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Angiopoietin-like proteins 3, 4 and 8 are linked to cardiovascular function in naïve sub-clinical and overt hypothyroid patients receiving levothyroxine therapy

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Abstract

Objective: Angiopoietin-like proteins (ANGPTL) 3, 4 and 8 are upcoming cardiovascular biomarkers. Experimental studies showed that thyroid hormones altered their levels. We assessed ANGPTL3, 4 and 8 as predictors of cardiovascular functions among naïve subclinical and naïve overt hypothyroidism (SCH and OH) and altered ANGPTL levels with levothyroxine replacement (LT4) and their association with improved cardiovascular risk factors and cardiovascular function.

Design and methods: The study was a prospective follow-up study that assessed ANGPTL3, 4 and 8 levels, vascular status (flow-mediated dilation% of brachial artery (FMD%), carotid intima-media thickness (CIMT), aortic stiffness index (ASI)), left ventricle (LV) parameters (ejection fraction (EF), myocardial performance index (MPI), and LV mass), well-known cardiovascular risk factors and homeostatic model for the assessment of insulin resistance, at two time points, that is, among naïve SCH, naïve OH, and healthy subjects groups; and at 6 months after achieving the euthyroid state with LT4 by calculating their increased or decreased delta changes (Δ↑ or Δ↓) in longitudinal arm among LT4-hypothyroid groups.

Results: Significantly elevated levels of ANGPTL3, 4 and 8 among hypothyroid groups than the healthy subjects were reduced with LT4. Multivariate analysis revealed ANGPTLs as independent predictors of cardiovascular functions and the contributors for ANGPTL level included ANGPTL3 and 4 for impaired FMD%, and ANGPTL8 for LV mass among naïve SCH; ANGPTL3 for EF% and ANGPTL8 for CIMT in naïve OH; Δ↓ANGPTL3 for Δ↓ASI meanwhile Δ↑freeT4 for Δ↓ANGPTL3, Δ↓fasting glucose, Δ↓triglyceride, and Δ↓thyroid peroxidase antibody for Δ↓ANGPTL4 among LT4-SCH. Δ↓ANGPTL4 for Δ↓MPI and Δ↓LV mass, meanwhile Δ↓TSH and Δ↓triglyceride for Δ↓ANGPTL3, Δ↑free T3 and Δ↓HOMA-IR for Δ↓ANGPTL4, and systolic blood pressure and waist circumference for Δ↓ANGPTL8 among LT4-OH.

Conclusion: Elevated ANGPTL3, 4 and 8 levels are differentially independent predictors of endothelial and cardiac function and are reduced with LT4 in SCH and OH.

Key Words

- ANGPTL3
- ANGPTL4
- ANGPTL8
- endothelial and cardiac function
- subclinical and overt hypothyroidism

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Introduction

Overt hypothyroidism (OH) and subclinical hypothyroidism (SCH) are characterized by the coexistence of traditional cardiovascular disease risk factors, insulin resistance (IR), elevated inflammatory markers, altered hemodynamic and morphological parameters of endothelial function of the peripheral muscular and central elastic arteries as assessed via flow-mediated dilation% (FMD%) of the brachial artery, carotid intima-media thickness (CIMT), and aortic stiffness index (ASI) as early atherosclerotic markers. These factors induce impaired cardiac function among the patients with hypothyroidism (1, 2).

Angiopoietin-like proteins (ANGPTLs) are a family of secreted proteins, including ANGPTL3, ANGPTL4 and ANGPTL8 (ANGPTL3, 4 and 8). They are proposed to inhibit lipoprotein lipase (LPL) enzyme activity in a coordinated manner to maintain metabolic homeostasis, which enhances the supply of fatty acid as a fuel source for many tissues such as the heart and the skeletal muscles during fasting. Moreover, metabolic homeostasis enhances adipose tissue storage during feeding. The inhibition of LPL activity by ANGPTL3 and ANGPTL4 is regulated by their interaction with ANGPTL8 with subsequent hypertriglyceridemia (3).

