

**ASSOCIATION BETWEEN THYROID HORMONES, LIPIDS AND OXIDATIVE STRESS MARKERS IN SUBCLINICAL HYPOTHYROIDISM**

## POVEZANOST IZMEĐU TIREOIDNIH HORMONA, LIPIDA I MARKERA OKSIDATIVNOG STRESA U SUBKLINIČKOJ HIPOTIREOZI

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**Summary**

**Background:** Oxidative stress plays a role in the pathogenesis of many chronic diseases. It is recognized in overt hypothyroidism while its existence in subclinical hypothyroidism (SCH) is not well established. The aim of this study was to determine whether there was increased oxidation of lipids and proteins in SCH, and examine their association with lipids and thyroid hormones.

**Methods:** Male adults (35–59 years) with SCH (n=467) and euthyroid controls (n=190) were studied. Anthropometric measurements, plasma lipids, thyroid stimulating hormone (TSH), free thyroxine (FT4), free triiodothyronine (FT3), total antioxidant capacity (T-AOC), lipid peroxidation products, malondialdehyde (MDA), advanced oxidation protein products (AOPP) and dityrosine concentrations were measured.

**Results:** Plasma concentrations of MDA were significantly higher ( $p < 0.05$ ) in SCH ( $8.11 \pm 1.39$  nmol/mL) compared with euthyroid controls ( $7.34 \pm 1.31$  nmol/mL) while AOPP, dityrosine and T-AOC levels were not different. MDA was not associated with TSH ( $\beta = -0.019$ ,  $P = 0.759$ ), FT4 ( $\beta = -0.062$ ,  $P = 0.323$ ) and FT3 ( $\beta = -0.018$ ,  $P = 0.780$ ) in SCH while levels increased with elevated total cholesterol ( $\beta = 0.229$ ,  $P = 0.001$ ), LDL ( $\beta = 0.203$ ,  $P = 0.009$ ) and triglycerides ( $\beta = 0.159$ ,  $P = 0.036$ ) after adjustment for age

**Kratak sadržaj**

**Uvod:** Oksidativni stres učestvuje u patogenezi mnogih hroničnih oboljenja. Ima ulogu u manifestnoj hipotireozu, dok njegovo prisustvo u subkliničkoj hipotireozu (SH) nije sasvim razjašnjeno. Cilj ove studije bio je da se odredi da li postoji povišena oksidacija lipida i proteina u SH i da se istraži njihova povezanost sa lipidima i tireoidnim hormonima.

**Metode:** Ispitivani su odrasli muškarci (35–59 godina) sa SH (n=467) i eutireoidne kontrolne osobe (n=190). Izmereni su antropometrijski parametri, koncentracije lipida u plazmi, tireostimulišućeg hormona (TSH), slobodnog tiroksina (FT4), slobodnog trijodtironina (FT3), ukupni antioksidantni kapacitet (T-AOC), proizvodi lipidne peroksidacije, malondialdehid (MDA), uznapredovali proizvodi proteinske oksidacije (AOPP) i ditirozini.

**Rezultati:** Koncentracije MDA u plazmi bile su značajno povišene ( $p < 0,05$ ) u SH ( $8,11 \pm 1,39$  nmol/mL) u poređenju sa eutireoidnim kontrolnim osobama ( $7,34 \pm 1,31$  nmol/mL) dok se nivoi AOPP, ditirozina i T-AOC nisu razlikovali. MDA nije bio povezan sa TSH ( $\beta = -0,019$ ,  $P = 0,759$ ), FT4 ( $\beta = -0,062$ ,  $P = 0,323$ ) i FT3 ( $\beta = -0,018$ ,  $P = 0,780$ ) u SH dok su nivoi rasli s povišenim vrednostima ukupnog holesterola ( $\beta = 0,229$ ,  $P = 0,001$ ), LDL ( $\beta = 0,203$ ,  $P = 0,009$ ) i triglicerida ( $\beta = 0,159$ ,  $P = 0,036$ ) posle prilago-

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**Abbreviations:** SCH, subclinical hypothyroidism; FT4, free thyroxine; FT3, free triiodothyronine; TC, total cholesterol; TG, triglycerides; AI, atherosclerosis index; MDA, malondialdehyde; AOPP, advanced oxidation protein products; T-AOC, total antioxidant capacity; ROS, reactive oxygen species.

and body mass index. T-AOC reduced ( $\beta=-0.327$ ,  $P=0.030$ ) with increased MDA in euthyroid controls and not in SCH ( $\beta=-0.068$ ,  $P=0.349$ ), while levels increased with elevated triglycerides in both groups.

