive protein (CRP), Netrin-1, Nesfatin-1, Adropin were measured. The BMI, WC, TSH, anti-TPO, FBG, TOTAL-C and LDL-C levels were significantly higher in the OH group than in the control group ($p=0.014$, $p=0.007$, $p<0.001$, $p<0.001$, $p<0.001$, $p=0.024$, $p=0.025$ respectively). There was no significant difference among the groups regarding all three molecules. Considering all cases, there was a relationship between adropin and TG, TOTAL-C, LDL-C and VLDL-C ($r=-0.314$, $p=0.007$; $r=-0.340$, $p=0.003$; $r=-0.305$, $p=0.009$; $r=-0.381$, $p=0.001$ respectively). The correlation analysis performed separately for the patient group revealed a correlation between nesfatin-1 and fT3 ($r=0.360$, $p=0.015$). This study provides new evidence on the relationship between netrin-1, nesfatin-1, and with thyroid hormone regulation.

Keywords: Adropin, Hypothyroidism, Nesfatin-1, Netrin-1, Thyroid.

Özet

Netrin-1, nesfatin-1 ve adropin; enerji homeostazı, inflamasyon, kardiyovasküler fonksiyon ve iştah düzenlemesi gibi fizyolojik süreçlerde rol oynayan düzenleyici peptitlerdir. Çalışmamız, aşikâr hipotiroidizm (AH) ve subklinik hipotiroidizm (SH) hastalarında serum netrin-1, nesfatin-1 ve adropin konsantrasyonlarını değerlendirerek; tiroid fonksiyon testleri ve metabolik parametrelerle ilişkilerini incelemek amacıyla tasarlanmıştır. Bu boşluğu ele alarak, araştırmamız bu moleküllerin tiroid ilişkili metabolik düzensizliklerdeki olası rolünün daha iyi anlaşılmasına katkıda bulunmayı amaçlamaktadır. Bu tek merkezli kesitsel çalışma prospektif olarak gerçekleştirilmiştir. Çalışmamıza 27 AH hastası, 29 SH hastası ve 27 kontrol grubu olmak üzere 83 kişi dahil edildi. Vücut kitle indeksi (VKİ), bel çevresi (BÇ), serbest triiodothyronin (sT3), serbest tiroksin (sT4), tiroid stimüle edici hormon (TSH), tiroid peroksidaz antikoru (anti-TPO), tiroglobulin antikoru (anti-TG), açlık kan şekeri (AKŞ), insülin, homeostatik model değerlendirmesi (HOMA-IR), trigliserid (TG), total kolesterol (TOTAL-K), düşük dansiteli kolesterol (LDL-K), çok düşük dansiteli kolesterol (VLDL-K), yüksek dansiteli kolesterol (HDL-K), C reaktif protein (CRP), Netrin-1, Nesfatin-1, Adropin ölçüldü. BKİ, BÇ, TSH, anti-TPO, AKŞ, TOTAL-K ve LDL-K düzeyleri AH grubunda kontrol grubuna göre anlamlı olarak daha yüksekti (sırasıyla $p=0,014$, $p=0,007$, $p<0,001$, $p<0,001$, $p<0,001$, $p=0,024$, $p=0,025$). Her üç molekül için de gruplar arasında anlamlı bir fark yoktu. Tüm vakalar değerlendirildiğinde, adropin ile TG, TOTAL-C, LDL-C ve VLDL-C arasında bir ilişki vardı (sırasıyla $r=-0,314$, $p=0,007$; $r=-0,340$, $p=0,003$; $r=-0,305$, $p=0,009$; $r=-0,381$, $p=0,001$). Hasta grubu için ayrı ayrı yapılan korelasyon analizi, nesfatin-1 ile sT3 arasında bir korelasyon olduğunu ortaya koydu ($r=0,360$, $p=0,015$). Bu çalışma, netrin-1, nesfatin-1 ve adropin ile tiroid hormonu regülasyonu arasındaki ilişkiye dair yeni kanıtlar sunması bakımından literatüre özgün bir katkı sağlamaktadır.

Anahtar Kelimeler: Adropin, Hipotiroidizm, Nesfatin-1, Netrin-1, Tiroid.

Introduction

Primary hypothyroidism is characterized by elevated serum thyroid-stimulating hormone (TSH) levels accompanied by reduced free thyroxine (fT4) concentrations (1). Subclinical hypothyroidism (SH) is defined by elevated TSH levels with normal fT4 concentrations and its diagnosis relies primarily on biochemical evaluation. While all patients with overt hypothyroidism (OH) require thyroid hormone replacement therapy (1), the management of SH remains controversial.

