

## Serum nitric oxide levels correlate with quality of life questionnaires scores of hypothyroid females



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### ARTICLE INFO

#### Keywords:

Hypothyroidism  
Nitric oxide  
Levothyroxine  
Quality of life

### ABSTRACT

Primary hypothyroidism can affect lipid metabolism, cardiovascular (CV) function, and overall patients' quality of life (QoL). Decrease in serum nitric oxide (NO) levels could promote the atherosclerosis acceleration in hypothyroid patients. Our hypothesis is that serum NO level is altered in hypothyroidism; more specifically, we hypothesize that the early vascular changes that can be observed in hypothyroidism could be due to these alterations and that serum NO levels are associated with lipid levels in female patients diagnosed with subclinical hypothyroidism (SCH) or clinical hypothyroidism (CH). Furthermore, since serum NO level is an early marker of atherosclerosis and related CV disorders, which are commonly present and follow hypothyroidism and greatly contribute to overall QoL, we further hypothesized that NO level would correlate with Thyroid Symptom Questionnaire (TSQ) and General Health Questionnaire 12 (GHQ12) scores in hypothyroid patients. A collateral of our hypothesis was that levothyroxine (LT4) treatment would affect serum NO levels as well as TSQ and GHQ12 scores. Therefore, we have analyzed lipid profile, the level of NO and QoL scores in female patients diagnosed with SCH and CH in order to determine the correlation between NO and generic and thyroid disease symptoms in treatment naïve SCH and CH patients and after LT4 treatment and laboratory euthyroidism achievement. As a consequence of our hypothesis is that measurement of serum NO level in SCH and CH patients may be an innovative way to improve LT4 treatment efficacy. This assumption could have a practical significance for future investigations regarding the management of hypothyroidism treatment protocols in current guidelines.

### Introduction

Insufficiency or inefficacy of peripheral thyroid hormones (TH) defined hypothyroidism. The mainstay of this syndrome is deceleration of numerous metabolic, mainly oxidative processes [1]. According to the clinical manifestations and biochemical definition, hypothyroidism is divided into latent, subclinical hypothyroidism (SCH) or overt, clinical hypothyroidism (CH) [2,3]. The prevalence of SCH is 1–20% of the general population and of CH expectedly lower, < 1% in non-endemic and > 5% in endemic areas [4–12].

Normal serum TH levels are necessary for the cardiovascular (CV) system (CVS) preservation [13,14]. The significant changes in CVS morphology and function are detected in both, SCH and CH [15], and

their extension depends on thyroid dysfunction severity and duration [16–18]. The SCH and CH are associated with an increased risk of atherosclerotic CV disease (CVD) and heart failure (HF) [19,20]. The presence of dyslipidemia, diastolic hypertension, increased arterial wall stiffness, endothelial dysfunction (ED), blood hypercoagulability, and elevated C-reactive protein (CRP) levels in patients with hypothyroidism could be the explanation of such an increased risk of atherosclerotic CVD, mainly presented as coronary heart disease (CHD), and HF [15,21]. Decreased nitric oxide (NO) bioavailability is one of the many possible causes or consequences of ED [22], and it is evidenced in hyperthyroid [23], SCH [24] and CH patients [23]. After levothyroxine (LT4) replacement and consequent achievement of euthyroidism in patients, some alterations in CVS function could be partially rather than

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<https://doi.org/10.1016/j.mehy.2019.109299>

Received 24 April 2019; Accepted 1 July 2019

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completely restored [25], including NO production [22].

The Quality of life (QoL) is the sense of well-being in the context of the culture and value system in which individuals live, in relation to their goals, expectations, standards and concerns' [26]. Different instruments used for its measurement can be divided into generic, disease-specific and for determined disease symptoms and signs assessment [26]. Generic instruments, such as General Health Questionnaire 12 (GHQ12) are suitable for the assessment of various aspects of health in different patients groups or general population [27]. The GHQ12 is a measure of current mental health and assesses psychiatric symptoms in different thyroid dysfunctions and the beneficial effects of administered LT4 [28–30]. Instruments for assessment of symptoms and signs of determined diseases, such as Thyroid Symptom Questionnaire (TSQ), pictured the presentation of thyroid disease in treatment-naïve patients as well efficacy and satisfaction with LT4 replacement [31,32].

The aims of this pilot study were: to determine serum NO levels in hypothyroid females, to evaluate the correlation between NO levels and generic and thyroid disease symptoms and signs-specific QoL scores, and to determine early effects of LT4 replacement on serum NO levels and QoL scores.