**Conclusions:** Oxidative stress was increased in subclinical hypothyroidism as evidenced by the elevated lipid peroxidation product, malondialdehyde, while protein oxidation was absent. Thus, reduction of oxidative stress may be beneficial in patients with subclinical hypothyroidism.

**Keywords:** hyperlipidemia, oxidative stress, subclinical hypothyroidism, thyroid hormones, thyroid stimulating hormone

## Introduction

Oxidative stress, caused by an imbalance in reactive oxygen species (ROS) produced during normal cell metabolism and/or efficiency of scavenger antioxidant defense, is implicated in the pathogenesis of many chronic diseases including cardiovascular diseases (CVDs) (1). It is well recognized in thyroid disorders, mainly hyperthyroidism and overt hypothyroidism (2, 3). However, few studies have reported enhanced oxidative stress in subclinical hypothyroidism (SCH). SCH is a common thyroid disorder characterized by mild elevation in thyroid stimulating hormone (TSH) while thyroid hormones are within normal levels. It has recently attracted much attention as it exhibits the same cardiovascular consequences as overt hypothyroidism (4). The prevalence in populations is influenced by factors such as TSH cutoff to define SCH, the race, sex, and age distribution of the population, pre-existing presence of autoimmune thyroid disorders, and iodine intake (5).

Thyroid hormones have a considerable impact on oxidative stress (6, 7), ascribed to their role in cellular metabolism and oxygen consumption (8, 9). They are kept within normal levels in the body via the endocrine negative feedback mechanism. However, changes in their levels could alter redox environment (10), via causing changes in the number and activity of mitochondrial respiratory chain components resulting in increased generation of ROS which are often attenuated by antioxidants (11). The overproduction of ROS results in increased oxygen consumption by thyroid hormones which disturbs the pro-oxidant/antioxidant balance leading to oxidative stress, and consequent damage to cellular structures, lipids, proteins, and DNA (12).

In the recent past, though information is still scanty, reports indicate that patients with SCH have altered redox status (13, 14) as evidenced by increased lipid peroxidation. While some studies show concomitant reduction in antioxidant status (15), others demonstrate an unaltered antioxidant system (16). Hypothyroid states may confer protective effects on lipid and protein oxidation via interception of ROS in the mitochondria by T3, or by activation of the antioxidant system (17). Unlike lipid peroxidation,

gođavanja za starost i indeks telesne mase. Porast nivoa MDA bio je praćen sniženjem nivoa T-AOC ( $\beta=-0,327$ ,  $P=0,030$ ) kod eutireoidnih kontrolnih osoba, ali ne i u SH ( $\beta=-0,068$ ,  $P=0,349$ ), dok su u obe grupe nivoi rasli s porastom triglicerida.

**Zaključak:** Oksidativni stres bio je povišen u subkličičkoj hipotireozii, što su pokazali i povišeni nivoi proizvoda lipidne peroksidacije, malondialdehida, dok je oksidacija proteina bila odsutna. Stoga, snižavanje oksidativnog stresa može biti korisno kod pacijenata sa subkličičkom hipotireozom.

**Ključne reči:** hiperlipidemija, oksidativni stres, subkličička hipotireoza, tireoidni hormoni, tireostimulišući hormon

studies reporting protein oxidation in SCH are currently limited. Haribabu et al. (18) recently reported that patients with SCH have increased MDA and protein carbonyls.

Lipid peroxidation products could also facilitate further generation of free radicals by forming adducts with proteins and exacerbate the effects of direct free radical-induced protein oxidation (19). In addition to protein carbonyls, advanced oxidation protein products (AOPP) are useful markers for estimating the degree of oxidant-mediated protein damage (20). They have been used to measure protein oxidation in many conditions including renal and cardiovascular diseases (21, 22). AOPPs, particularly albumin and its aggregates are abundant in dityrosine which allows cross-linking, forming disulphide bridges and carbonyl groups (23). Given that SCH increases cardiovascular disease risks and mortality, oxidative damage to lipids and proteins results in oxidative stress which could further enhance and aggravate risks. Therefore, the objective of this study was to determine whether there was increased oxidation of lipids and proteins in SCH, and examine their association with lipids and thyroid hormone levels.

