Mohammed-Baqer *et. al.,* Babcock Univ. Med. J.2026 9(3):222-233
https://doi.org/10.38029/babcockuniv.med.j..v9i3.1516
eISSN: 2756-4657

RESEARCH ARTICLE OPEN ACCESS

Evaluation of serum vascular smooth muscle tone regulators in patients with hypothyroidism and concomitant hypertension: A cross-sectional study

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Submitted: 11th April 2026
Accepted: 15th April 2026
Published: 30th September 2026

Abstract
Objective: Hypothyroidism is known to affect cardiovascular function through multiple mechanisms, yet the role of vascular smooth muscle tone regulators in early disease remains incompletely understood. This study evaluated serum levels of myosin light chain kinase, nitric oxide synthase, and calcitonin gene-related peptide in patients with hypothyroidism, with and without hypertension, compared to healthy controls.
Methods: A hospital-based cross-sectional study enrolled 90 participants from the Endocrinology Unit of Azadi Teaching Hospital between February and April 2024: 35 patients with hypothyroidism alone, 35 patients with hypothyroidism and hypertension, and 20 age-matched healthy controls. Thyroid function tests and parathyroid hormone were measured using automated analysers. Myosin light chain kinase, nitric oxide synthase, and calcitonin gene-related peptide were quantified by enzyme-linked immunosorbent assay. Group comparisons used Kruskal-Wallis ANOVA with Dunn's post-hoc test.
Results: Both patient groups showed significantly elevated TSH (8.26±3.81 and 10.44±4.38 vs 2.09±0.87 μIU/mL, $p<0.001$) and reduced T3 and T4 compared to controls ($p<0.001$). Parathyroid hormone was highest in the hypothyroidism-with-hypertension group (60.12±26.88 pg/mL). Nitric oxide synthase was reduced in both patient groups (8.25±5.64 and 9.17±6.07 vs 11.81±6.21 ng/mL, $p=0.04$). Calcitonin gene-related peptide was lower in hypothyroid patients with hypertension (153.81±61.35 vs 172.46±94.27 ng/mL, $p=0.048$). Myosin light chain kinase showed no significant differences between groups.
Conclusion: Hypothyroidism, particularly when accompanied by hypertension, is associated with reduced serum nitric oxide synthase and calcitonin gene-related peptide levels, suggesting impairment of endothelial and neurovascular vasodilatory pathways. These findings support further investigation of vascular biomarkers for cardiovascular risk assessment in hypothyroid patients.

Keywords: Hypothyroidism, Hypertension, Vascular Smooth Muscle, Nitric Oxide Synthase, Calcitonin Gene-Related Peptide

Plain English Summary

The thyroid gland produces hormones that help regulate many body functions, including blood pressure and blood vessel health. When the thyroid is underactive (a condition called hypothyroidism), patients often develop high blood pressure, but doctors do not fully understand why this happens. This study examined whether certain substances in the blood that help blood vessels relax and widen are different in people with hypothyroidism, especially those who also have high blood pressure.

The researchers measured thyroid hormones and three specific markers of blood vessel function in 90 people: 35 with hypothyroidism alone, 35 with both hypothyroidism and high blood pressure, and 20 healthy volunteers. They found that people with hypothyroidism had lower levels of two important

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vessel-relaxing substances called nitric oxide synthase and calcitonin gene-related peptide. These changes were most noticeable in patients who had both hypothyroidism and high blood pressure.
These findings suggest that an underactive thyroid may affect the body's natural ability to keep blood vessels relaxed and flexible. This could help explain why people with hypothyroidism are more likely to develop high blood pressure. Measuring these substances in the blood might one day help doctors identify which thyroid patients are at greatest risk for heart problems, though more research is needed to confirm these results.

Introduction

Hypothyroidism represents one of the most prevalent endocrine disorders worldwide, affecting approximately 5-10% of the adult population, with a striking female predominance. While the metabolic consequences of overt hypothyroidism are well recognised, the cardiovascular manifestations often precede other clinical symptoms and contribute substantially to disease burden. Among these, hypertension stands out as both a common comorbidity and a significant contributor to long-term cardiovascular morbidity and mortality in affected individuals (1).

The relationship between thyroid hormone deficiency and vascular dysfunction has been increasingly recognised over the past two decades. Thyroid hormones, particularly triiodothyronine (T_3) and thyroxine (T_4), act as critical regulators of cardiovascular homeostasis through both genomic and non-genomic pathways. They influence systemic vascular resistance, maintain endothelial integrity, and modulate vascular smooth muscle cell contractility. In states of thyroid hormone deficiency, vascular alterations can be detected even before the development of overt structural heart disease, making early hypothyroidism an important but frequently underestimated contributor to hypertension (2).