Aside from their role in lipid metabolism, human genetic studies highlighted the association of inactivating mutation in ANGPTL3, 4 and 8 with reduced risk of atherosclerosis and coronary artery disease (CAD) (4, 5, 6). Also, the knockout of any of these ANGPTLs can prevent the development and progression of atherosclerosis in mice (7, 8, 9). They are associated with obesity, dyslipidemia, and IR, as well as cardiovascular risk and atherosclerosis. Moreover, anti-ANGPTL3, anti-ANGPTL4, and anti-ANGPTL8 provide new opportunities for the therapeutic intervention for dyslipidemia, obesity, atherosclerosis, and CAD, but safety must be further evaluated before they can be effectively used in clinical setting (6, 10, 11). One pilot study demonstrated the regulatory role of ANGPTL4 upon endothelial and cardiac function parameters in chronic obstructive pulmonary disease (COPD) (12).

One preliminary study revealed that circulating ANGPTL3 and 8, but not ANGPTL4, were elevated in SCH and OH (13). Thyroid hormones (THs) upregulate ANGPTL8 gene expression in the cell line and suppress ANGPTL3 mRNA but not ANGPTL4 mRNA in hypothyroid rat (14, 15).

Based on the above data and to the best of our knowledge, our study was the first to: (i) evaluate the role of levothyroxine (LT4) replacement therapy on ANGPTL level after 6 months follow-up of achieved euthyroid state among naïve SCH and naïve OH groups in longitudinal arm; (ii) assess the association of ANGPTL3, 4 and 8 with the morphological and hemodynamic parameters of functional state of both the endothelium and left ventricle (LV) as early predictors of atherosclerosis and cardiac dysfunction, respectively and (iii) evaluate the role of THs, studied cardiovascular disease risk factors, and inflammatory parameters as contributors to the levels of these ANGPTLs at two time points, that is, at the baseline among hypothyroid groups in cross arm and at the end of longitudinal arm as we calculated the increased or decreased delta change ($\Delta\uparrow$or $\Delta\downarrow$) of these parameters.

Subjects and methods

We conducted this prospective, observational, cohort study with cross-sectional and longitudinal follow-up arms at the Endocrinology and Diabetes Unit, Internal Medicine Department, Minia University, Egypt, from February 2018 to June 2020. The cross-sectional arm included healthy euthyroid (HS), naïve SCH, and naïve OH groups ($n=36$ for each group, with female/male ratios of 30/6, 32/4, and 34/2, respectively).

Study design

The present study was conducted on a consecutive sample of naïve patients with SCH and OH who were referred to Endocrinology Unit, Minia University Hospital during the year 2018. Upon further analysis, 45 SCH and 45 OH patients met the diagnostic and exclusion criteria of the study. Diagnosis of SCH and OH was made according to the American Association of Clinical Endocrinologists and the American Thyroid Association Guidelines 2012 (16). Diagnosis of SCH was based on persistent, elevated thyroid-stimulating hormone (TSH) >4.5 IU/L with normal serum-free T4 (FT4) at least 3 months prior to the commencement of the study. The subjects that regained euthyroid state within this period were excluded. Diagnosis of OH was made among subjects with TSH >4.5 IU/mL and FT4 <0.9 ng/dL. Age- and sex-matched euthyroid healthy subjects were selected as controls. The subjects that met any of the following exclusion criteria were not included in the study: with cardiovascular disorder, diabetes mellitus, hypothalamic–pituitary axis problem, chronic illness, organ dysfunction, and malignancy anywhere or taking

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medications that may influence the studied laboratory and cardiovascular parameters.

Ethical aspects

This study was approved by the Institutional local Ethics Committee, Faculty of Medicine, Minia School, Egypt; and was conducted in accordance with the Declaration of Helsinki. All the subjects gave their informed consent for contribution after full explanation of the study design.

Cross arm of the study

Initially, through history, anthropometric, biochemical, and cardiovascular parameters were assessed among the three studied groups.