## Methods

### *Participants and research design*

The study participants were university employees on routine annual clinical examination at the Jiangnan University hospital. In our previous work (24), we found high prevalence of metabolic syndrome and its components among male workers compared to females ( $n=2428$ ) that was attributed to occupation type and SCH (25). In the present study, we further examined whether there was increased oxidative stress in randomly selected males (35–59 years) with SCH ( $n=467$ ) compared with individuals with normal thyroid function ( $n=190$ ). Patients with hyperthyroidism, overt hypothyroidism, or taking thyroxine or antithyroid drugs for treatment, or taking medications affecting thyroid function or with history of thyroid disease or taking lipid lowering drugs and antioxidant vitamin supplements were excluded from the study. Participants consented to participate in the

study. Approval was obtained from Jiangnan University Ethical Committee, and the University hospital management. The study was conducted in accordance with the Declaration of Helsinki from 1975, as revised in 2000.

#### Physical body assessment and biochemical analysis

Weight (kg) and height (m) were taken using a height and weight machine (HW-700, Zhengzhou, China) and used to compute the body mass index (BMI). After overnight fasting, venous blood samples were drawn in the morning between 8.00 a.m. and 11.00 a.m. Plasma was separated after centrifugation (KDC-1044, Hangzhou, China) at  $1000 \times g$ ,  $4^\circ\text{C}$  for 10 minutes and stored at  $4^\circ\text{C}$  for immediate analysis of lipids. The remaining plasma was stored at  $-80^\circ\text{C}$  for further analysis. Plasma lipids were analyzed by enzymatic methods using diagnostic kits from Nanjing Jiancheng Bioengineering Institute, Nanjing, PR China (Automatic Biochemical Analyzer, HF 400, Shanghai, China) at the Jiangnan University hospital laboratory.

#### Assessment of thyroid function

Thyroid stimulating hormone (TSH), free thyroxine (FT4) and free triiodothyronine (FT3) were determined by radioimmunoassay (xh6080, Xi'an) at Beijing Sino-uk Institute of Biological Technology, China. Normal thyroid function (euthyroidism) was defined as normal TSH levels (0.4–4.5 mU/L), FT4 (19–25.60 pmol/L) and FT3 (3.20–9.20 pmol/L). TSH levels of 4.5–10 mU/L with normal FT4 and FT3 were diagnosed as SCH.

#### Evaluation of oxidative stress

Total antioxidant capacity (T-AOC) and lipid peroxidation products, malondialdehyde (MDA) were determined using corresponding diagnostic kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, PR China) following manufacturer's instructions. T-AOC and the pink chromogen produced by the reaction of thiobarbituric acid with MDA were measured spectrophotometrically (SpectraMax M5, Molecular Devices) at 520 nm and 532 nm, respectively. Protein oxidation was assessed by measuring AOPP and dityrosine. AOPP was measured spectrophotometrically (SpectraMax M5, Molecular Devices) according to the method by Witko-Sarsat et al. (20) while dityrosine was determined by fluorescence (Fluoro Max4, Horiba JY) at excitation and emission wavelength of 320 nm and 400 nm, respectively.

#### Statistical analysis

All data were analyzed using SPSS software for Windows, 18 (SPSS, Inc. Chicago, USA). Data were expressed as means  $\pm$  standard deviations. Independent samples t-test was used to compare two means. Association between lipids, TSH, thyroid hormones and oxidative stress biomarkers was analyzed by multiple linear regressions. P values  $<0.05$  were considered statistically significant.

