Diabetes & Metabolic Syndrome: Clinical Research & Reviews 13 (2019) 312–316

Contents lists available at ScienceDirect

Diabetes & Metabolic Syndrome: Clinical Research & Reviews

journal homepage: www.elsevier.com/locate/dsx

Original Article

The influence of subclinical hypothyroidism on serum lipid profile, PCSK9 levels and CD36 expression on monocytes

M. Fazaeli, A. Khoshdel*, M. Shafiepour, M. Rohban

Rafsanjan University of Medical Sciences, Faculty of Medicine, Clinical Biochemistry Department, Rafsanjan, Iran

ARTICLE INFO

Article history:
Received 27 July 2018
Accepted 21 August 2018

Keywords:
Subclinical hypothyroidism
Thyroid stimulating hormone
Low-density lipoprotein cholesterol
Proprotein convertase subtilisin kexin 9
Cluster of differentiation 36

ABSTRACT

Background: Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a serine protease and a secreted protein which increases cholesterol levels in plasma via inducing degradation of low-density lipoprotein receptor (LDLR). Cluster of differentiation 36 (CD36) is a member of a family of cell surface proteins in many cells. CD36 is known as fatty acid translocase (FAT) because it imports fatty acids inside cells and participate in triglyceride storage. It has been suggested that PCSK9 regulates CD36 in some tissues.
Methods: Data and serum levels of TSH, FT4, lipid profile and PCSK9 and the expression of CD36 on monocytes from 40 new untreated patients with subclinical hypothyroidism (SH) and 40 age- sex- and BMI-matched euthyroid controls were analyzed in a cross-sectional study. Then the relationships between these parameters were examined.
Results: Patients with SH had higher TSH, FT4, total cholesterol (TC) and triglyceride (TG) Low-density lipoprotein (LDL) and PCSK9 levels than controls. There were significant and positive correlations between serum TSH levels and lipid parameters except HDL-C. PCSK9 had a significant and negative correlation with FT4. No significant correlation could be found in relation to PCSK9 and CD36.
Conclusions: PCSK9 inhibitors are used to reduce blood cholesterol levels as drugs. If it will be proven that PCSK9 can induce CD36 degradation, taking these drugs may have unwanted side effects. This study showed that serum PCSK9 and lipid profile levels increase in patients with subclinical hypothyroidism and there is no relationship between PCSK9 and CD36 in these patients.

1. Introduction

Thyroid hormone (TH) has an important role in regulation of a wide array of metabolic parameters. The association between TH and lipid metabolism has been well established [1]. Overt hypothyroidism is associated with hypercholesterolemia by alterating lipoprotein metabolism and is recognized as an important risk factor for atherosclerosis and cardiovascular disease (CVD) [2]. Subclinical hypothyroidism (SH) characterized by the finding of elevated serum thyroid stimulating hormone (TSH) concentrations with normal levels of free thyroxine (FT4) and free triiodothyronine (FT3). This condition is more common than overt hypothyroidism, nevertheless the nature and etiology of SH and overt hypothyroidism are identical [3,4]. Although the relationships between SH and lipid profile in serum have been investigated in several studies over the last 20 yr, the associations between SH and lipid status are still under debate [5]. Some research have shown that lipid profile is significantly increased in SH patients [6,7], and other studies reported contradictory results in the alteration of serum lipid profile under SH [8,9].

Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a secreted protein and enzyme which is expressed mainly in liver, kidney, intestine, and central nervous system. PCSK9 induces low-density lipoprotein receptor (LDLR) degradation and reduced LDLR levels on the plasma membrane. The catalytic domain of circulating PCSK9 binds to the epidermal growth factor precursor homology domain-A (EGF-A) of the LDLR in a calcium dependent manner and after internalization of this complex PCSK9 prevents LDL-R recycling to the cell surface and then inhibits low-density lipoprotein-cholesterol (LDL-C) clearance from plasma. So PCSK9 serum levels correlate positively with the plasma LDL-C [10]. Some studies have been suggested that thyroid function may regulate cholesterol metabolism via PCSK9, because a positive correlation between TSH and PCSK9 levels has been reported [11].