Medical treatment and longitudinal arm of the study

The SCH and OH subjects consequently received the initial dose of LT4 which was 25–75 μg/kg and 1.5–1.6 μg/kg, respectively. Then, TSH and FT4 were measured every 6 weeks with adjustment of the LT4 dose until euthyroid state and TSH target range (0.4–2.5 IU/mL) were achieved, and dosage was modified to reach the target by maintaining the TSH level in the target range during this period. Later, the THs were assessed every 3 months. Eight patients dropped out of the study as they neither came back for the follow-up nor followed the instructions of the study protocol. Six patients were excluded from the analysis because they developed concomitant disease ($n=4$) or failed to achieve the reference TSH level during the 3 months of treatment ($n=2$). After 6 months of achieving established laboratory euthyroid state on LT4 substitution therapy in the diseased groups (labeled as LT4-SCH and LT4-OH groups), the same anthropometric, laboratory, and cardiovascular parameters were assessed. We calculated the increased or decreased delta change (Δ↑or Δ↓) of these parameters to evaluate the effect of LT4 therapy on ANGPTLs in the context of these changes.

Measurement of biochemical parameters

An overnight fasting and 12-h fasting blood samples were drawn from each subject and prepared for the measurement of fasting plasma glucose (FPG), complete lipogram (total cholesterol and HDL-c, triglycerides, and calculated LDL-c) using standard laboratory methods. THs (TSH, freeT3 (FT3), and FT4) were assessed using Mini Vidas via enzyme-linked fluorescence immunoassay (BioMerieux, Marcy-l'Etoile, France). Specimens stored at –80°C were used to measure the anti-thyroid peroxidase (anti-TPO) antibodies (ORGENTEC, Mainz, Germany), high sensitive C-reactive protein ((hsCRP) (BIOS microwell ELISA Diagnostic Systems Kit, San Francisco, CA, USA), fasting plasma insulin (Epitope Diagnostics, Inc., San Diego, CA, USA), and plasma ANGPTL3, 4 and 8 (ELBA Science, Wuhan, China) via ELISA. Homeostatic model for the assessment of insulin resistance (HOMA-IR) was calculated (17).

Assessment of cardiovascular status

For endothelial function: (i) high-resolution, color-coded Doppler ultrasonography (Toshiba) with an 8- to 12-MHz linear array was employed for the measurement of common CIMT according to the recommendations of the Mannheim CIMT Consensus (18), and the FMD% of the brachial artery was calculated as follows: (post occlusion diameter – baseline diameter) × 100/baseline diameter according to the method described by Celermajer *et al.* (19). (ii) M-mode echocardiography of the aortic root was performed to calculate the ASI = 100 × (natural (systolic blood pressure (SBP))/diastolic blood pressure (DBP)) / ((aortic maximum systolic diameter – aortic end-diastolic diameter) / aortic end-diastolic diameter) (20).

According to the 2015 ASE/ESCI guidelines (21), we measured the LV ejection fraction (EF) and fractional shorting (FS) and the isovolumetric contraction time (IVCT) as markers of systolic function. The ratio of the peak velocity of the early E-wave to the atrial A-wave (E-wave/A-wave), deceleration time of the early transmitral flow (DT), isovolumetric relaxation time (IVRT), and early diastolic pulsed-wave tissue Doppler velocities at the mitral annulus (é) were measured as markers of diastolic function (22). The myocardial performance index (MPI) was calculated using the Tie index (23), which is the total of the IVCT and IVRT divided by the ejection time (ET) as marker of global cardiac function. LV mass was assessed using the Devereux formula (24).

Statistical analysis

In all the statistical analyses, the SPSS software version 22.0 was used. Prior to the study, the sample size was determined after a power calculation according to the data obtained by the pilot study. In that study, the post-treatment ANGPTL8 levels were 371.56 ± 96.29 and 459.44 ± 105.75 in the SCH and OH groups, respectively. A sample size of 36 patients in each group was determined

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to provide 95% power for independent samples t-test at the level of <0.05 significance. Data for continuous normally and non-normally distributed data were expressed as mean ± S.D. and median (interquartile range) and compared using Student's t-test and the Mann–Whitney *U*-test, respectively. Categorical variables were expressed as percentage and compared using the chi-squared test between two different groups. Paired sample t-test and the Wilcoxon signed rank test were used to compare pre-treated and post-treated values of studied parameters in the same group. The following tests were conducted for baseline variables among naïve subjects and for the delta changes (Δ) of these variables among the LT4 subjects. Spearman's or Pearson's correlation as bivariate analysis determined the relationship between ANGPTLs, cardiovascular disease risk factor, and cardiovascular function parameters. To assess the role of ANGPTLs as independent predictors of cardiovascular function parameters and to determine the independent predictors of ANGPTLs, variables significantly correlated with cardiovascular parameters and variables significantly correlated with ANGPTLs, respectively, were analyzed via multiple regression analysis using an appropriate method (enter or stepwise) and best model after the exclusion of collinear variables. A *P*-value <0.05 was considered statistically significant.