## Results

#### Lipid parameters and thyroid function

The SCH group had higher ( $P<0.05$ ) BMI, total cholesterol (TC), low density lipoprotein cholesterol (LDL), triglycerides (TG) and TC-HDL/HDL ratio

**Table I** Anthropometric and biochemical parameters of study participants.

	Euthyroidism TSH (0.4–4.5 mU/L, n=190)	Subclinical hypothyroidism TSH (4.5–10 mU/L, n=467)	P
Age (years)	46.60 $\pm$ 7.20	47.10 $\pm$ 6.80	0.334
BMI (kg/m <sup>2</sup> )	23.78 $\pm$ 2.39	24.38 $\pm$ 2.60	0.017*
TC (mmol/L)	5.20 $\pm$ 0.96	5.37 $\pm$ 0.91	0.040*
LDL (mmol/L)	2.81 $\pm$ 0.77	2.95 $\pm$ 0.75	0.037*
Triglycerides (mmol/L)	1.438 $\pm$ 0.956	1.642 $\pm$ 0.935	0.008*
HDL (mmol/L)	1.407 $\pm$ 0.349	1.403 $\pm$ 0.388	0.912
TC-HDL/HDL	2.87 $\pm$ 0.89	3.05 $\pm$ 0.98	0.030*
TSH (mU/L)	4.06 $\pm$ 0.31	5.31 $\pm$ 0.70	0.001**
FT4 (pmol/L)	15.00 $\pm$ 2.39	14.74 $\pm$ 2.23	0.187
FT3 (pmol/L)	5.13 $\pm$ 0.86	5.09 $\pm$ 0.84	0.583

Data are mean  $\pm$  standard deviations; BMI – body mass index; TC – total cholesterol, LDL – low density lipoprotein cholesterol; HDL – high density lipoprotein cholesterol; TSH – thyroid stimulating hormone; FT4 – free thyroxine; FT3 – free triiodothyronine.

\*\*  $P<0.01$ , \* $P<0.05$  significant by independent samples t-test

**Table II** Association of lipid parameters as dependent variables with thyroid function tests.

		Euthyroidism (n=190)						Subclinical hypothyroidism (n=467)					
		TSH		FT4		FT3		TSH		FT4		FT3	
	Model	$\beta$	P	$\beta$	P	$\beta$	P	$\beta$	P	$\beta$	P	$\beta$	P
TC (mmol/L)	1	-0.139	0.074	0.061	0.439	-0.085	0.279	0.023	0.645	0.069	0.269	0.123	0.017*
	2	-	-	-	-	-	-	-	-	-	-	0.133	0.017*
LDL (mmol/L)	1	-0.111	0.156	0.002	0.982	-0.092	0.243	-0.024	0.641	0.118	0.026*	0.167	0.001**
	2	-	-	-	-	-	-	-	-	0.111	0.042*	0.162	0.004*
TG (mmol/L)	1	-0.062	0.428	0.040	0.612	0.224	0.004*	0.106	0.034*	0.039	0.473	0.180**	0.001**
	2	-	-	-	-	0.177	0.040*	0.115	0.026*	-	-	0.168**	0.001**
HDL (mmol/L)	1	-0.076	0.326	0.045	0.562	-0.119	0.123	-0.017	0.739	-0.137	0.009*	-0.144*	0.004*
	2	-	-	-	-	-	-	-	-	-0.294	0.002*	-0.143*	0.006*
TC-HDL/ HDL	1	-0.043	0.581	0.019	0.813	0.114	0.140	0.022	0.664	0.177	0.001**	0.252**	0.001**
	2	-	-	-	-	-	-	-	-	0.199	0.001**	0.246**	0.001**

Values of  $\beta$  are standardized regression coefficients; TSH—thyroid stimulating hormone (mU/L); FT4—free thyroxine (pmol/L); FT3—free triiodothyronine (pmol/L); TC—total cholesterol; LDL—low density lipoprotein cholesterol; TG—triglycerides; HDL—high density lipoprotein cholesterol.