SH has been associated with increased diastolic blood pressure and elevated levels of total cholesterol (TOTAL-C)___and low-density lipoprotein cholesterol (LDL-C). In addition, plasma homocysteine levels, the homeostatic model assessment of insulin resistance (HOMA-IR), C-reactive protein (CRP), and factor VII concentrations have been reported to be higher in patients with SH compared to euthyroid subjects (2). These alterations contribute to impaired myocardial contractility, increased vascular stiffness, and endothelial dysfunction. Elevated TSH levels have also been linked to an increased risk of cardiovascular disease (2,3). Nevertheless, the cardiovascular consequences and underlying pathophysiological mechanisms of SH remain incompletely understood.

Netrin-1, a member of the neuronal guidance protein (NGP) family, regulates axonal migration and neuronal growth during central nervous system development. Beyond its neurotrophic functions, NGPs also exert non-neuronal effects that are relevant to atherosclerosis (4,5). Experimental findings regarding Netrin-1 are conflicting: some studies suggest an atheroprotective role through inhibition of leukocyte recruitment to lesion-prone sites, whereas others demonstrate pro-atherogenic effects by restricting macrophage egress from atherosclerotic plaques and enhancing cytokine production (4).

Netrin-1 can be derived from endothelial cells, macrophages, or exogenous sources. Endothelial-derived Netrin-1 appears to exert protective effects against atherosclerosis, whereas macrophage-derived Netrin-1 promotes pro-inflammatory and pro-atherogenic processes (4). Moreover, overexpression of Netrin-1 has been shown to prevent diabetes-induced vascular endothelial dysfunction and suppress inflammatory and apoptotic pathways (6).

Nesfatin-1, an anorexigenic peptide, is widely expressed in the central nervous system, including the hypothalamic, supraoptic, and paraventricular nuclei, the arcuate nucleus, and the tractus solitarius. It regulates food intake (7), and has been shown to suppress feeding even in leptin-deficient models (8). Owing to its anatomical distribution, nesfatin-1 is believed to influence multiple physiological processes, including feeding behavior, neuroendocrine regulation, autonomic control, mood, sleep, and pain perception. In hyperglycemic mice intravenous nesfatin-1has been shown to reduce blood glucose levels (9, 10).

Adropin, encoded by the Enho gene, is expressed in multiple tissues including the liver, pancreas, heart, vascular tissue, kidney, and brain. It plays a key role in energy homeostasis and glucose metabolism. Experimental data indicate that adropin ameliorates insulin resistance by reducing hepatic glucose production (11). Low circulating adropin concentrations have been correlated with metabolic syndrome, suggesting a potential protective role (12). Reduced adropin levels have also been associated with endothelial dysfunction, supporting its utility as a biomarker of vascular impairment in metabolic syndrome (13). In animal models, both hypothyroidism and hyperthyroidism have been associated with decreased serum adropin concentrations (14).

Both hyperthyroidism and hypothyroidism are associated with increased oxidative stress in humans and animals (15). TSH may also modulate thermogenesis, appetite, lipolysis, and lipogenesis, thereby influencing basal metabolic rate and BMI (16). Given the interplay between hypothyroidism, chronic inflammation, altered energy metabolism, and obesity, the present study aims to evaluate the roles of nesfatin-1, netrin-1, and adropin in these pathways. This study therefore seeks to determine their potential associations with subclinical and overt hypothyroidism.

Material and Method

Patient Selection and Study Design

This study was conducted between March 2017 and October 2017 in patients who consulted the Department of Endocrinology and Metabolism, Mugla Sitki Kocman University School of Medicine, Mugla, Türkiye. A total of 83 participants were enrolled, including 29 SH patients, 27 OH patients and 27 controls. Patients in the SH group were defined by serum TSH levels of 4.2–10 mIU/L with normal fT4 concentrations (1). OH was defined by elevated TSH levels with

decreased serum fT4 concentrations (1). The control group included individuals without thyroid disease or chronic systemic disorders. Exclusion criteria included type 2 diabetes mellitus, acute renal failure, coronary artery disease, heart failure, cerebrovascular accident, malignancy, rheumatic disease, and hepatic disease. Patients receiving levothyroxine therapy in either the OH or SH groups were also excluded. At baseline, demographic and anthropometric parameters were recorded, including age, weight, height, body mass index (BMI) [BMI; weight (kilograms)/ height (meters) ^{2}] and hip circumferences and waist circumference (WC), (waist: midway between the lower rib margin and the iliac crest). Fasting blood glucose (FBG), fasting insulin and lipid profile were measured. HOMA-IR [fasting insulin (μU/ml) x FPG (mmol/L)] / 22.5) and LDL-C (TOTAL-C- [high density lipoprotein cholesterol (HDL-C) + (triglyceride (TG)/5)]) were calculated. Patients with HOMA-IR value 2.7 and above are considered to have insulin resistance (17). Netrin-1, nesfatin, and adropin levels, as well as BMI, WC, fT3, fT4, TSH, anti-TPO, anti-TG, FBG, HOMA-IR, TG, TOTAL-C, LDL-C, VLDL-C, HDL-C, and CRP values were compared among the SH, OH, and control groups.