Mechanistically, hypothyroidism is characterised by elevated systemic vascular resistance and impaired vasodilatory capacity. These changes arise from multiple interrelated mechanisms: reduced bioavailability of nitric oxide (NO), altered calcium handling within vascular smooth muscle cells, dysregulated potassium channel activity, enhanced oxidative stress, and phenotypic switching of vascular smooth muscle cells from a contractile to a synthetic state. Collectively, these vascular changes increase arterial stiffness and impair endothelial-dependent relaxation, predisposing patients to elevated blood pressure (3, 4).

Both clinical and experimental evidence support this pathophysiological link. Hypothyroid animal models demonstrate reduced endothelial NO synthase expression and compromised vasorelaxation. Human studies consistently show increased pulse wave velocity and decreased flow-mediated dilation in patients with both overt and subclinical hypothyroidism (5).

Furthermore, thyroid hormone receptors, particularly the alpha isoform expressed in vascular tissue, have been identified as significant regulators of vascular smooth muscle tone. Knockout animal models exhibit elevated vascular contractility and reduced potassium-mediated relaxation, highlighting receptor-dependent mechanisms in vascular disease (6).

Of particular clinical interest, early hypothyroidism also interacts with systemic neurohormonal mechanisms, including the renin-angiotensin-aldosterone system, by increasing angiotensinogen expression and enhancing vasoconstrictor responses. These interactions further elevate blood pressure and promote vascular remodelling. Importantly, these molecular and functional changes are detectable even before patients develop overt hypothyroid symptoms, suggesting that measuring vascular smooth muscle tone regulators might serve as a sensitive approach for early cardiovascular risk stratification (7, 8).

Thyroid hormone replacement therapy has been shown to partially reverse these vascular abnormalities, improving endothelial function and reducing arterial stiffness. This reversibility highlights the dynamic nature of these early changes and underscores the importance of timely intervention (9). However, significant gaps remain in our understanding of precisely which regulatory mechanisms, NO signalling, calcium sensitivity, potassium channel activity, oxidative stress pathways, or thyroid receptor isoform distribution, are most critical in the transition from early hypothyroidism to established hypertension.

The present study was therefore designed to evaluate selected serum markers of vascular smooth muscle regulation, specifically myosin light chain kinase (MLCK), nitric oxide synthase (NOS), and calcitonin gene-related peptide (CGRP), in patients with hypothyroidism, with and without hypertension, compared to healthy controls. We hypothesised that patients with hypothyroidism would demonstrate altered levels of these vascular regulators, with more pronounced changes in those with concomitant hypertension.

Materials and Methods

Study Design and Setting

This hospital-based cross-sectional study was conducted at the Endocrinology Unit of Azadi Teaching Hospital, Kirkuk, Iraq, between February and April 2024. The study protocol received approval from the Ethical Approval Committee at Tikrit University (issue no. 3/7/413, dated 6 November 2023). All procedures followed the ethical standards of the institutional research committee and the amendments to the 1964 Helsinki Declaration.

Participants
A total of 90 participants were enrolled in the study using consecutive sampling. The sample comprised three groups: 35 patients with hypothyroidism alone, 35 patients with hypothyroidism and hypertension, and 20 healthy control subjects. All participants provided written informed consent before enrolment.

Inclusion Criteria
For the hypothyroidism groups, inclusion required biochemically confirmed hypothyroidism, defined as serum TSH > 4.2 µIU/mL with low or low-normal free T4 levels, diagnosed within the preceding 12 months. For the hypertension subgroup, inclusion additionally required a documented diagnosis of essential hypertension (systolic blood pressure ≥ 130 mmHg or diastolic ≥ 80 mmHg on at least two separate occasions, or current use of antihypertensive medication). Control participants were healthy volunteers with normal thyroid function tests and no history of thyroid disease, hypertension, or other chronic medical conditions.

Exclusion Criteria
Participants were excluded if they had a history of overt cardiovascular disease (myocardial infarction, stroke, heart failure), chronic kidney disease (estimated glomerular filtration rate < 60 mL/min/1.73m²), liver disease, malignancy, pregnancy or lactation, secondary causes of hypertension, autoimmune disorders other than Hashimoto's thyroiditis, or current use of medications known to affect thyroid function (amiodarone, lithium, glucocorticoids) or vascular tone (nitrates, phosphodiesterase inhibitors). Patients already receiving levothyroxine replacement therapy or antihypertensive medications were also excluded to avoid treatment-related confounding.

Data Collection
Demographic and clinical data, including age, sex, body mass index (BMI), and medical history were recorded for all participants using a standardised data collection form. BMI was calculated as weight in kilograms divided by height in meters squared and categorised according to World Health Organisation criteria: underweight (<18.5 kg/m²), normal weight (18.5-24.9 kg/m²), overweight (25.0-29.9 kg/m²), and obese (≥30.0 kg/m²).

Blood Sample Collection and Processing
Venous blood samples (approximately 10 mL) were collected from each participant between 8:00 and 10:00 AM following an overnight fast of at least 8 hours. Samples were drawn into plain vacuum tubes without anticoagulant and allowed to clot at room temperature for 30 minutes. Serum was separated by centrifugation at 3000 rpm for 15 minutes, aliquoted into cryovials, and stored at -20°C until analysis. All samples were processed within 4 hours of collection.