### Hypothesis

Thyroid function significantly affects lipid metabolism [33], CV function [21,34] and overall QoL [1,35]. Lipid metabolism is altered in both SCH and CH patients [33]. Beside TH, TSH has also independent, direct effects on lipid metabolism and an increased TSH level may be associated with a decrease of myocardial contractility and increased morbidity from CVD [36]. TH receptors are present in both myocardial and vascular endothelial tissues, so even minor changes in the levels of biologically active peripheral TH can affect the functioning of CVS [37]. Alterations in serum NO level are reported in several diseases; such are diabetes [38], metabolic syndrome [39], and CVD [40].

Moreover, SCH and CH patients have lower NO level and higher total cholesterol (TC), low-density lipoproteins-cholesterol (LDL-C), and CRP compared with euthyroid subjects [41–43]. Reduced NO bioavailability is accompanied by numerous modifiable atherosclerotic risk factors. All of these CV changes related to NO bioavailability in both types of hypothyroidism are reversible with LT4 replacement [21]. Experimental studies detected an increase in vascular NO production in hyperthyroid rats or after 3–4 months triiodothyronine (T3) treatment [44]. It was demonstrated that T3 via PI3K/Akt signaling pathway induces endothelium-independent rapid NO synthesis, presumably from vascular smooth muscle cells, with subsequent vasodilation [45,46]. The specific QoL questionnaires, TSQ and GHQ12, are used to determine the overall wellbeing of the hypothyroid patient and can be a useful tool when there is doubt to introduce LT4 replacement in SCH patient. Also, an important use of QoL questionnaires is the evaluation of LT4 therapy effects [47].

Thus, in the present pilot study, we hypothesized that serum NO level is altered in hypothyroidism; more specifically, we hypothesize that the early vascular changes that can be observed in hypothyroidism could be due to these alterations and that serum NO levels are associated with lipid levels in female patients diagnosed with SCH and CH. Furthermore, since serum NO level is an early marker of atherosclerosis and related CV disorders, which are commonly present and follow hypothyroidism and greatly contribute to overall QoL, we further hypothesized that NO level would correlate with TSQ and GHQ12 scores in hypothyroid patients/treatment naïve SCH and CH patients. A collaterally of our hypothesis was that LT4 treatment would affect serum NO levels as well as TSQ and GHQ12 scores (Fig. 1).

### Patients and methods

The pilot study included 82 female subjects recruited from Zemun Clinical Hospital, Serbia, who fulfilled basic inclusion criteria: to have

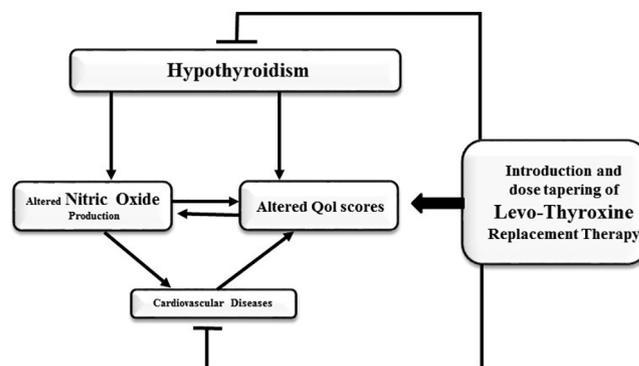


Fig. 1. Schematic representation of correlation of serum NO level with overall QoL in hypothyroidism and the beneficial effect of LT4 treatment on serum NO levels as well as on QoL. NO- nitric oxide; QoL- The Quality of Life; LT4- Levo-thyroxin.

performed biochemical analysis [TSH (thyroid stimulation hormone) and free thyroxine (FT4)] and that was LT4 treatment-naïve. They were divided into two groups: a group of patients (44 subjects) with newly diagnosed hypothyroidism and control group, consisting of 38 euthyroid subjects (normal values of TSH and FT4), gender-matched with the patient group. Hypothyroid group was further classified in SCH ( $3.40 < \text{TSH} < 10.00 \text{ mIU/ml}$ ,  $7.8 < \text{FT4} < 14.3 \text{ pmol/L}$ ) and CH ( $\text{TSH} > 10.00 \text{ mIU/ml}$ ,  $\text{FT4} < 7.8 \text{ pmol/L}$ ) group, each with 22 patients. Exclusion criteria were: LT4- or amiodarone-induced hyperthyroidism, the history of hypertension [ $> 140/90 \text{ mm}$  of mercury], diabetes [blood glucose  $> 6.1 \text{ mmol/L}$ , glycated hemoglobin (HbA1c) level  $> 6.5\%$ ], obesity [Body Mass Index (BMI)  $> 30 \text{ kg/m}^2$ ], inflammatory and malignant diseases, chronic renal and heart failure, chronic diarrhea, use of medicines [LT4, antithyroid drugs, statins, antiplatelet drugs, oral contraceptive pills, digitalis, beta- and angiotensin receptor blockers, inhibitors of ACE, corticosteroids, nitrates], age [ $< 18$  and  $> 70$  years], smoking and drinking habits. Control subjects were healthy volunteer subjects, recruited after a physical exam, with no history of any disease. None of the controls was taking any drugs affecting the levels of serum TH and lipid levels or the acceleration of atherosclerosis. From each subject, multiple serum samples were obtained after an overnight fast and diet (a list food enriched with nitrates, such as spinach, green salad, and beetroot to be avoided  $\geq 16 \text{ h}$  before blood sampling) [48]. The study was approved by the Ethics Committee of the School of Medicine, University of Belgrade, and informed consent was obtained from all subjects who participated in this pilot study.