Ethics committee approval

The study was conducted with the approval of Muğla Sıtkı Koçman University Faculty of Medicine Clinical Research Ethics Committee dated 16/03/2017 and numbered 5.

Biochemical analysis

4 cc venous blood was taken from the patients into the biochemical tube after about 8 hours fasting. The samples were centrifuged for 5 minutes at 4000xg after being kept at room temperature for 30 minutes, and the obtained sera were taken into ependorfs and stored at -80 °C until analysis. When the mentioned numbers of patients and controls had been obtained, samples stored at -80 °C were dissolved in sufficient amounts from ependorfs and thawed at room temperature to study serum netrin-1, nesfatin-1 and adropin levels. The molten samples were measured using the ELISA method using an Austrian made Molecular Devices SpectraMax i3 Multi-Mode, Microplate Reader with serial number 35 370-1448. The study was conducted according to the kit prospectus. To measure serum netrin-1 levels, Biont brand (catolog number:YLA1764HU) netrin-1 ELISA kit, to measure serum nesfatin-1 levels, Biont brand (catolog number:YLA0715HU) nesfatin-1 ELISA kit, to measure serum adropin levels, Biont brand (catolog number:YLA0019HU) adropin ELISA kit were used. Serum netrin-1 level was measured in units of pg/ml, nesfatin-1 level in ng/ml and adropin level in ng/L. The kits were stored at -20 °C before the study. Thyroid function tests and thyroid antibodies were studied by Roche brand Cobas c 8000 electrochemiluminescence assay. Serum glucose and lipid levels were measured by Roche Cobas c 8000 spectrophotometric method.

Statistical Analysis

Welch t-test or Wilcoxon-Mann-Whitney Test was applied in the comparison of healthy individuals with patient groups taking the distribution of data into account. The summary statistics of the data corresponding to the Welch t-test were expressed as mean ± standard deviation, and those of others are expressed as median, minimum, and maximum. Associations between the groups with sex and HOMA-IR were assessed by the Fisher's Exact Test. Correlation of adropin, nesfatin-1, and netrin-1 with free triiodothyronine (fT3), fT4, TSH, anti-thyroid peroxidase (anti-TPO) and anti thyroglobulin (anti-TG) were performed by Spearman analysis. A p-value less than 0.05 was considered statistically significant. To account for multiple comparisons among the three groups (OH, SH, and Control), a Bonferroni correction was applied, and a corrected p-value of less than 0.0167 was considered statistically significant.

Results

A total of 83 people were enrolled, including 29 (26 women and 3 men) SH patients, 27 (24 women and 3 men) OH patients and 27 (20 women and 7 men) control groups. The comparison of the biochemical and clinical characteristics of the study groups is depicted in Table 1. No significant difference was observed in the groups between the ages.

The BMI, WC, TSH, anti-TPO, FBG, TOTAL-C and LDL-C levels were significantly higher in the OH group than in the control group ($p=0.014$, $p=0.007$, $p<0.001$, $p<0.001$, $p<0.001$, $p=0.024$, $p=0.025$, respectively; Table 1). fT3 and fT4 levels were significantly higher in the control group than in the OH group ($p=0.004$, $p<0.001$, respectively; Table 1).

Table 1. Comparison of clinical and biochemical characteristics of OH, SH, and control groups