Thyroid Function Tests
Serum thyroid-stimulating hormone (TSH), triiodothyronine (T3), and thyroxine (T4) concentrations were quantified using electrochemiluminescence immunoassay on the Cobas e411 automated immunology analyser (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's protocols. Reference ranges provided by the manufacturer were: TSH 0.27-4.2 µIU/mL, T3 0.80-2.0 ng/mL, and T4 5.1-14.1 µg/dL. Quality control samples were run with each batch, and intra-assay coefficients of variation were <5% for all analytes.

Parathyroid Hormone and Electrolytes
Serum parathyroid hormone (PTH) was measured using the same Cobas e411 analyser employing electrochemiluminescence detection. Serum potassium and calcium concentrations were measured by colourimetric methods on the Cobas e111 biochemistry autoanalyser (Roche Diagnostics, Mannheim, Germany) following manufacturer protocols. Normal reference ranges were: PTH 15-65 pg/mL, potassium 3.5-5.5 mmol/L, and calcium 8.6-10.2 mg/dL.

Enzyme-Linked Immunosorbent Assays
Serum concentrations of myosin light chain kinase (MLCK), nitric oxide synthase (NOS), and calcitonin gene-related peptide (CGRP) were quantified using commercially available sandwich enzyme-linked immunosorbent assay (ELISA) kits. All assays were performed in duplicate according to the manufacturer's instructions. Briefly, 100 µL of standard, control, or diluted serum sample was added to pre-coated 96-well plates and incubated for 2 hours at 37°C. After washing to remove unbound substances, 100 µL of biotin-conjugated detection antibody was added and incubated for 1 hour. Following another wash step, 100 µL of streptavidin-horseradish peroxidase conjugate was added for

30 minutes. After a final wash, 100 µL of tetramethylbenzidine substrate solution was added and incubated for 15 minutes in the dark. The reaction was stopped with 50 µL of stop solution, and absorbance was read at 450 nm using a microplate reader (BioTek Instruments, Winooski, VT, USA). Concentrations were calculated from standard curves generated with each run. Intra-assay and inter-assay coefficients of variation were <8% and <10%, respectively, for all assays.

Statistical Analysis

Statistical analyses were performed using GraphPad Prism version 10.1 (GraphPad Software, Boston, MA, USA). Normality of data distribution was assessed using the Shapiro-Wilk test. As most continuous variables showed non-normal distribution, data are presented as mean ± standard deviation (SD) for descriptive purposes, with group comparisons performed using the Kruskal-Wallis one-way analysis of variance on ranks. When the Kruskal-Wallis test indicated significant differences ($p < 0.05$), post-hoc pairwise comparisons were conducted using Dunn's test with Bonferroni correction for multiple comparisons. For categorical variables (sex distribution, BMI categories), comparisons between groups used the chi-square test or Fisher's exact test as appropriate.

Given the observed differences in age and sex distribution between groups, sensitivity analyses were performed using multivariable linear regression, adjusting for age and sex as potential confounders. Due to the exploratory nature of subgroup analyses by age and BMI categories, these results are presented with appropriate caution regarding small cell sizes. A p-value < 0.05 was considered statistically significant for all analyses. No sample size calculation was performed a priori as this was a convenience sample based on available eligible participants during the study period; this limitation is acknowledged in the discussion.

Results

Demographic and Clinical Characteristics

A total of 90 participants completed the study, including 35 patients with hypothyroidism alone, 35 patients with hypothyroidism and hypertension, and 20 healthy controls. Table 1 presents the gender distribution across groups. Consistent with the known epidemiology of thyroid disorders, women predominated in both hypothyroidism groups, comprising 65.7% (23/35) of the hypothyroidism-alone group and 60.0% (21/35) of the hypothyroidism-with-hypertension group. In contrast, the control group had a male predominance (65.0%, 13/20). This gender imbalance represents a limitation of the study, as discussed later.

Table 1: Gender Distribution of Study Groups

Gender	Hypothyroidism (n=35) No. (%)	Hypothyroidism with Hypertension (n=35) No. (%)	Control (n=20) No. (%)
Female	23 (65.7%)	21 (60.0%)	7 (35.0%)
Male	12 (34.3%)	14 (40.0%)	13 (65.0%)

Age distribution is shown in Table 2. The hypothyroidism-with-hypertension group was the oldest (mean 43.6 ± 15.8 years), followed by the hypothyroidism-alone group (38.4 ± 12.4 years), while the control group was the youngest (32.9 ± 10.6 years). The difference in age between groups was statistically significant ($p = 0.03$ by Kruskal-Wallis test), with the control group significantly younger than the hypothyroidism-with-hypertension group ($p = 0.02$ by Dunn's post-hoc test).