Initially, at the time of the confirmation of thyroid dysfunction (by repeated TSH and FT4 measurement) in the hypothyroid group and the consequent initiation of LT4 treatment and in the control-euthyroid group, anthropometric, clinical, biochemical, and QoL parameters were assessed. The dose of LT4 was calculated according to the body mass (BM),  $< 1 \mu\text{g/kg BM}$  for subjects with TSH level  $< 10 \text{ mIU/mL}$  or  $1\text{--}1.5 \mu\text{g/kg BM}$  for subjects with TSH level  $> 10 \text{ mIU/mL}$ . In the cases of higher calculated doses, LT4 was gradually increased in weekly intervals. After three to six months of LT4 replacement therapy and established laboratory euthyroidism, to all treated patients serum NO levels and QoL scores were assessed.

### Anthropometric and clinical measurements

BMI was calculated as a BM (kg) divided by the square of height ( $\text{m}^2$ ). BM measurements were performed using a calibrated beam-type balance with the subject wearing light indoor clothes and no shoes and recorded to the nearest 0.1 kg. Body height was measured using Harpenden Anthropometer (Holtain Ltd., Crosswell, UK). Values for systolic blood pressure (SBP), and diastolic blood pressure (DBP) were

obtained using the same sphygmomanometer (HS 201C1 Palm Type Sphygmomanometer, Wenzhou Hongshun Industries and Trade Co.), a standard mechanical pressure gauge, with measurement on the left upper arm. The same person performed the same procedure for each subject: triple measurements with intervals of 10 min were obtained. Values are expressed in millimetres of mercury (mmHg). From obtained values, the mean value was calculated and used for further statistical analysis.

#### Determination of serum TSH and FT4 concentrations

Measurements of TSH and FT4 serum levels were determined by Immulite 2000 [49], by using a chemiluminescent enzyme immuno-metric assay. Briefly, the serum sample and a ligand-labelled tracer are added to a test unit containing a polystyrene bead coated with an antibody specific to the analyte to be measured. The tested samples underwent to a washing step after incubation. Further, an anti-ligand enzyme is introduced, and the test samples underwent to the second incubation after an unbound enzyme is removed. In the final step, a substrate is added, which in the presence of the enzyme produces emission of photons, measured by the Immulite instrument, and converted into concentration. Reference values for TSH and FT4 were 0.4–4.0 mIU/L and 7.8–14.3 pmol/L, respectively.

#### Determination of serum glucose, HbA1C, CRP, TC, triglycerides (Tg), high-density lipoproteins-cholesterol (HDL-C) and LDL-C levels

Serum glucose levels were measured by Dialab glucose GOD-PAP kit according to the manufacturer's manual and expressed as mmol/L. Serum concentrations of HbA1C and CRP were determined by a turbidometric inhibition immunoassay using the DXC 800 Beckman and Coulter apparatus (Beckman Coulter, USA). The levels of HbA1C and CRP were expressed in % and mg/L, respectively. The reference values for CRP and HbA1C (for non-diabetics) were < 7.0 mg/L and < 6.5% respectively. Serum concentrations of TC, triglyceride (Tg) and high-density lipoproteins-cholesterol (HDL-C) were assessed on Instrumentation laboratory autoanalyzer using enzymatic assays (Instrumentation Lab, MA, USA) [50,51] and the values of LDL-C were calculated using Friedewald's equation [52] [ $LDL-C = TC - HDL-C - 0.2 * TG$  (mmol/L)]. Concentrations of TC, Tg, HDL-C and LDL-C were expressed as mmol/L. The reference values were 3.6–6.1; 3.6–5.1; < 1.7, > 1.1; and < 3.2 in mmol/L for glucose, TC, Tg, HDL-C and LDL-C, respectively.