Results

Cross arm of the study

(a) The baseline anthropometric, laboratory, and cardiovascular function characteristics of the OH, SCH, and HS groups who were age- and sex-matched are summarized and compared in Tables 1 and 2. A significant increase in ANGPTL3, 4 and 8 levels was observed when OH was compared with SCH and when both groups were compared with the HS group. Also, most of the other studied cardiovascular disease risk factors demonstrated similar patterns (Table 1). Furthermore, most of the echocardiographic parameters were in the normal reference range in all analyzed groups, except for significantly impaired diastolic function% in the hypothyroid groups. Compared with healthy subjects, hypothyroid groups also had significantly impaired relative systolic, diastolic, global LV function, and significantly ↑LV mass in addition to impaired endothelial function markers as presented in Table 2.

(b) Bivariate correlation of the studied ANGPTLs with the cardiovascular disease risk factors and cardiovascular function parameters in the entire study population and among SCH and OH patients are presented in Tables 3 and 4.

Among SCH patients, ANGPTL3 was negatively correlated with age, obesity parameters, blood pressure, triglyceride, FMD%, IVRT, and DT and positively correlated with insulin, HOMA-IR, cholesterol, é septal, and é lateral. ANGPTL4 was positively correlated with SBP, insulin, HDL, ET, and IVCT and negatively correlated with TPO, hsCRP, FMD%, and FS%. ANGPTL8 was positively correlated with FPG, HDL, and LV mass and negatively correlated with waist circumference, triglyceride, and IVRT.

Of note, ANGPTL3, 4 and 8 were positively correlated with ASI and CIMT and negatively correlated with FMD% in a group of hypothyroid patients.

Among OH patients, ANGPTL3 was negatively correlated with age, BMI, EF%, and FS%. ANGPTL4 was positively correlated with BMI and cholesterol and negatively correlated with DT. ANGPTL8 was negatively correlated with HDL-c, CIMT, and IVCT.

ANGPTL3, ANGPTL4 and ANGPTL8 were not found to be correlated with diastolic function grade among either SCH or OH patients.

(c) Variables significantly correlated with ANGPTL-related cardiovascular function parameters among hypothyroid subjects are summarized in Table 5.

(d) Multivariate regression: The use of the best method and model showed that:

- Among naïve SCH patients: The independent predictors of the levels of ANGPTLs were as follows: age, cholesterol, and waist circumference for ANGPTL3; TPO and SBP for ANGPTL4; and only waist circumference for ANGPTL8. The independent predictors of the cardiovascular function parameters: both ANGPTL3 and ANGPTL4 were contributors for FMD%; both age and HOMA-IR (not ANGPTL4) were independent predictors of FS%; finally ANGPTL8 was independent predictors of LV mass (Supplementary 1, see section on supplementary materials given at the end of this article).
- Among naïve OH patients: Age and HDL were independent predictors of ANGPTL3 and ANGPTL8, respectively, ANGPTL3 was contributor to EF% while ANGPTL8 and FPG were independent predictors of CIMT (Supplementary 2).

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Table 1 Studied clinical and biochemical parameters.