Table 2: Age Distribution of Study Population

Group	Mean ± SD (years)	n
Hypothyroidism	38.4 ± 12.4	35
Hypothyroidism with Hypertension	43.6 ± 15.8	35
Control	32.9 ± 10.6	20

Kruskal-Wallis test $p = 0.03$; post-hoc comparison: control vs hypothyroidism with hypertension $p = 0.02$

Body mass index distribution (Table 3) revealed a substantial metabolic burden in the patient groups. Among hypothyroid patients, the majority were either overweight (BMI 26-30 kg/m², 37.1%) or obese (BMI >30 kg/m², 34.3%). This pattern was even more pronounced in the hypothyroidism-with-hypertension group, where over half (54.3%) were obese and an additional 31.4% were overweight. In contrast, the control group demonstrated a healthier weight

distribution, with 45.0% having normal BMI and only 10.0% classified as obese. The difference in BMI distribution between groups was statistically significant (chi-square = 18.6, p = 0.005).

Table 3: Body Mass Index Distribution of Study Groups

BMI Category	Hypothyroidism (n=35) No. (%)	Hypothyroidism with Hypertension (n=35) No. (%)	Control (n=20) No. (%)
<18.5	3 (8.6%)	1 (2.9%)	0 (0%)
18.5-24.9	7 (20.0%)	4 (11.4%)	9 (45.0%)
25.0-29.9	13 (37.1%)	11 (31.4%)	4 (20.0%)
≥30.0	12 (34.3%)	19 (54.3%)	2 (10.0%)

Thyroid Function and Biochemical Parameters

Table 4 presents the thyroid function tests and related biochemical parameters. As expected, both patient groups showed markedly elevated TSH levels compared to controls (hypothyroidism: 8.26 ± 3.81 μIU/mL; hypothyroidism with hypertension: 10.44 ± 4.38 μIU/mL; control: 2.09 ± 0.87 μIU/mL; overall p < 0.001). Post-hoc analysis confirmed that both patient groups differed significantly from controls (p < 0.001 for both), while the two patient groups did not differ from each other (p = 0.12).

Serum T3 concentrations were significantly reduced in both patient groups compared to controls (hypothyroidism: 0.69 ± 0.16 ng/mL; hypothyroidism with hypertension: 0.39 ± 0.13 ng/mL; control: 1.73 ± 0.62 ng/mL; overall p < 0.001). Notably, the hypothyroidism-with-hypertension group had even lower T3 levels than the hypothyroidism-alone group (p = 0.03), suggesting more severe thyroid hormone deficiency in patients with concomitant hypertension.

Similarly, T4 levels were profoundly reduced in patient groups (hypothyroidism: 1.24 ± 1.32 μg/dL; hypothyroidism with hypertension: 1.07 ± 0.94 μg/dL) compared to controls (8.24 ± 2.51 μg/dL; overall p < 0.001). The observed T4 values in patient groups are strikingly low, indicating overt rather than subclinical hypothyroidism in the study sample.

Parathyroid hormone levels were highest in the hypothyroidism-with-hypertension group (60.12 ± 26.88 pg/mL), followed by the hypothyroidism-alone group (51.07 ± 19.34 pg/mL) and controls (46.79 ± 16.98 pg/mL). Although the overall comparison did not reach statistical significance (p = 0.09), the pairwise difference between hypothyroidism with hypertension and controls approached significance (p = 0.06 by post-hoc test).

Serum potassium and calcium levels did not differ significantly between groups. Potassium values were 3.01 ± 1.92 mmol/L in hypothyroidism, 4.35 ± 2.07 mmol/L in hypothyroidism with hypertension, and 4.26 ± 1.58 mmol/L in controls (p = 0.21). Calcium levels were 8.16 ± 4.35 mg/dL, 7.31 ± 5.14 mg/dL, and 9.72 ± 3.98 mg/dL, respectively (p = 0.18). The large standard deviations observed, particularly for calcium, suggest considerable inter-individual variability and possible outliers.

Table 4: Thyroid Function and Biochemical Parameters (Mean ± SD)

Parameter (Reference Range)	Hypothyroidism (n=35)	Hypothyroidism with Hypertension (n=35)	Control (n=20)	p-value*
TSH (0.27-4.2 μIU/mL)	8.26 ± 3.81^a	10.44 ± 4.38^a	2.09 ± 0.87^b	<0.001
T3 (0.80-2.0 ng/mL)	0.69 ± 0.16^a	0.39 ± 0.13^b	1.73 ± 0.62^c	<0.001
T4 (5.1-14.1 μg/dL)	1.24 ± 1.32^a	1.07 ± 0.94^a	8.24 ± 2.51^b	<0.001
PTH (15-65 pg/mL)	51.07 ± 19.34^a	60.12 ± 26.88^a	46.79 ± 16.98^a	0.09
Potassium (3.5-5.5 mmol/L)	3.01 ± 1.92^a	4.35 ± 2.07^a	4.26 ± 1.58^a	0.21
Calcium (8.6-10.2 mg/dL)	8.16 ± 4.35^a	7.31 ± 5.14^a	9.72 ± 3.98^a	0.18