	OH ***n =27***	**SH** ***n =29***	**C** ***n =27***	**C vs OH** **p value**	**C vs SH** **p value**	**OH vs SH** **p value**
Age	34.95±8.84	33.11±9.59	30.48±7.05	0.184	0.502	0.750
BMI	28.27± 5.57	24.82± 5.65	23.79± 4.49	**0.014***	0.755	0.075
WC (cm)	93.40±12.27	85.75±14.40	80.74±13.89	**0.007***	0.381	0.151
fT3 (pmol/L)	3.89 (0.40 -8.32)	4.69 (4.00-5.38)	4.77 (3.90-5.72)	**0.004***	0.947	**0.009***
fT4 (ng/dL)	9.60 (0.40-14.66)	14.56 (8.83-20.31)	16.21 (11.80-20.86)	**<0.001***	0.120	**<0.001***
TSH (mIU/L)	34.00 (10.05-100.00)	5.90 (4.33-9.86)	2.01 (0.45- 3.69)	**<0.001***	0.643	**<0.001***
anti-TPO (IU/mL)	288.45 (5.73-600.00)	122.26 (5.00-600.00)	9.68 (5.00- 20.43)	**<0.001***	**0.045***	**0.004***
anti-TG (IU/mL)	537.23 (10.00-4000.00)	329.45 (10.00-2927.00)	76.61 (10.00-1192.00)	0.074	0.381	0.577
FBG (mg/dL)	94.26 (80.00-177.00)	88.26 (76.00-113.00)	82.30 (60.00-107.00)	**<0.001***	0.077	0.111
Insulin (µIU/mL)	10.31 (3.43- 30.20)	8.87 (1.87- 39.57)	9.24 (2.22 -46.50)	0.901	0.985	0.831
HOMA-IR	2.46 (0.68- 8.72)	2.02 (0.50- 11.04)	2.23 (0.42- 16.88)	0.953	0.958	0.847
TG (mg/dL)	95.61 (40.00-258.80)	118.58 (56.10-418.00)	92.64 (35.00-337.00)	0.987	0.319	0.473
TOTAL-C (mg/dL)	206.57±66.76	189.34± 39.20	169.17±34.36	**0.024***	0.262	0.441
LDL-C (mg/dL)	124.45±61.30	110.15± 34.23	91.26±28.70	**0.025***	0.228	0.490
VLDL-C (mg/dL)	17.54 (4.98- .76)	23.68 (11.22- 83.60)	18.43 (7.00- 67.4)	0.971	0.307	0.263
HDL-C (mg/dL)	63.00±14.37	57.03± 16.26	59.18±11.18	0.637	0.843	0.343
CRP (mg/L)	2.24 (0.00- 14.15)	2.33 (0.14- 13.05)	1.55 (0.22- 6.75)	0.648	0.541	0.993
Netrin-1 (pg/mL)	463.71 (257.48-1128.36)	440.30 (248.89-1888.98)	490.84 (272.10-808.72)	0.924	0.730	0.944
Nesfatin-1 (ng/mL)	19.76 (4.14- 53.99)	16.65 (4.03- 52.69)	15.96 (4.24- 53.56)	0.619	0.982	0.728
Adropin (ng/L)	144.65 (11.73-363.65)	159.84 (18.67-349.35)	192.77 (45.49-374.37)	0.208	0.423	0.854

OH: Overt hypothyroidism, SH: Subclinical hypothyroidism, C: Control, BMI:Body Mass Index, WC:Waist Circumference, fT3:Free T3, fT4:Free T4, TSH:Thyroid Stimulating Hormone, anti-TPO:Anti-thyroid Peroxidase Antibodies, anti-TG:Anti-thyroglobulin Antibodies, FBG:Fasting Blood Glucose , HOMA-IR:Homeostatic Model of Assessment Insulin Resistance, TG:Triglyceride, TOTAL-C:Total Cholesterol, LDL-C:LDL Cholesterol, VLDL-C:VLDL Cholesterol, HDL-C:HDL Cholesterol, CRP:C Reactive Protein, *:p≤0.05

Anti-TPO levels were significantly higher in the SH group than in the control group (p=0.045, Table 1).

When the SH and OH groups were compared, fT3 and fT4 values were statistically significantly higher in the SH group, while TSH and anti-TPO values were found to be statistically significant higher in the OH group (p=0.009, p<0.001, p<0.001, p=0.004, respectively).

There were no significant differences among the group in serum netrin-1, nesfatin-1, or adropin levels. In pairwise comparisons (C vs OH; C vs SH; OH vs SH), p-values for netrin-1 were 0.924, 0.730, and 0.944, respectively; for nesfatin-1, 0.619, 0.982, and 0.728, respectively; and for adropin, 0.208, 0.423, and 0.854 (Table 1).

Considering all cases, there was a relationship between adropin and TG, TOTAL-C, LDL-C and very low-density lipoprotein cholesterol (VLDL-C) (r=-0.314, p=0.007; r=-0.340, p=0.003; r=-0.305, p=0.009; r=-0.381, p=0.001, respectively) (Table 2).

The correlation analysis performed separately for the patient group revealed a correlation between nesfatin-1 and fT3 (r=0.360, p=0.015) (Table 3).

Table 2. Spearman's correlation analysis between netrin-1, nesfatin-1, adropin and WC, BMI, lipid levels , CRP and age for all included cases (n = 83)

		Netrin-1	Nesfatin-1	Adropin
WC (cm)	r	-0.008	0.078	-0.187
	p-value	0.945	0.510	0.114
BMI	r	0.071	0.085	-0.204
	p-value	0.548	0.473	0.084
TG	r	0.095	0.126	-0.314
(mg/dL)	p-value	0.425	0.425	0.007*
TOTAL- C	r	-0.178	-0.081	-0.340
(mg/dL)	p-value	0.134	0.500	0.003*
HDL-C	r	-0.098	-0.147	0.028
(mg/dL)	p-value	0.413	0.218	0.815
LDL-C	r	-0.168	-0.089	-0.305
(mg/dL)	p-value	0.157	0.460	0.009*
VLDL-C	r	0.026	0.057	-0.381
(mg/dL)	p-value	0.830	0.635	0.001*
CRP	r	0.089	-0.178	-0.096
(mg/L)	p-value	0.470	0.146	0.436