* Corresponding author. Department of Clinical Biochemistry, Faculty of Medicine, and Pistachio Safety Research Center, Rafsanjan University of Medical Sciences, Rafsanjan, Iran.
E-mail address: sedaname7@gmail.com (A. Khoshdel).

https://doi.org/10.1016/j.dsx.2018.08.021
1871-4021/

Cluster of differentiation 36 (CD36; scavenger receptor class B type 3 (SRB3)) is a member of the scavenger receptor, class B, type I (SRBI), a family of cell surface proteins in many cells like monocytes, macrophages, hepatocytes, and adipocytes that binds ligands including thrombospondin (TSP-1), oxidized LDL and collagen. CD36 is also known as fatty acid translocase (FAT) because it imports fatty acids inside cells [12]. Therefore CD36 involves in regulation of lipid metabolism by facilitating cellular uptake of fatty acids and participating in triglyceride storage. It has been suggested that PCSK9 regulates CD36 in some tissues [13].

It is established that high lipid profile is a risk factor of coronary heart disease (CHD) [14], and investigating the association between SH and lipid profile is important to prevent CHD in SH patients. Therefore, the aim of this study was to investigate the association of SH with cholesterol and lipoprotein metabolism in human and the correlation between PCSK9 and CD36 expression in this disease.

2. Subjects and methods

2.1. Subject selection

Forty new untreated patients (8 men and 32 women; age, 39.4 ± 14.8 yr; range, 17–61 yr; body mass index (BMI), 26.7 ± 5.2 (kg/m^2)) with SH were studied. Patients were selected among the subjects referred to the clinic of Rafsanjan hospital for the first medical examination in spring 2018. SH was diagnosed by biochemical findings. SH patients had normal FT4 and increased TSH concentrations (>6.3 mIU/l). The cause of SH was autoimmune lymphocytic thyroiditis (Hashimoto's thyroiditis) and autoantibodies to thyroid peroxidase (anti TPO) were positive in patients. Forty euthyroid subjects matched to the patients group for sex (8 men, 32 women), age (39.0 ± 11.8 yr, range 18–63 yr), and BMI (25.3 ± 3.4 kg/m^2) were used as controls. FT4 and TSH concentrations were normal in controls. None of the participants were taking drugs known to affect lipid metabolism or had diseases (e.g. dyslipidemia, diabetes mellitus, renal and hepatic failure, or other systemic diseases). All study subjects signed the consent sheet, which was approved by the institutional ethical committee.

2.2. Laboratory analysis

Blood was collected in the morning between 08·00 and 09·00 a.m. after an overnight fasting for the determination of serum TSH (normal range 0.36–6.3 μIU/ml), FT4 (normal range 0.8–2 ng/dl) and PCSK9 by enzyme-linked immunosorbent assay (ELISA) on a Biotek analyzer (Biochemistry Laboratory, Medical University, Rafsanjan). TSH and PCSK9 ELISA assay was performed using sandwich ELISA. In sandwich ELISA the wells are coated with specific monoclonal antibodies for the analyte (PCSK9, TSH). Analyte is attached to the antibody at the bottom of the well and then the second antibody attached to a peroxidase enzyme (HRP) is added to the well and capture the sample analyte in solution. After washing, 3,3′,5,5′-Tetramethylbenzidine (TMB) containing chromogen that is a substrate for peroxidase is added to the wells and generate blue coloration. By adding stop solution this reaction will stop and color change from blue to yellow that can be read at 450 nm. For FreeT4, ELISA is analyzed in a competitive manner. In competitive methods, the basis for assessing is the competition between two antigens (one of which is labeled) for ligand binding [15]. Anti-thyroid peroxidase (TPO) antibodies were analyzed for patients in the same way as before (Hormon Laboratory, Rafsanjan hospital). Antibody levels were considered negative when they were below 100 IU/ml.