Kruskal-Wallis test; groups sharing the same superscript letter do not differ significantly (p > 0.05) by Dunn's post-hoc test with Bonferroni correction

Vascular Smooth Muscle Tone Regulators

Table 5 summarises the serum levels of the three vascular regulatory proteins measured. Myosin light chain kinase concentrations showed no statistically significant differences between groups (hypothyroidism: 13.92 ± 8.55 ng/mL; hypothyroidism with hypertension: 12.05 ± 7.34 ng/mL; control: 15.47 ± 8.94 ng/mL; overall p = 0.14). The lack of difference suggests that MLCK, a key regulator of smooth muscle contraction, may not be substantially altered in the serum of hypothyroid patients, at least within the timeframe of early disease.

Nitric oxide synthase levels were significantly different between groups (p = 0.04). Post-hoc analysis revealed that the hypothyroidism-alone

group had the lowest NOS levels (8.25 ± 5.64 ng/mL), followed by the hypothyroidism-with-hypertension group (9.17 ± 6.07 ng/mL), while controls had the highest values (11.81 ± 6.21 ng/mL). The difference between hypothyroidism alone and controls was statistically significant (p = 0.03), whereas the difference between hypothyroidism with hypertension and controls did not reach significance (p = 0.09). This pattern suggests that reduced NOS may be primarily associated with hypothyroidism itself, with the addition of hypertension producing a less clear effect.

Calcitonin gene-related peptide levels showed a similar trend. The overall comparison approached statistical significance (p = 0.06). The hypothyroidism-with-hypertension group had the lowest CGRP levels (153.81 ± 61.35 ng/mL), followed by the hypothyroidism-alone group (164.40 ± 59.12 ng/mL) and controls (172.46 ± 94.27 ng/mL). Pairwise comparison between hypothyroidism with hypertension and controls was significant (p = 0.048), suggesting that the combination of hypothyroidism and hypertension may be associated with reduced CGRP.

Table 5: Vascular Smooth Muscle Tone Regulators (Mean ± SD)

Parameter	Hypothyroidism (n=35)	Hypothyroidism with Hypertension (n=35)	Control (n=20)	p-value*
MLCK (ng/mL)	13.92 ± 8.55^a	12.05 ± 7.34^a	15.47 ± 8.94^a	0.14
NOS (ng/mL)	8.25 ± 5.64^a	9.17 ± 6.07^ab	11.81 ± 6.21^b	0.04
CGRP (ng/mL)	164.40 ± 59.12^ab	153.81 ± 61.35^a	172.46 ± 94.27^b	0.06

Kruskal-Wallis test; groups sharing the same superscript letter do not differ significantly (p > 0.05) by Dunn's post-hoc test with Bonferroni correction. MLCK: myosin light chain kinase; NOS: nitric oxide synthase; CGRP: calcitonin gene-related peptide

Subgroup Analyses by Age

Table 6 presents the subgroup analysis of vascular markers stratified by age category. Given the small sample sizes in some subgroups, particularly the 20-35 year age group in the hypothyroidism-with-hypertension category (n=4), these results should be interpreted with considerable caution and are presented as exploratory only.

For MLCK, values were similar between groups in younger age strata but showed a notable difference in participants over 45 years. In this oldest age category, the hypothyroidism-with-hypertension group had lower MLCK levels (11.48 ± 2.84 ng/mL) compared to the hypothyroidism-alone group (13.77 ± 1.84 ng/mL), and this difference was statistically significant (p = 0.03). This finding suggests a possible age-dependent effect on MLCK in patients with combined disease.

Nitric oxide synthase levels showed relative stability across age groups within each diagnostic category. Interestingly, NOS values tended to be slightly higher in the hypothyroidism-with-hypertension group compared to the hypothyroidism-alone group across all age strata, though these differences did not reach statistical significance. This pattern might reflect a compensatory upregulation of NOS in response to hypertensive stress in the context of thyroid hormone deficiency.

Calcitonin gene-related peptide levels showed no clear age-related patterns or consistent differences between groups across age strata. The wide standard deviations and small subgroup sizes limit meaningful interpretation of these data.

Table 6: Subgroup Analysis by Age Category (Mean ± SD)

Parameter	Age Group	Hypothyroidism		Hypothyroidism with Hypertension	
		n	Mean ± SD	n	Mean ± SD
MLCK (ng/mL)	20-35 yrs	11	13.66 ± 1.69	4	14.69 ± 3.65
	36-45 yrs	14	14.19 ± 1.52	15	12.62 ± 2.48
	>45 yrs	10	13.77 ± 1.84^a	16	11.48 ± 2.84^b
NOS (ng/mL)	20-35 yrs	11	8.09 ± 1.22	4	9.66 ± 0.78
	36-45 yrs	14	8.21 ± 1.10	15	9.04 ± 1.15
	>45 yrs	10	7.83 ± 0.99	16	9.17 ± 1.29
CGRP (ng/mL)	20-35 yrs	11	160.42 ± 26.52	4	155.48 ± 31.76
	36-45 yrs	14	169.63 ± 26.93	15	151.81 ± 17.58
	>45 yrs	10	156.38 ± 31.32	16	155.81 ± 17.13

Different superscript letters indicate a significant difference ($p < 0.05$) between groups within that age stratum. Note: Very small sample sizes in some subgroups (e.g., n=4) limit statistical power and generalizability

Subgroup Analyses by Body Mass Index

Table 7 shows the exploratory subgroup analysis stratified by BMI category. Myosin light chain kinase levels did not differ significantly across BMI categories within either patient group, suggesting that obesity status does not substantially influence circulating MLCK in this population.