OH: Overt hypothyroidism, SH: Subclinical hypothyroidism, C: Control, BMI:Body Mass Index, WC:Waist, Circumference, TG:Triglyceride, TOTAL-C:Total Cholesterol,, LDL-C:LDL Cholesterol, VLDL-C:VLDL Cholesterol, HDL-C:HDL Cholesterol, CRP:C Reactive Protein, *:p≤0.05

The significance threshold was adjusted to alpha = 0.05 / 3 = 0.0167. While major clinical markers (TSH, etc.) remained highly significant, the differences in adropin, nesfatin-1, and netrin-1 remained non-significant under this more stringent threshold.

Table 3. Spearman's correlation analysis between netrin-1, nesfatin-1, adropin and fT3, fT4, TSH, anti-TPO, anti-TG, TG, TOTAL-C, LDL-C, VLDL-C, HDL-C for patient group. (n = 56)

		Netrin-1	Nesfatin-1	Adropin
fT3 (pmol/L)	r	0.163	0.360	0.242
	p-value	0.283	0.015*	0.109
fT4 (ng/dL)	r	-0.104	0.027	0.087
	p-value	0.493	0.857	0.565
TSH (mIU/L)	r	0.099	0.027	0.087
	p-value	0.512	0.857	0.565
anti-TPO (IU/mL)	r	-0.093	-0.021	0.029
	p-value	0.545	0.889	0.848
anti-TG (IU/mL)	r	-0,157	-0.119	-0.039
	p-value	0.314	0.449	0.806
TG (mg/dL)	r	0.239	0.060	-0.071
	p-value	0.113	0.695	0.643
TOTAL- C (mg/dL)	r	-0.093	-0.275	-0.255
	p-value	0.542	0.068	0.090
LDL-C (mg/dL)	r	-0.098	-0.286	-0.253
	p-value	0.522	0.057	0.930
VLDL-C (mg/dL)	r	0.137	-0.053	-0.182
	p-value	0.369	0.728	0.230
HDL-C (mg/dL)	r	-0.135	-0.100	-0.096
	p-value	0.376	0.512	0.533

OH: Overt hypothyroidism, SH: Subclinical hypothyroidism, C: Control, fT3:Free T3, fT4:Free T4, TSH:Thyroid Stimulating Hormone, anti-TPO:Anti-thyroid Peroxidase Antibodies, anti-TG:Anti-thyroglobulin Antibodies, TG:Triglyceride, TOTAL- C:Total Cholesterol, LDL-C:LDL Cholesterol, VLDL-C:VLDL Cholesterol, HDL-C:HDL Cholesterol, VIT-D:Vitamin D, CRP:C Reactive Protein, , *:p≤0.05.
p-values for multiple comparisons were adjusted using Bonferroni correction. Statistical significance was set at $p < 0.0167$ (0.05/3).

Discussion

In this study, we aimed to determine the levels of netrin-1, nesfatin-1 and adropin levels in patients with OH and SH and to investigate the relationship between these proteins and the clinical and biochemical features of subclinic and overt hypothyroidism. Our findings demonstrate that BMI, WC, FBG, TOTAL-C and LDL-C levels were statistically significantly higher in the OH group compared to the C group. No differences were observed in the serum levels of these molecules among the groups. A negative correlation was observed between Adropin levels and TG, TOTAL-C, and LDL-C levels, while a positive correlation was found between Nesfatin-1 levels and fT3 levels.

Recent evidence has shown that thyroid hormones have a variety of effects on energy homeostasis, as well as lipid and glucose metabolism. OH is clearly associated with body weight gain, predominantly caused by edema and increased adiposity. Previous studies have observed a positive correlation between TSH levels and anthropometric measurements such as BMI and WC (18). In the Norway HUNT study involving 15.000 participants, for each mU/l increase in TSH among women, weight increased 0.9 kg, BMI 0.3 kg/m2 and WC 0.6 cm. The corresponding figures for men were 0.8 kg, 0.2 kg/m2 and 0.5 cm (19). Consistent with these data, our study demonstrated higher BMI and WC values in patients with OH compared to healthy subjects. The stimulatory effect of thyroid hormones on metabolic rate could explain these increases in body mass measures. Several hypotheses have been proposed to explain the relationship between body weight and TSH. The first suggests that increased TSH represents an adaptive mechanism aimed at counteracting weight gain. Alternatively, it has been proposed that TSH is partially inactive in obesity or that TSH resistance is increased. Finally, adipokines may directly affect serum TSH levels, the most important of which is leptin. Leptin, secreted by adipocytes, is known to stimulate TSH secretion by the pituitary (18).