Triglyceride (TG) and total cholesterol (TC) were analyzed by glycerol oxidase enzymatic and cholesterol oxidase enzymatic methods, respectively. Cholesterol enzymatic methods analyze cholesterol directly in plasma or serum through a series of reactions. Initially, cholesterol ester converts into cholesterol by cholesterol esterase. Then Cholesterol Oxidase catalyzes the oxidation of 3-OH group of cholesterol and one of reaction products is hydrogen peroxide (H2O2) that is quantified enzymatically and created color was detected by spectrophotometer at 540–570 nm [16]. The method has been used to evaluate plasma triglycerides is based on the hydrolysis of triglycerides and evaluation of glycerol that is released in reaction. Glycerophosphate that is formed by glycerokinase reaction is oxidase and resulting H2O2 is analyzed as described previously in cholesterol method [17]. HDL-C was evaluated directly after precipitation of apolipoprotein B-containing lipoproteins with magnesium chloride and dextran sulfate and then the cholesterol of HDL is analyzed by cholesterol assay [18]. All these analyses were performed on the serum and by autoanalyser (Mindray) (Biochemistry Laboratory, Rafsanjan hospital). Low density lipoprotein-cholesterol (LDL-C) was calculated using the Friedewald formula; TC − (HDL-C + TG/5) [19].

Quantification of CD36 expression on monocytes was done by flow cytometry. At first Whole-blood samples with citrate were immediately moved to the lab and processed. Red blood cells lysed after adding Lysis Buffer. Fluorescein isothiocyanate (FITC) conjugated CD14 antibody was used to identify monocytes and phycoerythrin (PE) conjugated CD36 antibody to identify CD36 receptor on the monocyte surface. For isotype matched negative controls we used FITC conjugated mouse IgG1 and PE conjugated mouse IgM (BioLegend, USA). Then the remaining white blood cells were washed twice with phosphate-buffered saline [20]. We used a FACSCalibur cytometer (Partec, Germany) to determine the surface intensity of antigens on peripheral blood monocytes and the dot plot (the two-dimensional histogram that show two parameters) was used for evaluating FACS data (Fig. 1).

2.3. Statistical analysis

Data were analyzed using SPSS 21.0 software. Numeric variables are presented as the mean ± standard deviation (SD). Independent-samples *t*-test was used to compare age, BMI, thyroid function and lipid profile between SH patients and controls. p-value of 0.05 or lower was assumed to be statistical significance. Comparisons among two groups were carried out by using Pearson's correlation coefficients.

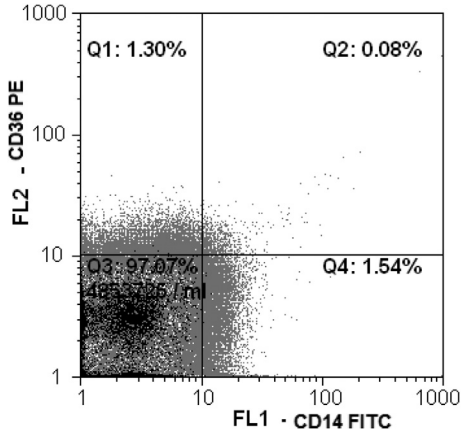

Fig. 1. Assay for CD36 expression on monocytes. Q4 region represents cells that have been tagged with FITC (monocytes). Cells that have CD36 receptor on their surface and marked with PE are located in the Q1 region. The cells stained by both antibody markers (Monocytes that express CD36 receptor on their surface) are placed in Q3 region. The Q4 region refers to cells that have been negative for both PE and FITC.