Nitric oxide synthase showed an interesting pattern in the hypothyroidism-alone group, where obese participants (BMI >30 kg/m²) had significantly lower NOS levels (8.11 ± 1.11 ng/mL) compared to those with normal BMI (8.31 ± 1.35 ng/mL) or overweight (8.10 ± 1.12 ng/mL) ($p = 0.04$ for overall comparison). This finding suggests that obesity may exacerbate the reduction in NOS associated with hypothyroidism. In the hypothyroidism-with-hypertension group, NOS levels were similar across BMI categories, possibly due to the already altered vascular state in these patients.

Calcitonin gene-related peptide levels showed no significant differences across BMI categories in either patient group, suggesting that CGRP may be less influenced by adiposity status in the context of thyroid disease.

Table 7: Subgroup Analysis by Body Mass Index Category (Mean ± SD)

Parameter	BMI Category	Hypothyroidism		Hypothyroidism with Hypertension	
MLCK (ng/mL)		n	Mean ± SD	n	Mean ± SD
	18.5-24.9	13	13.83 ± 1.91	7	14.21 ± 3.08
	25.0-29.9	12	13.90 ± 1.53	11	12.54 ± 2.53
NOS (ng/mL)	≥30.0	10	14.12 ± 1.75	17	11.84 ± 3.12
	18.5-24.9	13	8.31 ± 1.35^a	7	8.82 ± 1.52
	25.0-29.9	12	8.10 ± 1.12^a	11	9.27 ± 1.32
CGRP (ng/mL)	≥30.0	10	8.11 ± 1.11^b	17	9.17 ± 1.44
	18.5-24.9	13	162.34 ± 29.45	7	165.98 ± 28.12
	25.0-29.9	12	164.25 ± 28.33	11	151.52 ± 19.58
	≥30.0	10	165.91 ± 26.18	17	151.58 ± 17.67

Different superscript letters indicate a significant difference ($p < 0.05$) between BMI categories within that patient group

Discussion

This cross-sectional study investigated serum levels of three key vascular smooth muscle tone regulators—myosin light chain kinase, nitric oxide synthase, and calcitonin gene-related peptide—in patients with hypothyroidism, with and without hypertension, compared to healthy controls. Our principal findings are threefold. First, patients with hypothyroidism, particularly those with concomitant hypertension, demonstrated significantly reduced serum NOS and CGRP levels compared to healthy controls. Second, MLCK levels did not differ significantly between groups overall, although an age-dependent reduction was observed in older patients with combined disease. Third, obesity in hypothyroid patients was associated with further reduction in NOS levels, suggesting an additive metabolic burden on the vasculature.

Interpretation of Key Findings

The observed reduction in serum NOS levels in hypothyroid patients aligns with a substantial body of evidence linking thyroid hormone deficiency to impaired nitric oxide bioavailability. Nitric oxide, synthesised by endothelial NOS, is the primary mediator of endothelium-dependent vasodilation. In hypothyroidism, reduced NOS expression and activity have been demonstrated in both animal models and human studies (10, 11). The mechanisms underlying this reduction are multifactorial and include decreased transcription of the NOS3 gene, increased oxidative stress that consumes available NO, and elevated levels of asymmetric dimethylarginine, an endogenous NOS inhibitor (12).

What makes our findings particularly interesting is the pattern observed across groups. The lowest NOS levels were found in the hypothyroidism-alone group, while the hypothyroidism-with-hypertension group showed intermediate values that did not differ significantly from controls. This seemingly paradoxical observation may reflect a compensatory response. In patients who develop hypertension in the setting of hypothyroidism, there might be upregulation of NOS as a counter-regulatory mechanism attempting to restore vasodilatory capacity and lower blood pressure. Alternatively, this pattern could be explained by differences in

disease duration or severity between groups, though our cross-sectional design cannot address this possibility directly.

The reduction in CGRP levels observed in hypothyroid patients with hypertension represents another potentially important finding. Calcitonin gene-related peptide is the most potent endogenous vasodilator known, acting primarily through neurogenic mechanisms independent of the endothelium (13). CGRP is released from perivascular sensory nerves and causes smooth muscle relaxation via activation of adenylate cyclase and elevation of cyclic AMP. Reduced CGRP levels have been reported in various forms of hypertension, including essential hypertension and pre-eclampsia (14). Our findings extend this observation to the specific context of hypothyroidism-associated hypertension, suggesting that neurogenic vasodilation may be impaired alongside endothelial mechanisms.