Variables	Baseline groups			Levothyroxine replacement groups	
	Healthy (n = 36)	SCH ((n = 36)	OH (n = 36)	SCH (n = 36)	OH (n = 36)
Age (years)[a]	35.6 ± 9.9	40.2 ± 8.6	41.1 ± 9.9	41.2 ± 8.5	41.9 ± 10
Sex: female, n (%)[b]	34 (94.4)	32 (88.9)	30 (83.3)	32 (88.9)	30 (83.3)
BMI (kg/m^2)[a]	23.3 ± 0.84	26.7 ± 2[f]	26.7 ± 2[f]	25.9 ± 1.9[f, i]	26.1 ± 2[f, i]
Systolic blood pressure (mmHg)[a]	109.4 ± 10.6	124.4 ± 8.7[f]	125.8 ± 8.8[f]	123.3 ± 9.9[f]	120.3 ± 11[f, i]
Diastolic blood pressure (mmHg)a	67.8 ± 4.3	75.8 ± 4.9[f]	75.8 ± 6.2[f]	73.3 ± 5.7[f, g]	74.4 ± 5.9[f]
Waist circumference (cm)[a]	75.1 ± 4.9	78.3 ± 7.9	78.1 ± 8.9	75.8 ± 7.7[i]	76.9 ± 7.3[g]
TSH (IU/mL)[c]	2.1 (1.4–3.1)	11 (10–17)[f]	17.5 (8.4–53.2)[f, k]	2.2 (1.9–2.4)[i]	2.1 (1.6–2.4)[i]
Free T3 (pg/mL)[a]	2.6 ± 0.2	2.5 ± 0.2	1.1 ± 0.5[f, l]	2.6 ± 0.4[h]	2.6 ± 0.2[i, j]
FreeT4 (ng/dL)[a]	1.3 ± 0.2	1.2 ± 0.1[f]	0.47 ± 0.1[f, l]	1.4 ± 0.2[i]	1.2 ± 0.2[i, k]
Anti-thyroid peroxidase (IU/mL)[c]	12 (8.8–14.8)	127 (80–192)[f]	141 (102–284)[f, l]	70 (40–105)[i]	82 (44–155)[i, j]
Fasting plasma glucose (mg/dL)[a]	76.7 ± 3.5	79 ± 5[d]	81.6 ± 6.6[e]	77.2 ± 5.1[g]	78.4 ± 6.4[h]
Insulin (mU/mL)[c]	4 (2.5–4.6)	7.1 (3.2–8.9)[f]	8.2 (5.5–10.6)[f]	2.7 (1.9–3.6)[g, h]	3.7 (2.3–4.6)[i]
HOMA-IR[c]	0.7 (0.5–0.9)	1.4 (0.6–1.7)[f]	1.7 (1.2–2.1)[f, j]	0.5 (0.4–0.7)[i]	0.7 (0.4–0.9)[i]
High density lipoprotein (mg/dL)[a]	52.6 ± 5	48.4 ± 5.3[e]	46.8 ± 5.3[f]	54.5 ± 4.7[i]	53.9 ± 4.4[i]
Low density lipoprotein (mg/dL)[a]	83 ± 9	125 ± 13[f]	153 ± 28[f, l]	90.2 ± 9[i]	118 ± 27[i, l]
Total cholesterol (mg/dL)[a]	142 ± 13	197 ± 11[f]	234 ± 25[f, l]	177 ± 15[i]	184 ± 16[i, j]
Triglyceride (mg/dL)[a]	95.6 ± 30	141 ± 21[f]	176 ± 22[f,k]	120 ± 23[i]	128 ± 22[i,k]
hs-C-reactive protein (mg/L)[c]	1.03 (0.9–1.3)	4.1 (3.7–4.9)[f]	5 (4.9–9.4)[f,k]	1.2 (0.8–2)[f,i]	2.8 (2.1–3.6)[i,k,f]
ANGPTL3 (ng/mL)[c]	18 (12–22)	41 (32–51)[f]	58 (48–72)[f, l]	26 (18–31)[f, i]	38 (30–49)[f, i, l]
ANGPTL4 (ng/mL)[a]	45 ± 13	74 ± 16[f]	92 ± 11[f, l]	48 ± 13[i]	72 ± 13[f, i, l]
ANGPTL8 (pg/mL)[a]	191 ± 12	470 ± 70f	630 ± 62[f, l]	371 ± 96[f, i]	459 ± 105[f, i, j]

[a]Normally distributed quantitative data are expressed as mean ± s.d. and compared using Student's t-test between each two different groups and paired t-test when compared basal and post-treated within the same group; [b]Qualitative variables are expressed as frequency and compared by χ^2 test; [c] Non-parametric quantitative data are expressed as median and interquartile (25–75%) and compared using Mann–Whitney U-test between each two different groups and Wilcoxon signed rank test when compared basal and post-treated within same group. Statistical significance vs healthy subjects; [d]P < 0.05, [e]P < 0.01, [f]P < 0.001. Statistical significance vs disease respective baseline group [g]P < 0.05, [h]P < 0.01, [i] P < 0.001; Statistical significance when comparing either basal SCH vs basal OH group or treated SCH vs treated OH [j]P < 0.05, [k]P < 0.01, [l]P < 0.001.
ANGPTL, angiopoietin-like protein; HOMA-IR, homeostatic model for the assessment of insulin resistance; OHT, overt hypothyroidism; SCH, subclinical hypothyroidism.