#### Determination of serum NO levels

Serum nitrate (NO<sub>3</sub><sup>-</sup>) and nitrite (NO<sub>2</sub><sup>-</sup>) concentrations were measured as end products of NO, since it is difficult to measure NO concentration directly, because of its short half-life. The concentrations of NO were measured at the initial visit in the overall study population and at control visit after laboratory euthyroidism was achieved in the hypothyroid treated group. Serum NO levels were measured by Nitrate/Nitrite Colorimetric Assay Kit (Cayman Chemical I.N. 780001) according to the manufacturer's manual through two-steps reaction. The first reaction included nitrate-reductase activity, and the second one

was the Griess reaction [48,53]. The absorbance at 540 nm was measured in an automated microplate reader (Perkin Elmer, Wallac 1420 Victor) and the nitrate concentrations were calculated from a NaNO<sub>3</sub> standard curve and expressed as μmol/L.

#### QoL scores

To all examinees, TSQ and GHQ12 were delivered on the initial visit (TSQi, GHQ12i) and in treated patients on control visit (TSQeu, GHQ12eu), when laboratory euthyroidism according to TSH level was achieved. There was no bias during the Questionnaires fulfilling. The same format of structured question and answers determined the use of those in the present study. After the Questionnaires fulfilling, total scores were calculated. Each item is rated on a four-point scale (less than usual, no more than usual, rather more than usual, or much more than usual). The selected scoring method was standard (Likert) [the answers are transformed into ranks: a to 0, b to 1, c to 2 and d to 3]. Maximal total scores of both study questionnaires are 36. The total scores are then classified in clusters (< 15- no distress; 16–25- minor distress; > 25- major distress) [26,27,54].

#### Statistical analyses

The Statistical Package for the Social Sciences (SPSS) 18.0 (SPSS Inc., Chicago, Illinois) statistical software package was used for all statistical calculations. Data are presented as the mean ± standard deviation for continuous variables, or as the median for heterogeneous variables. Skewed data were logarithmically transformed were needed for further analysis. Differences between two groups were analyzed by chi-square, Wilcoxon and Mann-Whitney test. Differences between the three groups were analyzed by one-way ANOVA and Kruskal-Wallis test. To test correlations, Spearman (ρ) and Pearson's (r) test were used. A linear regression analysis model (presented as b coefficient) was used to test the prediction of anthropometric, biochemical and hormonal variables for serum NO concentration. The values of p < 0.05 (2-tailed) were considered statistically significant.

#### Preliminary results and discussion

In the present study, we have analyzed lipid profile and the level of NO in female patients diagnosed with SCH and CH in order to determine the correlation between NO and generic and thyroid disease symptoms and signs-specific QoL scores in treatment naïve SCH and CH patients and after LT4 treatment and laboratory euthyroidism achievement. To the best of our knowledge, our pilot study is the first report that investigates the connection between NO, lipids and QoL scores in SCH and CH patients.

The anthropometric and clinical parameters of SCH and CH patients and control subjects are presented in Table 1. SCH and CH patients have significantly higher BMI (p < 0.01), while SBP and DBP were not found to be significantly different compared with control subjects (Table 1.). The level of TSH was significantly increased (p < 0.01) in SCH and CH patients compared with control subjects (Table 2.). Level of FT4 was within the reference range for both SCH and control groups and elevated in the CH group.

**Table 1**  
Anthropometric and clinical characteristics of the study population.

Parameters	SCH	CH	Control	p	Intergroup difference
Age [year]	43.0 ± 14.0	44.4 ± 10.8	36.3 ± 8.0	0.007	CH vs. Cs
BMI [kg/m <sup>2</sup> ]	25.3 ± 3.5	25.3 ± 3.5	22.9 ± 3.0	0.006	SCH vs.Cs; CH vs. Cs
SBP [mmHg]	116.8 ± 15.2	116.4 ± 11.1	117.1 ± 9.6	0.967	n.s
DBP [mmHg]	70.9 ± 8.7	73.0 ± 9.7	73.9 ± 6.8	0.385	n.s

SCH- subclinical hypothyroid group; CH- clinical hypothyroid group; BMI- Body Mass Index; SBP- systolic blood pressure; DBP- diastolic blood pressure; p- the statistical significance level; n.s.-non significant.