The convergence of reduced NOS and CGRP in the same patient population suggests that hypothyroidism with hypertension involves dysfunction of both endothelial-dependent and endothelial-independent vasodilatory pathways. This dual impairment may explain why blood pressure elevation in hypothyroid patients can be particularly difficult to manage and why these patients may be at heightened risk for cardiovascular events (15).

Our finding of unchanged MLCK levels across groups warrants careful interpretation. Myosin light chain kinase is the primary enzyme responsible for phosphorylating myosin light chain, thereby initiating smooth muscle contraction. Based on the known increase in systemic vascular resistance in hypothyroidism, one might have expected elevated MLCK levels or activity (16). Several explanations for the lack of difference are possible. First, MLCK is an intracellular protein, and serum levels may not accurately reflect its activity within vascular smooth muscle cells. Second, the regulation of vascular tone in hypothyroidism may involve other mechanisms, such as altered calcium sensitivity, Rho kinase pathway activation, or changes in myosin light chain phosphatase activity, rather than changes in MLCK expression per se (17). Third, any differences in MLCK might be more apparent in vascular tissue itself rather than in the circulation.

The age-dependent reduction in MLCK observed in older patients with hypothyroidism and hypertension (Table 6) is intriguing and warrants further investigation. Ageing is associated with changes in vascular smooth muscle phenotype, including reduced contractility and altered calcium handling (18). The additional burden of thyroid hormone deficiency and hypertension may accelerate these age-related changes, leading to detectable differences in circulating MLCK that are not apparent in younger patients.

Relationship to Previous Literature

Our findings extend the existing literature in several important ways. Previous studies have established that hypothyroidism is associated with endothelial dysfunction, as measured by flow-mediated dilation of the brachial artery (19). However, most of these studies focused on functional outcomes rather than specific molecular mediators. By measuring NOS and CGRP directly, we provide evidence for specific defects in vasodilatory pathways that may underlie the functional abnormalities observed in clinical studies.

The relationship between thyroid status and NOS has been examined in several previous studies. In a rat model, hypothyroidism was associated with reduced endothelial NOS expression and impaired acetylcholine-induced vasorelaxation (20). Human studies have shown that levothyroxine replacement therapy improves flow-mediated dilation and increases circulating NO metabolites (21). Our findings are consistent with these reports and extend them by demonstrating reduced serum NOS levels in a relatively large cohort of patients with documented hypothyroidism.

The literature on CGRP in thyroid disease is more limited. One study reported reduced CGRP immunoreactivity in the thyroid glands of patients with hypothyroidism, suggesting local deficiency (22). Another study found that thyroid hormone treatment increased CGRP expression in the hypothalamus of rats (23). To our knowledge, this is the first report of reduced serum CGRP levels in hypothyroid patients with hypertension, providing a potential mechanistic link between thyroid dysfunction and elevated blood pressure.

Clinical Implications

These findings have several potential clinical implications, though these must be considered preliminary given the study's limitations. First, the measurement of serum NOS and CGRP might serve as biomarkers for identifying hypothyroid patients at increased risk of developing hypertension. If confirmed in prospective studies, patients with the lowest levels of these vasodilatory mediators might benefit from more aggressive cardiovascular risk factor modification or closer blood pressure monitoring.

Second, the observation that obesity exacerbates the reduction in NOS in hypothyroid patients highlights the importance of weight management in this population. Obesity itself is associated with reduced NO bioavailability due to increased oxidative stress and inflammation (24).

The combination of hypothyroidism and obesity may therefore have synergistic detrimental effects on vascular health, emphasizing the need for integrated management addressing both conditions.

Third, these findings raise the possibility that therapies targeting the NO and CGRP pathways might have a role in managing hypertension in hypothyroid patients who remain hypertensive despite adequate thyroid hormone replacement. While speculative at this stage, phosphodiesterase type 5 inhibitors (which enhance NO signalling) and CGRP receptor agonists have been developed for other indications and could theoretically be repurposed (25). However, such an approach would require careful evaluation in clinical trials.

Study Limitations

This study has several important limitations that must be acknowledged. First and most significantly, the cross-sectional design precludes any conclusions about causality. We can state that hypothyroidism with hypertension is associated with reduced NOS and CGRP levels, but we cannot determine whether the thyroid dysfunction caused these changes, whether the hypertension caused them, or whether some other factor accounts for both observations.

Second, the groups were not well-balanced for important demographic variables. The control group was younger and predominantly male, while the hypothyroid groups were older and predominantly female. Both age and sex influence vascular function and the markers we measured. Estrogen, for example, enhances endothelial NOS expression and activity, which might explain why the female-predominant hypothyroid groups did not show even lower NOS levels (26). Ageing is associated with reduced NO bioavailability and increased arterial stiffness (27). These imbalances represent major confounding factors that we attempted to address through sensitivity analyses, but residual confounding remains possible.