Thyroid dysfunction and diabetes mellitus are closely related. Serum TSH has been reported to be positively associated with hyperglycemia and insulin resistance in euthyroid subjects in several studies (20). TSH increases leptin secretion in adipose tissue, stimulates hepatic glucose production. In addition, TSH decreases insulin secretion and synthesis from pancreatic β-cells and consequently increases serum blood glucose levels. Glucose utilization is slowed in the peripheral tissues, and the rates of glucose oxidation and glycogen synthesis are decreased in hypothyroidism. The inability of insulin to

sufficiently maintain glucose utilization by the muscles leads to insulin resistance (20). The Rotterdam Study, which included 8452 participants, showed that higher TSH levels, even within the normal range, are associated with a higher risk of diabetes (21). However, in our study, no significant difference was observed in insulin resistance parameters among the groups, despite significantly higher FBG levels in patients with OH.

Thyroid hormones play an important role in the synthesis, mobilization and metabolism of lipids. They regulate receptor-mediated LDL degradation by increasing the binding of the lipoprotein to its cell-surface receptor. Hypothyroidism is associated with hypercholesterolemia and hypertriglyceridemia (22). Therefore, current guidelines recommend screening for hypothyroidism before starting treatment in newly diagnosed hyperlipidemia (1). Consistent with these data, our findings revealed significantly higher TOTAL-C and LDL-C levels in patients with OH.

There are some contradictory findings regarding the functions of nesfatin-1. However, several studies have shown that nesfatin-1 plays a role in diabetic hyperphagia, mood disorders, epilepsy, stress, sleep, anxiety, and reproductive functions. Recent studies have also demonstrated its effects on insulin secretion, energy expenditure, and the regulation of gastrointestinal functions (9). In one study, serum nesfatin-1 levels were shown to increase in hyperthyroidism and return to levels similar to controls after euthyroidism was achieved (23). In a cross-sectional study, no significant differences were found in nesfatin-1 levels among patients with OH, SH, and healthy individuals, consistent with our results (24). Thyroid hormones are tightly regulated by the hypothalamic-pituitary-thyroid axis, whose main component is the hypothalamic paraventricular nucleus, containing TRH-producing neurons that regulate TSH secretion in the anterior pituitary. Yang et al. (25) demonstrated that nesfatin-1 can increase the phosphorylation of insulin receptor/insulin receptor substrate-1/AMPK/Akt/rapamycin complex-2, resulting in an increase in Phos immunoreactivity in hypothalamic nuclei. These findings suggest that nesfatin-1 may play a role in the regulation of thyroid hormone functions. It has also been reported that nesfatin1 immunopositive neurons are associated with TRH neurons in the paraventricular nucleus, and central nesfatin-1 affects the membrane potential of TRH neurons (26). In our study, we found a positive correlation between fT3 levels and nesfatin-1 levels. This relationship may contribute to the weight loss effect of thyroid hormones, as well as the anorexigenic effect of nesfatin-1. Our finding suggests a possible interaction between thyroid hormones and nesfatin-1 in energy balance and appetite regulation. fT3, as the biologically active thyroid hormone, increases basal metabolic rate and promotes energy expenditure. Nesfatin-1, on the other hand, has anorexigenic effects and influences glucose homeostasis through hypothalamic pathways (7,9). The observed positive correlation may indicate that higher fT3 levels enhance nesfatin-1 secretion, thereby amplifying appetite suppression and energy utilization. This interaction could represent one of the mechanisms by which thyroid hormones contribute to body weight regulation. fT3 may potentiate central nesfatin-1 signaling, explaining our positive nesfatin-1–fT3 correlation despite no group-wise differences in serum levels. Since much of nesfatin-1's action is neuronal and synaptic, central activity can be high while peripheral concentrations remain within a narrow range.(27). However, the absence of correlation with fT4 and TSH may reflect either the relatively small sample size or the complex neuroendocrine regulation of nesfatin-1 that is not solely dependent on thyroid status.

Adropin is a hormone which has significant roles in energy homeostasis. In one study, correlation has been found between low adropin levels and metabolic syndrome which suggests adropin is a potential protective agent against metabolic syndrome (12). Another study reported that low circulating adropin levels are associated with endothelial dysfunction, highlighting its potential utility in evaluating vascular impairment in patients with metabolic syndrome. (13). A recent study revealed that plasma adropin levels may vary with diet in humans, with the knowledge of carbonhydrate rich diet causes low adropin levels and high fructose involved diet increases the risk of having high cholesterol levels and metabolic syndrome, suggesting there may be a relation among adropin and fructose, TG metabolism (28). Adropin has also been shown to prevent obesity-related hyperinsulinemia and hepatosteatosis (29). In a recent meta-analysis including 15 studies, serum adropin concentrations were found significantly lower in patients with T2DM compared to control group (30). Yu et al.(31) demonstrated that patients with stable

ischemic heart disease had lower levels of adropin than the control group.