Model 1, after adjustment for age

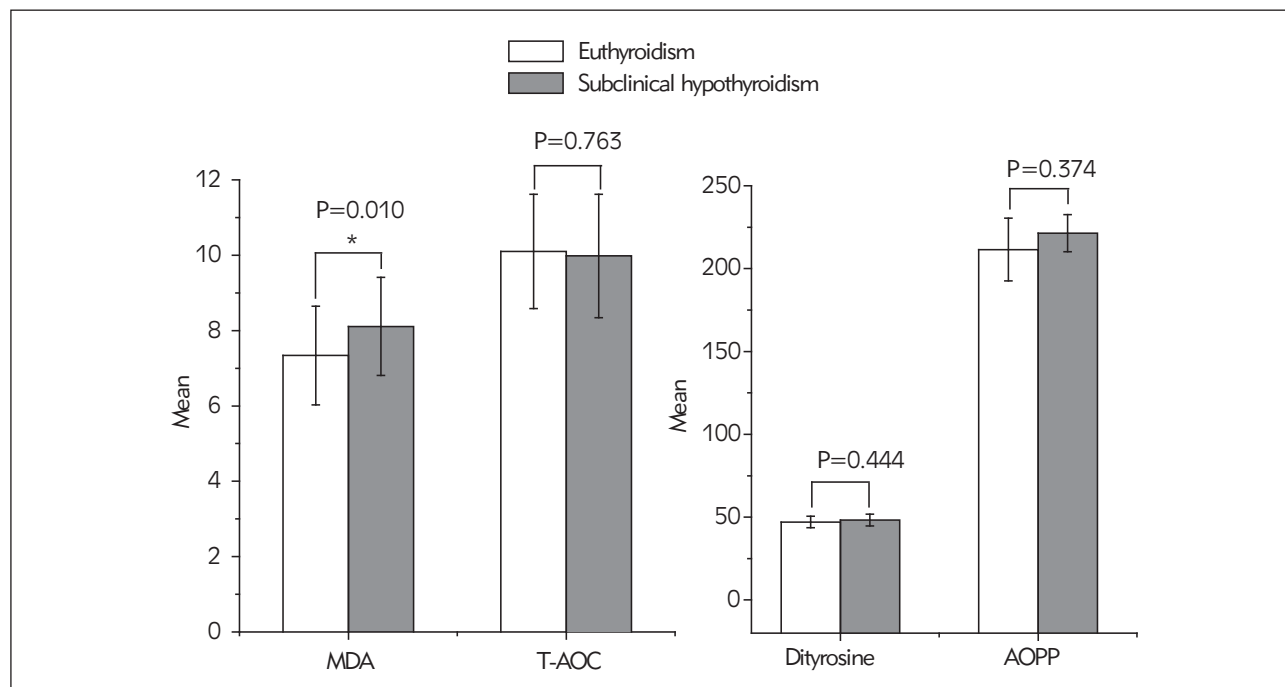
Model 2, further adjustment for body mass index

\*\* P<0.01, \*P<0.05 significant by multiple linear regression

compared with euthyroid controls whereas age and high density lipoprotein cholesterol (HDL) were not different (Table I). As expected, TSH levels were higher in SCH compared to euthyroids while FT4 and FT3 were the same. After adjustments for age and BMI, a positive relation between TSH and TG ( $\beta=0.115$ ,  $P=0.026$ ) was found in SCH whereas no association was found in the euthyroid group (Table II). FT4 correlated positively with LDL ( $\beta=0.111$ ,  $P=0.042$ ), TC-HDL/HDL ( $\beta=0.199$ ,  $P=0.001$ ) and inversely with HDL ( $\beta=-0.294$ ,  $P=0.002$ ), while FT3 associated ( $p<0.05$ ) with all the lipids. In the euthyroid group, only FT3 correlated positively with TG ( $\beta=0.177$ ,  $P=0.040$ ).

#### Oxidative stress markers and thyroid function

As illustrated in Figure 1, the SCH group showed elevated MDA ( $8.11\pm 1.30$  nmol/mL) levels compared to euthyroid controls ( $7.34\pm 1.31$  nmol/mL), whereas AOPP, dityrosine and T-AOC were not different ( $P>0.05$ ). The corresponding levels in euthyroids were  $211\pm 18.87$  mmol/L,  $47.08\pm 3.44$  pg/mL and  $10.10\pm 1.52$  U/mL while levels in SCH were  $221.39\pm 11.21$  mmol/L,  $48.27\pm 3.61$  pg/mol and  $9.98\pm 1.64$  U/mL, respectively. MDA was not associated ( $P>0.05$ ) with either TSH or thyroid hormones in SCH (Table III). After age and BMI adjustment, TSH correlated positively with AOPP ( $\beta=0.186$ ,



**Figure 1** Oxidative stress markers in euthyroidism and subclinical hypothyroidism. Data are mean  $\pm$  standard deviations; \* $p < 0.05$  significant using independent samples t-test; AOPP- advanced oxidation protein products (mmol/L); MDA-malondialdehyde (nmol/mL); T-AOC-total antioxidant capacity (U/mL); dityrosine (pg/mL).

**Table III** Association of oxidative stress markers as dependent variables with thyroid function tests and total antioxidants.