**Table 2**  
Hormonal, biochemical and inflammatory parameters of the study population.

Parameters	SCH	CH	Control	p	Intergroup difference
Ln TSH [mIU/ml]	2.75 ± 0.11	3.52 ± 0.53	2.43 ± 0.06	< 0.001	SCH vs. Control; CH vs. Control;
FT4 [pmol/L]	9.35 ± 1.5	6.1 ± 1.7	10.9 ± 1.2	< 0.001	SCH vs. CH SCH vs. Control; CH vs. Control;
Glycaemia [mmol/L]	5.3 ± 0.6	5.0 ± 0.5	4.7 ± 0.4	< 0.001	SCH vs. CH SCH vs. Control; CH vs. Control
HbA1c [%]	5.3 ± 0.4	5.2 ± 0.6	5.1 ± 0.3	0.121	n.s
TC [mmol/L]	5.7 ± 1.3	6.3 ± 1.9	5.0 ± 0.8	0.005	CH vs. Control
HDL-C [mmol/L]	1.0 ± 0.2	1.1 ± 0.3	1.5 ± 0.3	< 0.001	SCH vs. Control; CH vs. Control
LDL-C [mmol/L]	4.3 ± 1.2	4.4 ± 1.5	3.1 ± 0.8	< 0.001	SCH vs. Control; CH vs. Control
Ln Tg [mmol/L]	2.40 ± 0.07	2.44 ± 0.12	2.40 ± 0.04	0.291	n.s/
Ln NO [µM]	2.99 ± 0.34	3.17 ± 0.44	3.22 ± 0.59	0.128	n.s
CRP [mg/L]	0 (1.33)	0.2(0.7)	0.1 (0.4)	0.902	n.s

TSH- thyroid stimulating hormone; FT4- free thyroxin; HbA1c- glycated hemoglobin; TC- total cholesterol; HDL-C- HDL-cholesterol; LDL-C- LDL-cholesterol; Tg- triglycerides; NO- nitric oxide; CRP- C-reactive protein. Other abbreviations are the same as under Table 1.

Results of biochemical, hormonal and inflammatory parameters of the same groups are presented in Table 2. Significantly higher glucose level ( $p < 0.001$ ) in SCH and CH patients compared with control subjects, but no significant difference in the level of HbA1c were observed. Results obtained for lipid profile in our pilot study are similar to the results reported from other studies [55–58]. TC concentration was significantly higher only in the CH group compared with controls ( $p < 0.01$ ), while the levels of HDL-C were significantly lower ( $p < 0.001$ ) and LDL-C ( $p < 0.001$ ) significantly higher in both SCH and CH group of patients compared with the results obtained for the control subjects. No significant changes in Tg between SCH and CH patients and control subjects were observed. The LDL-receptor expression is upregulated by TH, suggesting that hypercholesterolemia is a frequent consequence of hypothyroidism [59]. In contrary to results showing elevated serum lipid levels, there are also studies indicating no significant difference in lipid profile between SCH and CH patients and euthyroid controls [60,61], and some other studies have yielded conflicting findings showing an association of SCH and CH with lipid variables other than TC and LDL-C [62–64]. In addition, it has been demonstrated that increased LDL-C in SCH and CH is reversible with LT4 replacement [58,65,66]. LT4 replacement therapy contributed to the correction of hypercholesterolemia in 11 of 13 performed studies as well as the correction of elevated LDL-C levels in 7 of 9 studies [67]. Mostly serum Tg is not changed in SCH patients as in our study, but sometimes the level of Tg can be slightly increased [12].

Dyslipidemia associated with hypothyroidism is a common risk factor for CVD [58,68]. In patients with SCH, the most frequent CV abnormalities are diastolic dysfunction and an increase in systemic vascular resistance. All these abnormalities are due to impaired ventricular filling and relaxation, and ED [36,69,70]. Decreased NO bioavailability is associated with ED [22]. There are several studies that show decreases in NO production in patients suffered from hyperthyroidism [23], as well as SCH and CH [23,24]. However, we did not find any significant decrease in NO production in SHT and CH patients in comparisons with euthyroid controls (Table 2.), as it was expected [23,24,71]. We have observed a non-significant decrease in NO concentrations in SCH and CH patients. In addition, no significant change in the observed inflammatory marker, CRP between SHT and CH patients, and control subjects were observed (Table 2.). The study performed by Kumar et al. [71] shows that NO levels were significantly decreased in SCH patients compared with controls. Vicinanza et al. reported that reduced endothelial NO production in hypothyroidism could be due to inhibition of NO production mediated by non-genomic effects of TH [72]. Besides other studies also indicated the lower

concentrations of NO in the SCH group compared with healthy euthyroid controls [22,24,41]. Reasons for controversial results could be probably because of different study population ethnicity, the relatively small sample of the examined population, and differences in the study design.