There are conflicting data on whether Netrin-1 slows or accelerates the progression of atherosclerosis, depending on its cellular source. While macrophage-derived Netrin-1 exerts pro-atherogenic and pro-inflammatory effects, the secreted isoform appears to have a protective role in the pathogenesis of atherosclerosis (4). Thyroid function has been associated with the presence and severity of atherosclerosis. (3). Although we hypothesized that netrin-1 may play a role in the etiology of atherosclerosis in hypothyroidism, we did not find a significant difference in netrin-1 levels among the OH, SH, and control groups in our study.

Although Netrin-1, Nesfatin-1, and Adropin are implicated in energy homeostasis, vascular health, and inflammatory regulation, our study did not detect significant differences in their serum levels between OH, SH and C groups. Several explanations may account for this apparent paradox. First, these molecules may exert their effects in a tissue-specific manner rather than being reflected in circulating levels. For example, Netrin-1 derived from endothelial cells and macrophages may influence local vascular inflammation and atherosclerotic processes without causing measurable systemic changes. Similarly, Nesfatin-1 secretion in discrete hypothalamic nuclei may regulate appetite and neuroendocrine pathways, yet peripheral serum concentrations may not fully capture central activity.

Second, compensatory mechanisms in hypothyroidism could mask changes in these biomarkers. For instance, increased TSH and altered adipokine signaling might counterbalance fluctuations in Nesfatin-1 or Adropin, maintaining stable circulating levels despite metabolic alterations.

Third, the sample size limitation reduces statistical power and might obscure subtle differences that larger cohorts could reveal. Moreover, heterogeneity in body composition, diet, and comorbidities may introduce variability, diluting group-level differences.

Finally, the time course of thyroid dysfunction may be relevant. Acute versus chronic hypothyroid states might differentially influence these molecules, and cross-sectional assessment may fail to detect dynamic fluctuations. Longitudinal studies following biomarker changes before and after thyroid hormone replacement could better clarify causal relationships.

Our study has some limitations that should be acknowledged. First, the cross-sectional design prevents us from establishing a causal relationship between thyroid status and the levels of the investigated peptides. Second, the relatively small sample size (n=83) is a major constraint. Our post-hoc power analysis revealed that the study was underpowered to detect small-to-moderate effects, which may explain the lack of statistically significant differences in serum adropin, nesfatin-1, and netrin-1 levels across groups. Larger prospective studies with more participants are needed to confirm these findings. Lastly, although we excluded major co-morbidities, other metabolic factors not accounted for in this study might have influenced the serum peptide concentrations.

Conclusion

In conclusion, while hypothyroidism significantly affects metabolic and lipid parameters. the roles of netrin-1, nesfatin-1, and adropin are not yet fully understood. Further large-scale, longitudinal studies are required to clarify their potential systemic effects.

Acknowledgements

None.

Conflict of interest statement

All authors declare that there is no conflicts of interest related to this research.

Ethics Committee Approval

Ethical approval for this study was obtained from the Clinical Research and Ethics Committee of Muğla Sıtkı Koçman University (approval number: 250094/104, approval date: 06/02/2025).

Funding

The study was supported by the Muğla BAP unit. The project number is 17/100.

References

1. Jonklaas J, Bianco AC, Bauer AJ, et al. Guidelines for the Treatment of Hypothyroidism: Prepared by the American Thyroid Association Task Force on Thyroid Hormone Replacement. Thyroid. 2014;24(12):1670-1675.