3. Results

Table 1 summarizes the baseline characteristics in patients with SH disease and control subjects. TSH levels were significantly higher in SH patients than controls ($p < 0{\cdot}001$). FT4 levels in patients were within the normal range but significantly lower than controls ($p < 0{\cdot}001$). TC, LDL-C and TG levels were significantly higher in patients with SH compared with control group ($p < 0{\cdot}001$). HDLC levels in SH patients were not different from the controls ($p = 0.651$). PCSK9 was also significantly higher in patients ($p < 0{\cdot}001$). No differences could be found in CD36 expression between SH patients and control subjects ($p = 0.533$).

The correlation coefficients between thyroid function, lipid profile, PCSK9 and CD36 are shown in Table 2. TSH levels positively correlated with serum TC ($r = 0.425$; $p < 0{\cdot}001$), LDL-C ($r = 0.391$, $p < 0{\cdot}001$) and TG ($r = 0.323$; $p = 0.004$). FT4 levels negatively correlated with TC ($r = -0.552$; $p < 0{\cdot}001$), and LDL-C ($r = -0.502$, $p < 0{\cdot}001$) and TG ($r = -0.527$, $p < 0{\cdot}001$) but there was no statistically significant correlation between TSH, FT4 and HDL ($r = -0.013$, $p = 0.906$ and $r = 0.069$, $p = 0.544$, respectively). There were significant correlations between serum TSH, FT4 values and lipoprotein ratios (TC/HDL and LDL/HDL). PCSK9 was correlated with TSH positively and with FT4 negatively ($r = 0.324$; $p = 0.003$ and $r = -0.593$, $p < 0{\cdot}001$).

PCSK9 had a significant correlation with lipid profile that these results are available in Table 3, but there were no important correlation between PCSK9 and CD36 ($r = 0.006$; $p = 0.960$).

Table 2
Spearman's correlation coefficient (rho) between TSH, FT4, lipid profile, PCSK9 and CD36 for all subjects.

	TSH		FT4	
	r	p-Value	r	p-Value
TC	0.425**	<0·001	−0.552**	<0·001
LDL-C	0.391*	<0·001	−0.502**	<0·001
HDL-C	−0.013	0.906	0.069	0.544
TG	0.323**	0.004	−0.527**	<0·001
TC/HDL	0.386**	<0·001	−0.500**	<0·001
LDL/HDL	0.395**	<0·001	−0.498**	<0·001
PCSK9	0.324**	0.003	−0.593**	<0·001
CD36	−0.135	0.233	0.139	0.218

*Correlation is significant at the 0.05 level (2-tailed).
**Correlation is significant at the 0.01 level (2-tailed).

Table 3
Spearman's correlation coefficient (rho) between PCSK9 levels and lipid profile.

	PCSK9	
	r	p-Value
TC	0.338**	0.002
LDL-C	0.344**	0.002
HDL-C	−0.243*	0.030
TG	0.478**	<0·001
TC/HDL	0.502**	<0·001
LDL/HDL	0.505**	<0·001

*Correlation is significant at the 0.05 level (2-tailed).
**Correlation is significant at the 0.01 level (2-tailed).

4. Discussion

SH is the most common thyroid disorder with prevalence ranging from 3% to 15% of adult populations [21]. It increases with age and is more prevalent in women than men [22]. One of the most common thyroid diseases that cause thyroid dysfunctions is Hashimoto thyroiditis [23]. Overt hypothyroidism influences serum cholesterol and LDL levels in different ways such as decreasing LDLR, which mediates cholesterol uptake from the circulation and 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, which regulates cholesterol biosynthesis, and cholesterol 7-hydroxylase (CYP7AI) that participates in the synthesis of the bile acids [24–26]. In some reports no differences found between SH patients and euthyroid controls in LDL levels [27,28]. In our study and several cross-sectional studies, SH was found to be associated with increase in TC and LDL-C [29,30]. There are more contradictory results about the effects of SH on TG and HDL-C. Some articles believe that SH has an effect on TG level [31,32], Higher serum TG may be observed in hypothyroid patients because of their lower lipoprotein lipase activity [33]. Vice versa some studies suggested that the level of TG does not change in patients with SH [34]. In our study there was a significant different in serum TG levels between SH patients and controls. HDL-C was not significantly different between two groups, and there are articles that have achieved similar results [35,36]. While some researchers have reached the opposite findings [37] and they have been suggested that decreased plasma CETP concentrations and hepatic lipase activity in hypothyroidism may lead to alterations in serum HDL-C concentration [33]. In our study the average of TSH was statistically significantly elevated in SH patients and There is general consensus that individuals with serum TSH values greater than 10 mIU/liter have higher lipid levels [38].