Furthermore, multiple linear regression analysis of anthropometric, biochemical and hormonal predictors for NO concentration shows that the Tg level is the only independent predictor for serum NO concentration ( $p < 0.001$ ) in our pilot study population (Table 3). Nevertheless, the multiple linear regression models did not demonstrate a correlation between NO level and TSH and FT4 level in our pilot study (Table 3). However, there are other studies that show correlation between NO concentrations and TSH levels even after adjustment for other CVD risk factors, suggesting the effects of elevated TSH on atherosclerosis progress in patients with hypothyreosis [41,73]. In addition, the correlations analysis between NO and anthropometric and biochemical parameters show a statistically significant positive correlation between NO level and BMI ( $r = 0.279$ ;  $p < 0.01$ ) (Fig. 2A), HbA1C ( $r = 0.232$ ;  $p < 0.05$ ) (Fig. 2B) and Ln Tg ( $r = 0.409$ ;  $p < 0.001$ ) (Fig. 2C), however a much larger study population size is required in regard to showing real correlation significance; to show if this correlation is positive.

Average duration and overall weekly doses of LT4 therapy sufficient for the achievement of laboratory euthyroidism are shown in Table 4. The median time of laboratory euthyroidism achievement is the same for both groups of patients, but the weekly doses of LT4 were significantly higher in CH patients as expected. A positive effect of LT4 substitution on the level of lipid fractions and reduced risk of CV risk and CHD is shown in SCH and CH patients [68,74–78]. Lipid profile normalization and partial restoration of deranged endothelium-dependent vasodilation are registered in LT4 treated patients with

**Table 3**  
Anthropometric, biochemical and hormonal predictors for NO concentration in the observed population (Multiple linear regression analysis).

Parameters	b	SE	p
BMI [kg/m <sup>2</sup> ]	−0.010	0.016	0.545
HbA1c [%]	0.042	0.114	0.714
LnTg [mmol/L]	3.671	0.697	< 0.001
LnTSH [mIU/ml]	−0.112	0.169	0.510
FT4 [pmol/L]	0.056	0.039	0.156

b- regression coefficient; SE- standard error. Other abbreviations are the same as under Tables 1 and 2.

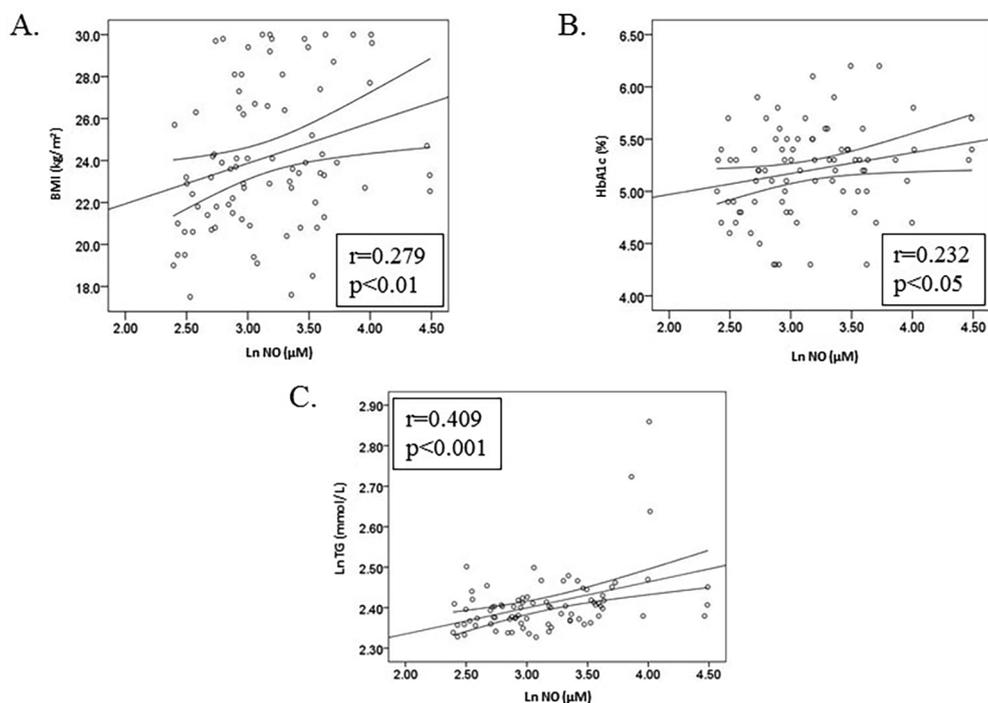


Fig. 2. Correlation between NO concentrations and BMI (A), HbA1c (B) and Ln Tg (C). *r*-coefficient; Other abbreviations are the same as under Tables 1 and 2.