Longitudinal arm of the study

(a) Changes in the anthropometric, laboratory, and cardiovascular function parameters for 6 months with the euthyroid state achieved among the LT4-SCH and LT4-OH patients revealed significant reductions in ANGPTL3, 4 and 8 and improvement in most of the studied cardiovascular disease risk factors and cardiovascular function parameters (Tables 1 and 2).

(b) Bivariate correlations of Δ↓ANGPTL with Δchanges of both cardiovascular disease risk factors and cardiovascular function parameters among LT4-SCH and LT4-OH patients are presented in Tables 3 and 4.

- Among LT4-SCH patients, Δ↓ANGPTL3 was positively correlated with Δ↑FT3, Δ↑FT4, Δ↓DBP, Δ↓ASI, Δ↑é septal, and Δ↑é lateral. Δ↓ANGTPL4 was positively correlated with Δ↓FPG and negatively correlated with Δ↓TPO, Δ↓triglyceride, and Δ↓ANGPTL8. Δ↓ANGPTL8 was negatively correlated with Δ↓BMI and Δ↓TSH, Δ↓IVRT, and Δ↑ET.
- Among LT4-OH patients, Δ↓ANGPTL3 was positively correlated with Δ↓DBP, Δ↓TSH, and Δ↓ANGPTL8 and negatively correlated with Δ↓WC, Δ↓triglyceride, and Δ↓DT. Δ↓ANGPTL4 was positively correlated with Δ↓MPI and Δ↓LV mass and negatively correlated with Δ↑FT3, Δ↓insulin, Δ↓HOMA-IR, and ↑ΔET. Δ↓ANGPTL8 was positively correlated with Δ↓SBP and negatively correlated with Δ↓BMI and Δ↓WC.

(c) Variables significantly correlated with Δ↓ANGPTLs related to the cardiovascular function parameters

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Table 2 Studied cardiovascular parameters.

Variables	Baseline groups			Levothyroxine replacement groups	
	Healthy (n = 36)	SCH ((n = 36)	OH (n = 36)	SCH (n = 36)	OH (n = 36)
Cardiac systolic markers					
Ejection fraction (%)[a]	71.9 ± 4.8	67.5 ± 5.6[d]	66.7 ± 6[e]	69.2 ± 4.7[c, h]	69.1 ± 5.1[c, h]
Fractional shortening (%)[a]	41.6 ± 5.4	35.7 ± 3.9[e]	35.7 ± 4.6[e]	37.8 ± 4.3[d, h]	37.9 ± 4.9[d, h]
IVCT (ms)[a]	31.7 ± 1.4	33.3 ± 2.5[d]	34.3 ± 2.7[d]	31.8 ± 2[g]	32.1 ± 2.3[h]
Ejection time (m)[a]	341.2 ± 41.3	259.8 ± 18.1[e]	250.4 ± 21.2[e, i]	334.9 ± 43.6[h]	324.5 ± 24.8[c, h]
Cardiac diastolic markers					
E/A[a]	1.3 ± 0.1	1 ± 0.3[e]	1.2 ± 0.3[i]	1.3 ± 0.1[h]	1.3 ± 0.2
é septal (cm/s)[a]	8.8 ± 0.4	7.7 ± 0.8[e]	7.9 ± 1.2[e]	8.3 ± 0.5[h]	8.4 ± 0.6[h]
é lateral (cm/s)[a]	10 ± 0	9.5 ± 0.6[e]	9.4 ± 0.8[e]	10 ± 0[h]	9.9 ± 0.3[h, i]
DD grades (%): G0/GI/GII[b]	100/0/0	55.6/44.4/0[e]	66.7/27.8/5.6[e]	100/0/0[h]	94.4/5.6/0[h]
IVRT (ms)[a]	71.7 ± 1.9	76.3 ± 4.3[e]	80.5 ± 4.8[e, k]	73.2 ± 3.1[c, h]	73.7 ± 2.8[c, g]
Deceleration time (ms)[a]	163 ± 8	202 ± 27[e]	192 ± 32[e]	186 ± 19[e, h]	17 3 ± 21[d, h, j]
Global cardiac function: MPI[a]	0.3 ± 0.03	0.43 ± 0.04[e]	0.46 ± 0.04[e, j]	0.31 ± 0.04[h]	0.33 ± 0.02[g, j]
LV mass (g)[a]	107 ± 7	115 ± 10[e]	120 ± 17[e]	111 ± 8[g]	113 ± 15[c, h]
Vascular function markers					
Aortic stiffness index[a]	3.3 ± 0.6	6.3 ± 1.6[e]	9.6 ± 2.4[e, h, k]	4 ± 0.8[e]	5.6 ± 1.6[e, h, k]
CIMT (mm)[a]	0.51 ± 0.06	0.56 ± 0.09[d]	0.78 ± 0.15[e, h]	0.49 ± 0.06[h]	0.57 ± 0.10[d, f, g, j]
Flow-mediated dilation (%)[a]	15.9 ± 3	10.8 ± 1.4[h]	7.7 ± 2.2[e, k]	15.8 ± 1.9[h]	12.1 ± 2.3[d, h, k]