Though thyroid hormones have various targets in organ systems, they can have significant effects on the cardiovascular system. Reports of cardiac disorders are different in patients with SH. The results of some studies show that myocardial infarction has increased [39], but some reported no change [40]. To predict vascular risk we used two lipoprotein ratios (total cholesterol/HDL cholesterol and LDL/HDL cholesterol ratios) that are important indicators for cardiovascular risk [41]. In our study serum TSH and PCSK9 levels had significant positive correlations with lipoprotein ratios (TC/HDL and LDL/HDL). These relationships may be a tendency to arterial stiffness and potentially increase the risk of atherosclerosis and coronary artery disease in SH patients. So screening for SH patients is worthwhile for because they have an increased risk of developing CHD.

Sterol regulatory element-binding protein-2 (SREBP-2) has been well established as a TH target. SREBP-2 stimulates intracellular cholesterol synthesis and promotes LDLR gene expression [42] and is also able to upregulate PCSK9 [43]. This makes it possible to postulate that thyroid function could play a role in PCSK9 regulation [11]. Some findings in euthyroid non-obese subjects and euthyroid subjects with stable CHD suggest that thyroid function status is a regulator for PCSK9 and also there is a negative association between TH and PCSK9 levels and a positive correlation between TSH and PCSK9 levels [11,44]. In contrast, in a study on

Table 1
Basal characteristics of subjects.

	Control (n = 40)	Patient (n = 40)	p-Value
Age (yr)	39.0 ± 11.8	39.4 ± 14.8	0.888
BMI (kg/m^2)	25.3 ± 3.4	26.7 ± 5.2	0.146
TSH (μIU/ml)	2.4 ± 0.8	31.5 ± 22.4	<0·001
FT4 (ng/dl)	1.5 ± 0.1	0.9 ± 0.1	<0·001
TC (mg/dl)	152.6 ± 15.7	203.8 ± 42.4	<0·001
HDLc (mg/dl)	44.7 ± 9.9	43.6 ± 12.5	0.651
LDLc (mg/dl)	89.0 ± 16.1	124.9 ± 36.0	<0·001
TG (mg/dl)	84.1 ± 42.7	227.5 ± 113.7	<0·001
TC/HDL	3.5 ± 0.6	4.9 ± 1.5	<0·001
LDL/HDL	2.0 ± 0.4	3.0 ± 1.1	<0·001
PCSK9(ng/ml)	176.5 ± 14.1	202.0 ± 19.5	<0·001
CD36 (%)	0.18 ± 0.11	0.16 ± 0.13	0.533

hyperthyroid patients although it has been suggested that TH reduced circulating PCSK9, there was no significant correlation between serum PCSK9 and TH levels [45]. The results of this study confirm that PCSK9 was significantly higher in patients than controls and PCSK9 had a positive correlation with TSH and a negative with FT4.

So far, in vitro studies have been done on the effects of PCSK9 on CD36 receptors and the results were different in various cell lines [46,47]. In a study on intestinal epithelial cells, the author concluded that with the increasing of PCSK9 activity, CD36 also increases to compensate for the reduction of LDLR [46]. On the other hand, an article suggested that PCSK9 induces the degradation of CD36, like its effect on LDLR. This is the result of the study on the liver and kidney cell lines and PCSK9 knockout mice [47]. In this study we intended to examine the effects of PCSK9 on CD36 expression on monocytes. Ultimately, we found no correlation between PCSK9 and the percentage of CD36 presence on the monocytes surface.