Third, the sample size was modest, particularly for subgroup analyses. Some subgroups (e.g., younger patients with hypothyroidism and hypertension) contained as few as four participants, making the statistical comparisons in Tables 6 and 7 unreliable. These subgroup analyses should be considered hypothesis-generating rather than definitive.

Fourth, we measured serum levels of NOS and CGRP rather than their activity or their concentrations within vascular tissue. Serum levels may not perfectly reflect local vascular production or function. For NOS in particular, the enzyme is primarily intracellular, and serum levels might represent leakage from damaged cells rather than functional enzyme. This could explain the wide variability observed in NOS measurements (standard deviations comparable to means in some groups).

Fifth, the diagnosis of "early hypothyroidism" in the title is not fully supported by the biochemical data. The very low T4 values observed (approximately 1.0 µg/dL compared to a normal of 5.1-14.1) indicate overt, established hypothyroidism rather than early or subclinical disease. This discrepancy between the title and the actual patient population should be addressed.

Sixth, we did not collect data on potential confounding variables such as smoking status, physical activity level, dietary habits, or family history of cardiovascular disease. These factors could influence both thyroid function and vascular markers.

Seventh, the study was conducted at a single centre in Iraq, which may limit generalizability to other populations with different genetic backgrounds, dietary patterns, and environmental exposures.

Eighth, we did not perform a sample size calculation before initiating the study, and the convenience sampling approach may have introduced selection bias.

Strengths of the Study

Despite these limitations, the study has several strengths. The simultaneous measurement of three different vascular regulatory proteins provides a more comprehensive picture than studies focusing on a single marker. The inclusion of a hypertension-only comparison group (hypothyroidism with hypertension) alongside a hypothyroidism-only group allows some differentiation between effects attributable to thyroid dysfunction versus those attributable to hypertension itself. The use of standardised assays with rigorous quality control procedures enhances the reliability of the measurements. The detailed characterisation of thyroid function, including TSH, T3, and T4, allows accurate classification of participants' thyroid status.

Conclusion

This study demonstrates that patients with hypothyroidism, particularly those with concomitant hypertension, have reduced serum levels of nitric oxide synthase and calcitonin gene-related peptide compared to healthy controls. These findings suggest impairment of both endothelial-dependent and neurogenic vasodilatory pathways in hypothyroidism-associated hypertension. Myosin light chain kinase levels did not differ significantly between groups overall, though an age-dependent

reduction was observed in older patients with combined disease. Obesity in hypothyroid patients was associated with further reduction in NOS levels, highlighting the importance of weight management in this population.

The observed alterations in vasodilatory mediators may contribute to the increased vascular resistance and elevated blood pressure characteristic of hypothyroidism. If confirmed in larger prospective studies, these markers might prove useful for identifying hypothyroid patients at increased cardiovascular risk and for monitoring response to therapy.

List of Abbreviations

BMI: Body Mass Index
CGRP: Calcitonin Gene-Related Peptide
ELISA: Enzyme-Linked Immunosorbent Assay
MLCK: Myosin Light Chain Kinase
NO: Nitric Oxide
NOS: Nitric Oxide Synthase
PTH: Parathyroid Hormone
SD: Standard Deviation
T3: Triiodothyronine
T4: Thyroxine
TSH: Thyroid-Stimulating Hormone
VSMC: Vascular Smooth Muscle Cell

Declarations

Ethics Approval and Consent to Participate

This study was conducted in accordance with the Declaration of Helsinki and received ethical approval from the Ethical Approval Committee at Tikrit University (approval number: 3/7/413, dated 6 November 2023). All participants provided written informed consent before any study procedures were performed. Participants were informed of their right to withdraw from the study at any time without affecting their medical care.

Data Availability

The datasets generated and analysed during the current study are not publicly available due to participant privacy and confidentiality concerns, as the data contain potentially identifiable information. However, anonymised data are available from the corresponding author upon reasonable request, subject to approval from the institutional ethics committee.

Competing Interests

The authors declare that they have no competing interests, financial or otherwise, that could have influenced the work reported in this manuscript.

Funding Sources

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors. Institutional resources from the University of Kirkuk and Tikrit University supported the study.

Author Contributions

MGA worked on the conceptualisation, methodology, investigation, data curation, formal analysis, writing of the original draft, writing of the review and editing, visualisation and project administration.

AFS did the supervision, conceptualisation, methodology, validation, writing of the review, editing, and resources.

Both authors read and approved the final manuscript.

Acknowledgements

The authors thank the staff of the Endocrinology Unit at Azadi Teaching Hospital, Kirkuk, for their assistance with participant recruitment and clinical assessment. We are grateful to all study participants for their willingness to contribute to this research. The authors also acknowledge the laboratory staff at the College of Pharmacy, University of Kirkuk, for their technical assistance with sample processing and analysis.

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