[a]Normally distributed quantitative data are expressed as mean ± s.d. and compared using Student's t-test between each two different groups and paired t-test when compared basal and post-treated within the same group. [b]Qualitative variables are expressed as frequency and compared by χ^2 test. Statistical significance vs healthy subjects, [c]P< 0.05, [d]P < 0.01, [e]P < 0.001. Statistical significance vs disease respective baseline group, [f]P <0.05, [g]P<0.01, [h]P < 0.001; Statistical significance when comparing either basal SCH vs basal OH group or treated SCH vs treated OH,[i]P < 0.05, [j]P < 0.01, [k]P < 0.001.
A, late transmitral flow velocity; CIMT, common carotid artery intimal media thickness; DD, diastolic dysfunction; E, early transmitral flow velocity; é, early diastolic pulsed-wave tissue Doppler velocities at the mitral annulus; G, grade; IVCT, isovolumetric contraction time; IVRT, isovolumetric relaxation time; LV=left ventricle; MPI, myocardial performance index; OHT, overt hypothyroidism; SCH, subclinical hypothyroidism.

among LT4-hypothyroidism patients are summarized in Table 5.

(d) Multivariate regression analysis:

- Among LT4-SCH patients, Δ↑FT4 was an independent predictor of Δ↓ANGPTL3; Δ↓FPG, Δ↓TPO and Δ↓triglyceride were contributors to Δ↓ANGPTL4; Δ↓ANGPTL3 and Δ↑HDL were independent contributors to ASI (Supplementary 1).
- Among LT4-OH patients, the independent predictors were Δ↓TSH and Δ↓triglyceride for Δ↓ANGPTL3, both Δ↓HOMA-IR and Δ↑FT3 for Δ↓ANGPTL4, and both Δ↓SBP and Δ↓BMI for Δ↓ANGPTL8. Moreover, Δ↓ANGPTL4 and Δ↓DBP were independent predictors of improved MPI. Δ↓ANGPTL4 was only a positive contributor to Δ↓LV mass (Supplementary 2).

Discussion

We demonstrated that ANGPTL3, 4 and 8 differentially contributed to cardiovascular function parameters among naïve and LT4-hypothyroid patients at 6 months follow-up with the achievement of euthyroid state, an aspect not mentioned before. Moreover, we were first to determine the predictors of ANGPTLs, and first to describe the significant reduction in levels of ANGPTL3, 4 and 8 after therapy.