5. Limitations

We did not analyze serum lipoprotein (a). Anti-TPO antibody was not carried out for controls. The effects of L-thyroxine therapy was not investigated in patients. The expression of PCSK9 and CD36 genes were not evaluated in the monocytes of the subjects.

6. Conclusion

Our data indicated that PCSK9 levels are affected by thyroid function in subclinical hypothyroidism and CD36 expression does not correlate with the PCSK9 level in these patients. Serum TC, LDL-C and TG levels increase in subclinical hypothyroidism. Therefore, there is a potential association between subclinical hypothyroidism and atherosclerosis.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgments

This work was funded by Rafsanjan University of Medical Sciences (Rafsanjan, Iran). Patients and controls selection and thyroid function and lipid profile analyzes supported in part by a grant from the Rafsanjan hospital.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.dsx.2018.08.021.

References

[1] Brent GA. Mechanisms of thyroid hormone action. JCI (J Clin Investig) 2012;122(9):3035.
[2] Cappola AR, Ladenson PW. Hypothyroidism and atherosclerosis. J Clin Endocrinol Metabol 2003;88(6):2438–44.
[3] Vierhapper H, Derfler K, Bratusch-Marrain P, Waldhäusl W. Determination of free thyroxin and triiodothyronine in "subclinical" hypothyroidism and hyperthyroidism. Clin Chem 1984;30(3):500–1.
[4] Golden SH, Robinson KA, Saldanha I, Anton B, Ladenson PW. Prevalence and incidence of endocrine and metabolic disorders in the United States: a comprehensive review. J Clin Endocrinol Metabol 2009;94(6):1853–78.
[5] Surks MI, Ortiz E, Daniels GH, Sawin CT, Col NF, Cobin RH, et al. Subclinical thyroid disease: scientific review and guidelines for diagnosis and management. Jama 2004;291(2):228–38.
[6] Walsh JP, Bremner AP, Bulsara MK, O'leary P, Leedman PJ, Feddema P, et al. Thyroid dysfunction and serum lipids: a community-based study. Clin Endocrinol 2005;63(6):670–5.
[7] Iqbal A, Jorde R, Figenschau Y. Serum lipid levels in relation to serum thyroid-stimulating hormone and the effect of thyroxine treatment on serum lipid levels in subjects with subclinical hypothyroidism: the Tromsø Study. J Intern Med 2006;260(1):53–61.
[8] Hernández-Mijares A, Jover A, Bellod L, Bañuls C, Solá E, Veses S, et al. Relation between lipoprotein subfractions and TSH levels in the cardiovascular risk among women with subclinical hypothyroidism. Clin Endocrinol 2013;78(5):777–82.
[9] Santos-Palacios S, Brugos-Larumbe A, Guillén-Grima F, Galofré JC. A cross-sectional study of the association between circulating TSH level and lipid profile in a large Spanish population. Clin Endocrinol 2013;79(6):874–81.
[10] Alborn WE, Cao G, Careskey HE, Qian Y-W, Subramaniam DR, Davies J, et al. Serum proprotein convertase subtilisin kexin type 9 is correlated directly with serum LDL cholesterol. Clin Chem 2007;53(10):1814–9.
[11] Kwakernaak AJ, Lambert G, Muller Kobold AC, Dullaart RP. Adiposity blunts the positive relationship of thyrotropin with proprotein convertase subtilisin-kexin type 9 levels in euthyroid subjects. Thyroid 2013;23(2):166–72.
[12] Febbraio M, Hajjar DP, Silverstein RL. CD36: a class B scavenger receptor involved in angiogenesis, atherosclerosis, inflammation, and lipid metabolism. J Clin Invest 2001;108(6):785.
[13] Gagnon A, Ooi TC, Cousins M, Favreau C, Henry K, Landry A, et al. The anti-adipogenic effect of peripheral blood mononuclear cells is absent with PCSK9 loss-of-function variants. Obesity 2016;24(11):2384–91.
[14] Levy RI. Cholesterol, lipoproteins, apoproteins, and heart disease: present status and future prospects. Clin Chem 1981;27(5):653–62.