		Euthyroidism (n=190)						Subclinical hypothyroidism (n=467)					
		MDA		AOPP		Dityrosine		TSH		FT4		FT3	
	Model	$\beta$	P	$\beta$	P	$\beta$	P	$\beta$	P	$\beta$	P	$\beta$	P
TSH (mU/L)	1	0.084	0.440	-0.141	0.290	0.069	0.618	-0.019	0.759	0.229	0.005*	-0.209	0.009*
	2	-	-	-	-	-	-	-	-	0.186	0.034*	-0.206	0.004*
FT4 (pmol/L)	1	-0.007	0.952	-0.046	0.737	-0.458	0.001**	-0.062	0.323	0.209	0.012*	0.070	0.383
	2	-	-	-	-	-0.397	0.015*	-	-	0.185	0.038*	-	-
FT3 (pmol/L)	1	0.004	0.037	0.099	0.458	-0.324	0.017*	-0.018	0.780	-0.010	0.907	0.018	0.825
	2	-	-	-	-	0.177	0.040*	0.115	0.026*	-	-	0.168**	0.001**
T-AOC (U/mL)	1	-0.456	0.001**	0.250	0.094	0.134	0.459	-0.068	0.349	0.136	0.148	-0.115	0.226
	2	-	-	-	-	-	-	-	-	-0.294	0.002*	-0.143*	0.006*
MDA (nmol/mL)	2	-0.043	0.581	0.019	0.813	0.114	0.140	0.022	0.664	0.177	0.001**	0.252**	0.001**
	1	-	-	0.200	0.254	-0.129	0.475	-	-	0.002	0.987	-0.041	0.683

Values of  $\beta$  are standardized regression coefficients; MDA-malondialdehyde (nmol/mL); AOPP-advanced oxidation protein products (mmol/L); dityrosine (pg/mL); T-AOC-total antioxidant capacity; TSH-thyroid stimulating hormone; FT4-free thyroxine; FT3-free triiodothyronine.

Model 1, after adjustment for age

Model 2, further adjustment for body mass index

\*\*  $P < 0.01$ , \* $P < 0.05$  significant by multiple linear regression

$P=0.034$ ) and inversely with dityrosine ( $\beta=-0.206$ ,  $P=0.004$ ), FT4 correlated positively with AOPP ( $\beta=0.185$ ,  $P=0.038$ ) while FT3 and T-AOC were not associated with lipid or protein oxidation markers. In euthyroids, dityrosine correlated negatively with FT4 ( $\beta=-0.397$ ,  $P=0.015$ ), while T-AOC reduced ( $\beta=-0.327$ ,  $P=0.030$ ) with increased MDA.

#### Oxidative stress markers and lipid parameters

Table IV shows the association between oxidative stress markers and lipid parameters. After adjustment for age and BMI, MDA increased ( $P<0.05$ ) with elevated lipids (except HDL) in SCH while no association was found in euthyroid controls. AOPP correlated positively with triglycerides ( $\beta=0.261$ ,  $P=0.003$ ), TC-HDL/HDL ratio ( $\beta=0.207$ ,  $p=0.015$ ), and inversely with HDL ( $\beta=-0.172$ ,  $P=0.045$ ). T-AOC positively associated with TG ( $\beta=0.135$ ,  $P=0.044$ ) and TC-HDL/HDL ratio ( $\beta=0.141$ ,  $P=0.030$ ) and inversely with TC, LDL and HDL. In the euthyroid group, AOPP correlated positively with TG ( $\beta=0.359$ ,  $P=0.010$ ), while T-AOC positively correlated with TG ( $\beta=0.227$ ,  $P=0.032$ ) and negatively with LDL (only age adjusted). Dityrosine was not associated ( $P>0.05$ ) with all lipid parameters in the euthyroid and SCH groups.