Table 4

Duration and a weekly dose of LT4 replacement needed to achieve laboratory euthyroidism.

Parameters	SCH		CH		<i>p</i>
	Median	Min-max	Median	Min-max	
Duration [months]	3	3–6	3	3–6	0.544
Weekly LT4 dose [µg]	175	175–525	525	225–875	< 0.001

LT4- Levo-thyroxin. Other abbreviations are the same as under Table 1.

hypothyroidism [22,79,80]. Better effects of LT4 on lowering significantly elevated TC levels (i.e. > 6.2 mmol/L) were shown in SCH patients [67]. The regular and proper use of LT4 replacement, decrease the requirement for lipid-lowering drug use [81].

Furthermore, LT4 reduces almost entirely CVS abnormalities, such as decreased myocardial contractility, systolic and diastolic dysfunction, and increased systemic vascular resistance. Patients with SCH, who are under substitution with LT4, have significantly reduced risk from heart failure, and reduced overall mortality rates in comparison with untreated patients [82,83]. In our previous study we show that after LT4 replacement in hypothyroid subjects, the values for intima-media complex thickness (IMCT) were significantly decreased compared with the values before LT4 treatment. A significant decrease in IMCT was probably accompanied by the fall of TC and TSH. The arterial wall changes occur early with an increase of TSH, and LT4 treatment leads to improvement of the lipid profile and decreases IMCT. The improvement of IMCT in hypothyroid patients after LT4 therapy could contribute to the reduction of CV risk [68].

Furthermore, six months after stable euthyroidism is achieved, as expected the levels of TSH were significantly decreased, and NO concentrations in comparison with the levels at the treatment-naïve visit were insignificantly decreased in both SCH and CH groups (Table 5). Even though after LT4 management serum NO levels decreased insignificantly, this change in NO concentration is an important early effect of LT4 substitution. The lowest NO levels were detected in patients with SCH, while the highest ones in euthyroid controls. Moreover, our results show that serum NO levels before and after LT4 replacement are in the

normal range for adult females. Even though FT4 levels were similar in SCH and euthyroid groups, the NO level was lower in SCH, thus could be concluded that the levels of NO are TSH dependent [41].

To evaluate the correlation between NO levels and TSQ and GHQ12 scores, and to determine the early effects of LT4 replacement therapy on QoL scores, we further measured TSQi, GHQ12i, TSQeu and GHQ12eu scores in SCH and CH patients and controls. As we expected [27], our results show increased TSQ and GHQ12 scores in both SCH and CH patients group compared with controls and a significant decrease of TSQ and GHQ12 scores after laboratory euthyroidism achievement in treated SCH and CH group of patients. There are conflicting results of QoL scores in SCH despite the expectations that they should be reduced based on the QoL scores for CH [84,85]. A very important use of questionnaires can be in SCH patients, particularly in those with significantly higher scores (“major-distress cluster”) either of general or of disease symptoms and signs expression tests. Considering improved QoL scores of laboratory euthyroid patients who feel like hypothyroid [86], some authors added T3 or increased LT4 dose to achieve lower reference or even slightly subnormal levels of TSH [87]. Median overall TSQi, GHQ12i, TSQeu and GHQ12eu scores are 14 (5–36), 12 (1–28), 12 (1–28) and 10 (4–31), respectively. Distribution of mean QoL scores through patients groups is presented in Table 6. TSQi and GHQ12i were significantly increased in SCH and CH patients group compared with controls. A significant difference is registered between overall TSQi and TSQeu ( $Z = -5.426$ ,  $p < 0.01$ ) and overall GHQ12i and GHQ12eu ( $Z = -4.239$ ,  $p < 0.01$ ), both TSQ and GHQ12 were significantly decreased after laboratory euthyroidism achievement in SCH and CH patients. Additionally, significant correlations are shown between TSQi and GHQ12i ( $\rho = +0.737$ ,  $p < 0.01$ ) and between TSQeu and GHQ12eu ( $\rho = +0.524$ ,  $p < 0.01$ ).

Jorde et al. documented a significantly better mental health status in males with SCH in comparison to gender and age-matched controls [88]. Furthermore, significant positive correlations are shown between TSQi and GHQ12i and between TSQeu and GHQ12eu in both SCH and CH patients group indicating that thyroid related psychological distress is closely related to the general psychological well-being of patients with SCH and CH before as well as after LT4 induced euthyroidism.