[15] Van Weemen B, Schuurs A. Immunoassay using antigen—enzyme conjugates. FEBS Lett 1971;15(3):232–6.
[16] Allain CC, Poon LS, Chan CS, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. Clin Chem 1974;20(4):470–5.
[17] Bachorik P, Cooper G, Lippel K, Ahmed S. Standardization of cholesterol and triglyceride measurements by twelve Lipid Research Clinics. Protides of the biological fluids; 1977.
[18] Warnick G, Benderson J, Albers J. Dextran sulfate-Mg2+ precipitation procedure for quantitation of high-density-lipoprotein cholesterol. Clin Chem 1982;28(6):1379–88.
[19] Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18(6):499–502.
[20] Porwit-MacDonald A, Janossy G, Ivory K, Swirsky D, Peters R, Wheatley K, et al. Leukemia-associated changes identified by quantitative flow cytometry. IV. CD34 overexpression in acute myelogenous leukemia M2 with t (8; 21). Blood 1996;87(3):1162–9.
[21] Veltri F, Rocha FO, Willems D, Praet J-P, Grabczan L, Kleynen P, et al. Prevalence of thyroid dysfunction and autoimmunity in the older population and implications of age-specific reference ranges. Clin Chim Acta 2017;465:34–9.
[22] Hoogendoorn EH, Hermus AR, De VegT F, Ross HA, Verbeek AL, Kiemeney LA, et al. Thyroid function and prevalence of anti-thyroperoxidase antibodies in a population with borderline sufficient iodine intake: influences of age and sex. Clin Chem 2006;52(1):104–11.
[23] Hiromatsu Y, Satoh H, Amino N. Hashimoto's thyroiditis: history and future outlook. Hormones (Basel) 2013;12(1):12–8.
[24] Mathe D, Chevallier F. Effects of the thyroid state on cholesterol metabolism in the rat. Biochim Biophys Acta Lipids Lipid Metabol 1976;441(1):155–64.
[25] Pandak W, Heuman D, Redford K, Stravitz R, Chiang J, Hylemon P, et al. Hormonal regulation of cholesterol 7alpha-hydroxylase specific activity, mRNA levels, and transcriptional activity in vivo in the rat. J Lipid Res 1997;38(12):2483–91.
[26] Gullberg H, Rudling M, Saltó C, Forrest D, Angelin B, Vennström BR. Requirement for thyroid hormone receptor β in T3 regulation of cholesterol metabolism in mice. Mol Endocrinol 2002;16(8):1767–77.
[27] Vierhapper H, Nardi A, Grösser P, Raber W, Gessl A. Low-density lipoprotein cholesterol in subclinical hypothyroidism. Thyroid 2000;10(11):981–4.
[28] Imaizumi M, Akahoshi M, Ichimaru S, Nakashima E, Hida A, Soda M, et al. Risk for ischemic heart disease and all-cause mortality in subclinical hypothyroidism. J Clin Endocrinol Metabol 2004;89(7):3365–70.
[29] Lindeman RD, Schade DS, LaRue A, Romero LJ, Liang HC, Baumgartner RN, et al. Subclinical hypothyroidism in a biethnic, urban community. J Am Geriatr Soc 1999;47(6):703–9.
[30] Marwaha RK, Tandon N, Garg M, Kanwar R, Sastry A, Narang A, et al. Dyslipidemia in subclinical hypothyroidism in an Indian population. Clin Biochem 2011;44(14–15):1214–7.
[31] Bandyopadhyay SK, Basu A, Pal SK, Roy P, Chakrabarti S, Pathak H, et al. A study on dyslipidaemia in subclinical hypothyroidism. J Indian Med Assoc 2006;104(11):622–4. 6.
[32] Asranna A, Taneja R, Kulshreshta B. Dyslipidemia in subclinical hypothyroidism and the effect of thyroxine on lipid profile. Indian journal of endocrinology and metabolism 2012;16(Suppl 2):S347.
[33] Pearce EN. Update in lipid alterations in subclinical hypothyroidism. J Clin Endocrinol Metabol 2011;97(2):326–33.
[34] Paoli M, Bellabarba G, Velazquez E, Mendoza S, Molina C, Wang P, et al. Sex steroids, lipids, and lipoprotein cholesterols in women with subclinical and overt hypothyroidism before and after L-thyroxine therapy. Clinica chimica acta; international journal of clinical chemistry 1998;275(1):81.
[35] Guntaka M, Hanmayyagari B, Rosaline M, Nagesh V. Lipid profile in subclinical