#### Discussion

Oxidative stress plays a role in the pathogenesis of many chronic diseases including cardiovascular diseases. Prospective studies indicate that patients with SCH have increased risk for all-cause and cardiovascular mortality (4), and thus the presence of oxidative stress in these patients could further enhance risks. In this study, oxidative stress was found to be increased in SCH due to elevated plasma lipids induced by low thyroid function. The SCH group presented a dyslipidemic pattern compared to euthyroid controls and was attributed to higher body weight. Obesity affects thyroid function via many mechanisms including increasing levels of leptin hormone (26) which subsequently affects TSH production (27). Leptin stimulates the hypothalamus-pituitary-thyroid axis, regulates the thyroid receptor gene expression, and is involved in the activation of T4 to T3 by deiodinase enzyme (28). It is possible that leptin caused a mild elevation in TSH which stimulated the thyroid to increase production of T4 and secretion of T3. T3 in turn affected lipids by increasing the expression of genes for key enzymes involved in lipid metabolism (8). T3 induces HMG-CoA reductase, which is the first step in cholesterol biosynthesis (9). It also upregulates LDL receptors that mediate uptake of cholesterol-rich LDL via directly binding to specific thyroid hormone responsive elements.

In the study, thyroid hormones affected lipids resulting in hypercholesterolemia, hypertriglyceridemia and reduced HDL levels in SCH. Hypercholesterolemia

**Table IV** Association of oxidative stress markers as dependent variables with lipid parameters.

	Model	Euthyroidism (n=190)								Subclinical hypothyroidism (n=467)							
		MDA		AOPP		Dityrosine		T-AOC		MDA		AOPP		Dityrosine		T-AOC	
		$\beta$	$P$	$\beta$	$P$	$\beta$	$P$	$\beta$	$P$	$\beta$	$P$	$\beta$	$P$	$\beta$	$P$	$\beta$	$P$
TC	1	-0.020	0.851	-0.016	0.910	0.042	0.762	-0.168	0.068	0.199	0.002*	0.029	0.732	0.046	0.570	-0.179	0.003*
	2	-	-	-	-	-	-	-	-	0.229	0.001**	-	-	-	-	-0.149	0.026*
LDL	1	-0.043	0.697	-0.129	0.336	-0.018	0.899	-0.228	0.012*	0.184	0.004*	0.067	0.415	0.070	0.390	-0.184	0.002*
	2	-	-	-	-	-	-	-0.180	0.102	0.203	0.009*	-	-	-	-	-0.155	0.019*
TG	1	0.075	0.484	0.348	0.006*	0.203	0.139	0.212	0.019*	0.197	0.015*	0.278	0.001**	0.030	0.711	0.147	0.016*
	2	-	-	0.359	0.010*	-	-	0.227	0.032*	0.159	0.036*	0.261	0.003*	-	-	0.135	0.044*
HDL	1	0.203	0.057	-0.186	0.156	-0.085	0.533	-0.124	0.174	-0.088	0.162	-0.211	0.008*	-0.122	0.903	-0.196	0.001**
	2	-	-	-	-	-	-	-	-	-	-	-0.172	0.045*	-	-	-0.195	0.003*
AI	1	-0.049	0.646	0.097	0.459	0.083	0.545	-0.067	0.467	0.151	0.016*	0.233	0.004*	0.026	0.749	0.120	0.043*
	2	-	-	-	-	-	-	-	-	0.159	0.036	0.207	0.015*	-	-	0.141	0.030*

Values of  $\beta$  are standardized regression coefficients; MDA—malondialdehyde (nmol/mL); AOPP—advanced oxidation protein products (mmol/L); dityrosine (pg/mL); T-AOC—total antioxidant capacity (U/mL); TC—total cholesterol (mmol/L); LDL—low density lipoprotein cholesterol (mmol/L); TG—triglycerides (mmol/L); HDL—high density lipoprotein cholesterol (mmol/L); AI—atherosclerosis index (TC-HDL/HDL ratio).

Model 1, after adjustment for age

Model 2, further adjustment for body mass index

\*\*  $P<0.01$ , \* $P<0.05$  significant by multiple linear regression



is due to decreased fractional clearance of cholesterol from the plasma and the reduced uptake by cells as a result of reduced number of LDL receptors (29). LDL receptors contain the SREBP-2 gene which is regulated by T3. Hence, LDL receptors could decrease when the SREBP-2 gene expression reduces. Likewise, hypertriglyceridemia in both the SCH and euthyroid group is due to decreased activity of lipoprotein lipase, also stimulated by T3 (30). Lipoprotein lipase lowers TG levels through hydrolysis of TG-rich lipoproteins, and augments transfer of cholesterol from these lipoproteins to HDL cholesterol. The reduction in HDL cholesterol is attributed to increased hepatic lipase which hydrolyses phospholipid-rich HDL2 to HDL3 and enhanced cellular uptake.