2. Yang N, Yao Z, Miao L, et al. Novel Clinical Evidence of an Association between Homocysteine and Insulin Resistance in Patients with Hypothyroidism or Subclinical Hypothyroidism. PLoS One. 2015;10(5):e0125922.
3. Akbaba G, Berker D, Isık S, et al. Changes in the before and after thyroxine treatment levels of adipose tissue, leptin, and resistin in subclinical hypothyroid patients. Wien Klin Wochenschr. 2016;128(15-16):579-585.
4. Yuan X, Shen G, Xiao H, et al. Netrin-1 and RGMa: Novel Regulators of Atherosclerosis-Related Diseases. Cardiovasc Drugs Ther. 2025;39(1):211-219.
5. Ziegon L, Schlegel M. Netrin-1: A Modulator of Macrophage Driven Acute and Chronic Inflammation. Int J Mol Sci. 2021;23(1):275.
6. Toque HA, Fernandez-Flores A, Mohamed R, et al. Netrin-1 is a novel regulator of vascular endothelial function in diabetes. PLoS One. 2017;12(10):e0186734.
7. Dore R, Levata L, Lehnert H, et al. Nesfatin-1: functions and physiology of a novel regulatory peptide. J Endocrinol. 2017;232(1):45-65.
8. Oh-I S, Shimizu H, Satoh T, et al. Identification of nesfatin-1 as a satiety molecule in the hypothalamus. Nature. 2006;443(7112):709-712.
9. Feijóo-Bandín S, Rodríguez-Penas D, García-Rúa V, et al. Nesfatin-1: a new energy-regulating peptide with pleiotropic functions. Implications at cardiovascular level. Endocrine. 2016;52(1):11-29.
10. Riva M, Nitert MD, Voss U, et al. Nesfatin-1 stimulates glucagon and insulin secretin and beta cell NUCB2 is reduced in human type 2 diabetic subjects. Cell Tissue Res. 2011;346(3):393-405.
11. Ali II, D'Souza C, Singh J, et al. Adropin's Role in Energy Homeostasis and Metabolic Disorders. Int J Mol Sci. 2022;23(15):8318.
12. Yosaee S, Khodadost M, Esteghamati A, et al. Metabolic Syndrome Patients Have Lower Levels of Adropin When Compared With Healthy Overweight/Obese and Lean Subjects. Am J Mens Health. 2017;11(2):426-434.
13. Oruc CU, Akpinar YE, Dervisoglu E, et al. Low concentrations of adropin are associated with endothelial dysfunction as assessed by flow-mediated dilatation in patients with metabolic syndrome. Clin Chem Lab Med. 2017;55(1):139-144.
14. Mogulkoc R, Dasdelen D, Baltaci SB, et al. The effect of thyroid dysfunction and treatment on adropin, asprosin and preptin levels in rats. Horm Mol Biol Clin Investig. 2020;42(1):37-42.
15. Mancini A, Di Segni C, Raimondo S, et al. Thyroid Hormones, Oxidative Stress, and Inflammation. Mediators Inflamm. 2016;2016:6757154.
16. Walczak K, Sieminska L. Obesity and Thyroid Axis. Int J Environ Res Public Health. 2021;18(18):9434.
17. Matthews DR, Hosker JP, Rudenski AS, et al. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28(7):412-419.
18. Tiller D, Ittermann T, Greiser KH, et al. Association of Serum Thyrotropin with Anthropometric Markers of Obesity in the General Population. Thyroid. 2016;26(9):1205-1214.
19. Svare A, Nilsen TI, Bjøro T, et al. Serum TSH related to measures of body mass: longitudinal data from the HUNT Study, Norway. Clin Endocrinol (Oxf). 2011;74(6):769-775.
20. Biondi B, Kahaly GJ, Robertson RP. Thyroid Dysfunction and Diabetes Mellitus: Two Closely Associated Disorders. Endocr Rev. 2019;40(3):789-824.
21. Chaker L, Ligthart S, Korevaar TI, et al. Thyroid function and risk of type 2 diabetes: a population-based prospective cohort study. BMC Med. 2016;14(1):150.
22. Laway BA, War FA, Shah S, et al. Alteration of lipid parameters in patients with subclinical hypothyroidism. Int J Endocrinol Metab. 2014;12(3):e17496.
23. Tohma Y, Akturk M, Altinova A, et al. Circulating Levels of Orexin-A, Nesfatin-1, Agouti-Related Peptide, and Neuropeptide Y in Patients with Hyperthyroidism. Thyroid. 2015;25(7):776-783.
24. Gungunes A, Ozbek M, Ginis Z, et al. Serum nesfatin-1 levels in overt and subclinical hyperthyroidism. Minerva Endocrinol. 2014;39(3):209-214.
25. Yang M, Zhang Z, Wang C, et al. Nesfatin-1 action in the brain increases insulin sensitivity through Akt/AMPK/TORC2 pathway in diet-induced insulin resistance. Diabetes. 2012;61(8):1959-1968.
26. Liu F, Yang Q, Gao N, et al. Decreased plasma nesfatin-1 level is related to the thyroid dysfunction in patients with type 2 diabetes mellitus. J Diabetes Res. 2014;2014:128014.
27. Schalla MA, Stengel A. Current understanding of the role of nesfatin-1. J Endocr Soc. 2018;2(10):1188-1206.
28. Stevens JR, Kearney ML, St-Onge MP, et al. Inverse association between carbohydrate consumption and plasma adropin concentrations in humans. Obesity (Silver Spring). 2016;24(8):1731-1740.
29. Kumar KG, Zhang J, Gao S, et al. Adropin deficiency is associated with increased adiposity and insulin resistance. Obesity (Silver Spring). 2012;20(7):1394-1402.
30. Soltani S, Beigrezaei S, Malekahmadi M, et al. Circulating levels of adropin and diabetes: a systematic review and meta-analysis of observational studies. BMC Endocr Disord. 2023;23(1):73.
31. Yu HY, Zhao P, Wu MC, et al. Serum adropin levels are decreased in patients with acute myocardial infarction. Regul Pept. 2014;190-191:46-49.