hypothyroidism: a biochemical study from tertiary care hospital. CHRISMED J Health Res 2014;1(4):266.
[36] Canaris GJ, Manowitz NR, Mayor G, Ridgway EC. The Colorado thyroid disease prevalence study. Arch Intern Med 2000;160(4):526–34.
[37] Pirich C, Müllner M, Sinzinger H. Prevalence and relevance of thyroid dysfunction in 1922 cholesterol screening participants. J Clin Epidemiol 2000;53(6):623–9.
[38] Duntas LH, Wartofsky L. Cardiovascular risk and subclinical hypothyroidism: focus on lipids and new emerging risk factors. What is the evidence? Thyroid 2007;17(11):1075–84.
[39] Klein I, Ojamaa K. Thyroid hormone and the cardiovascular system. N Engl J Med 2001;344(7):501–9.
[40] Vanderpump M, Tunbrldge W, French J, Appleton D, Bates D, Clark F, et al. The incidence of thyroid disorders in the community: a twenty-year follow-up of the Whickham Survey. Clin Endocrinol 1995;43(1):55–68.
[41] Millán J, Pintó X, Muñoz A, Zúñiga M, Rubiés-Prat J, Pallardo LF, et al. Lipoprotein ratios: physiological significance and clinical usefulness in cardiovascular prevention. Vasc Health Risk Manag 2009;5:757.
[42] Shin D-J, Osborne TF. Thyroid hormone regulation and cholesterol metabolism are connected through sterol regulatory element-binding protein-2 (SREBP-2). J Biol Chem 2003;278(36):34114–8.
[43] Awan Z, Baass A, Genest J. Proprotein convertase subtilisin/kexin type 9 (PCSK9): lessons learned from patients with hypercholesterolemia. Clin Chem 2014;2014, 225946. clinchem.
[44] Li S, Xu R-X, Guo Y-L, Zhang Y, Zhu C-G, Sun J, et al. Thyroid function and PCSK9 in euthyroid subjects with coronary artery disease. Clin Lipidol 2015;10(3):235–42.
[45] Bonde Y, Breuer O, Lütjohann D, Sjöberg S, Angelin B, Rudling M. Thyroid hormone reduces PCSK9 and stimulates bile acid synthesis in humans. J Lipid Res 2014;55(11):2408–15.
[46] Levy E, Ouadda ABD, Spahis S, Sane AT, Garofalo C, Grenier É, et al. PCSK9 plays a significant role in cholesterol homeostasis and lipid transport in intestinal epithelial cells. Atherosclerosis 2013;227(2):297–306.
[47] Demers A, Samami S, Lauzier B, Des Rosiers C, Sock ETN, Ong H, et al. PCSK9 induces CD36 degradation and affects long-chain fatty acid uptake and triglyceride metabolism in adipocytes and in mouse liver. Arterioscler Thromb Vasc Biol 2015;115, 306032. ATVBAHA.