Further, we evaluated the connection between NO level and TSQ

**Table 5**  
The concentrations of TSH and NO before and after LT4 replacement.

Parameters	SCH		p	CH		p
	Before LT4	After LT4		Before LT4	After LT4	
Ln TSH [mIU/ml]	2.75 ± 0.11	2.52 ± 0.07	< 0.001	3.52 ± 0.53	2.50 ± 0.09	< 0.001
Ln NO [µM]	2.99 ± 0.34	2.95 ± 0.41	0.725	3.17 ± 0.44	3.28 ± 0.43	0.242

Ln- natural logarithm. Other abbreviations are the same as under previous tables.

**Table 6**  
Mean QoL scores.

QoL scores	SCH	CH	Control	p
TSQi	15.5 (8–24)	16.5 (5–36)	11 (7–23)	p < 0.01
GHQ12i	13 (5–26)	14.5 (3–28)	7 (1–15)	p < 0.01
TSQeu	12 (3–20)	12.5 (1–28)	NA	p > 0.05
GHQ12eu	8 (4–31)	10 (6–20)	NA	p > 0.05

QoL- The Quality of Life; SCH- subclinical hypothyroid group; CH- clinical hypothyroid group; TSQi- The Thyroid Symptom Questionnaire on initial visit; GHQ12i- General Health Questionnaire 12 on initial visit; TSQeu- The Thyroid Symptom Questionnaire after euthyroidism achievement; GHQ12eu- General Health Questionnaire 12 after euthyroidism achievement; NA- non applicable; Other abbreviations are the same as under Table 1.

and GHQ12 scores in SCH and CH patients and controls. The results show that the initial serum NO level of overall study population negatively correlates with TSQi score ( $\rho = -0.259$ ,  $p < 0.05$ ), but that there is no correlation of serum NO level with GHQ12i score ( $\rho = +0.142$ ,  $p > 0.05$ ). Furthermore, there is neither significant correlation between NO level after LT4-induced laboratory euthyroidism achievement and TSQeu score ( $\rho = +0.181$ ,  $p > 0.05$ ) nor GHQ12eu score ( $\rho = +0.142$ ,  $p > 0.05$ ).

Based on the results from our study we suggest that the use of the initial serum NO level, together with QoL instruments (i.e. TSQ) could be another beneficial weapon in the hands of clinicians that could help them to evaluate the severity of hypothyroidism and make the decision to introduce LT4 substitution.

## Conclusions

In conclusion, our preliminary results presented in this paper suggest that serum NO level could be altered and associated with lipid levels in female patients diagnosed with SCH and CH. Furthermore, our preliminary results show that the initial serum NO concentration of the overall study population negatively correlates with TSQi score. Therefore, we assume that serum NO level could be a good predictor of the severity of hypothyroidism and that LT4 treatment will affect serum NO levels as well as TSQ and GHQ12 scores. As a consequence of our hypothesis is that measurement of NO level in SCH and CH patients may be an innovative way to improve LT4 treatment efficacy.

Based on our preliminary data we propose that NO level may be a useful tool in the hands of clinicians that could help them to evaluate the severity of hypothyroidism and make the decision to introduce LT4 substitution in patients with SCH, or to adjust the actual normal personal TSH threshold in SCH and CH patients, considering that TSH reference values in young people and women of reproductive age are already less than the normal adult TSH reference values. Thus, monitoring circulating NO levels in hypothyroidism may serve as an appropriate diagnostic measure and our preliminary results might be helpful for designing a clinical study on a larger population sample for initiating and optimizing LT4 therapy, especially in SHT and for a better understanding of the effects of LT4 substitution on serum NO level in the patients with SCH and CH as well as unraveling the relationship between NO and TSH, lipids and CVD and overall QoL. Considering the lack of statistically significant change in NO level after LT4 substitution,

it would be of benefit to design a study in regard to follow up later effects of LT4 treatment (for example 6 or 12 months after achievement of euthyroid TSH level) and to address their association with lipids and CVD and overall QoL parameters. Further research providing further arguments related to serum NO levels correlation with QoL scores of hypothyroid patients, is important to develop new therapeutic approaches to prevent and treat complications associated with hypothyroidism.

## Declaration of Competing Interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this article.

## Acknowledgements

This work was supported by grants funded by the Ministry of Science, Education and Technological development, Republic of Serbia, No. 173033 (to E.R.I.) Special thanks to all physician's and nurses' staff of Dept. of Endocrinology and Diabetes of Zemun Clinical Hospital for enormous endeavor and engagement towards study protocol realization.

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