Plasma MDA is an important biomarker of oxidative damage to lipids. We observed a marked increase in MDA in SCH compared with euthyroid controls which indicates increased oxidative stress. This increase in MDA was not associated with thyroid hormones but was related to elevated plasma lipids. Thus, lipid oxidation in SCH may not have been directly caused by low thyroid function, but was enhanced by the presence of elevated plasma cholesterol and LDL secondary to hypothyroidism. This was also observed by Santi et al. (13). Prolonged availability of oxidation substrates (mainly LDL) in the plasma increases their susceptibility to oxidative modification (31). Oxidized LDL impairs endothelial function leading to atherosclerosis, the first major cardiovascular disease event. It is plausible that thyroid hormones impacted first on lipids causing hyperlipidemia, and excess lipids acted as substrates for T3. Consequently, accelerated consumption of oxygen by T3 occurred resulting in enhanced generation of ROS, higher consumption of cellular antioxidants and inactivation of antioxidant enzymes (10, 12).

Interestingly, the total antioxidant capacity (T-AOC) reduced in euthyroid controls but remained unchanged in SCH, a finding similar to Torun et al. (16) but contrary to other studies (14, 15). It is also suggested that the depletion of antioxidant defense may be more severe in overt hypothyroidism (TSH  $\geq$  10 mU/L) than in SCH (32). In the present study, although hypercholesterolemia resulted in increased MDA and concomitant consumption of antioxidants, severe depletion of antioxidants may not have occurred. This could be due to high concentrations of lipid-soluble antioxidants such as vitamin A,  $\alpha$ -tocopherol,  $\beta$ -carotene, and carrier proteins (33). These antioxidants are usually distributed in plasma lipids and lipoprotein fractions (34), and thus individuals with elevated plasma lipids may have higher levels of antioxidants. It follows therefore that the oxidant imbalance due to LDL oxidation caused the cells to respond by increasing antioxidants to counter ROS generation. It is also probable that the plasma TG contained high concentrations of lipid soluble antioxidants from the diet: hypertriglyceridemia is often associated with high fat intake. In the study, TG asso-

ciated positively with T-AOC in both the SCH and euthyroid group. Non-concomitant reduction in T-AOC may also be attributed to the role of T3 as an antioxidant in hypothyroid states. T3 can facilitate scavenging of ROS by increasing availability of antioxidants to cells, and thus protects LDL from oxidation (17). However, persistent hyperlipidemia could overload the antioxidant system resulting in concomitant reduction in antioxidant defense. In our previous study, we observed that oxidative stress increases with progressive hyperlipidemia (35). Therefore, it might be suggested that, in SCH, the antioxidant mechanism in the cells may be capable of quenching ROS at initial stages of hyperlipidemia, and antioxidant capacity could eventually be reduced in the course of time.

Proteins are also sensitive to oxidative damage leading to alteration in structure and function (20–22). In the study, protein oxidation was not found in SCH attributable to differential time course of changes in oxidation processes as polyunsaturated fatty acids are more susceptible to free radical attack compared to proteins, and/or differences in efficiency of repair mechanisms (36). AOPPs are mostly derived from albumins, and to a lesser extent from fibrinogen and lipoproteins. They are abundant in dityrosine which allows cross-linking, forms disulphide bridges and carbonyl groups (23). Currently, there are not many data on the effect of subclinical hypothyroid state on protein oxidation. One more recent study (18) showed increased protein carbonyls in SCH patients, and similar to the present study, protein carbonyls were not associated with MDA. Thus, more studies are needed to elucidate the presence of oxidative stress in mild thyroid states. The increased oxidative modification of lipids in SCH suggests the need for reduction of oxidative stress as it may further increase CVD risk and mortality in these patients. A probable limitation of the study has to do with the cross-sectional design that does not explain causality, and thus the need remains for a well-designed prospective study.

## Conclusion

The study demonstrated that oxidative stress was increased in subclinical hypothyroidism as indicated by the elevated lipid peroxidation product, malondialdehyde, while protein oxidation was absent. Thus, reduction of oxidative stress may be beneficial in patients with subclinical hypothyroidism.

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## Conflict of interest statement

The authors stated that have no conflicts of interest regarding the publication of this article.

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