Only one study reported similar elevated levels of ANGPTL3 and 8 (but not ANGPTL4) among OH and SCH patients. ANGPTL3 and 8 were found to be negatively correlated with FT3 and FT4 but positively correlated with TSH among all the study subjects (13). Our study had similar findings, but there was difference in terms of the elevated levels of ANGPTL4 in the hypothyroid groups; higher levels of ANGPTL3, 4 and 8 were observed among OH patients than the SCH patients. Reduced levels of ANGPTL3, 4 and 8 among LT4-hypothyroid patients were related to the improvement of the TH levels. Δ↓ANGPTL3 was dependent on Δ↑FT4 among LT4-SCH patients, whereas Δ↓ANGPTL3 and Δ↓ANGPTL4 were dependent on Δ↓TSH and Δ↑FT4, respectively, among LT4-OH patients. Elevated ANGPTL4 level may be attributed to the role of THs, similar pattern to other IR conditions or stimulatory effect of relative hypoxia on ANGPTL4 in hypothyroidism (25, 26, 27). Moreover, THs suppressed ANGPTL3 mRNA but not ANGPTL4 mRNA in hypothyroid rats (14). We found that both initial and Δ↓anti-TPO were independent inhibitory contributors to the corresponding ANGPTL4 values among SCH patients. This may be an autoimmune protective mechanism similar to downregulated astrocytic ANGPTL4 expression in active demyelinating multiple

Table 3 Correlation of both baseline and DELTA changes of ANGPTL3, 4 and 8 with corresponding studied clinical and laboratory data among all baseline study groups and naïve and treated hypothyroid patients. Spearman's or Pearson's correlation determines the relation between ANGPTL and cardio-metabolic risk factor among naïve patients and LT4 (thyroxin replacement therapy) patients.

Naïve patients			**Age** (years)	**BMI** (kg/m^2)	**WC** (cm)	**SBP** (mmHg)	**DBP** (mmHg)	**TSH** (IU/mL)	**FT3** (pg/mL)	**FT4** (ng/dL)	**TPO** (IU/mL)	**FPG** (mg/dL)	**Insulin** (mU/mL)	**Homa-IR**	**HDL-c** (mg/dL)	**LDL-c** (mg/dL)	**Total C** (mg/dL)	**TG** (mg/dL)	**hsCRP** (mg/L)
ANGPTL3 (ng/ml)	all	r	0.02	0.52[b]	0.01	0.39[b]	0.44[b]	0.71[b]	−0.6[b]	−0.73[b]	0.74[b]	0.26[b]	0.54[b]	0.57[b]	−0.33[b]	0.78[b]	0.81[b]	0.69[b]	0.65[b]
	SCH	r	−0.65[b]	−0.27	−0.36[a]	−0.41[a]	−0.28	0.01	0.18	−0.01	−0.05	0.19	0.39[a]	0.44[b]	0.32	0.23	0.45[b]	−0.38[a]	−0.03
	OH	r	−0.48[b]	−0.38[a]	0.19	0.31	−0.02	0.04	0.16	−0.12	−0.02	−0.11	0.20	0.23	0.08	0.12	−0.01	−0.22	−0.04
ANGPTL4 (ng/mL)	all	r	0.3[b]	0.65[b]	0.18	0.6[b]	0.52[b]	0.7[b]	−0.7[b]	−0.78[b]	0.71[b]	0.30[b]	0.50[b]	0.53[b]	−0.34[b]	0.77[b]	0.82[b]	0.81[b]	0.68[b]
	SCH	r	0.16	0.31	0.13	0.46[b]	0.05	0.05	0.22	−0.01	−0.47[b]	0.11	0.37[a]	0.31	0.42[a]	0.02	0.02	−0.03	−0.33[a]
	OH	r	0.30	0.33[a]	0.19	0.15	−0.05	0.22	−0.11	0.03	−0.05	−0.03	−0.29	−0.29	−0.03	0.26	0.35[a]	0.29	0.09
ANGPTL8 (pg/mL)	all	r	0.21[a]	0.61[b]	0.12	0.48[b]	0.5[b]	0.67[b]	−0.7[b]	−0.77[b]	0.76[b]	0.31[b]	0.49[b]	0.52[b]	−0.42[b]	0.76[b]	0.82[b]	0.77[b]	0.71[b]
	SCH	r	−0.01	−0.01	−0.52[b]	−0.28	0.14	−0.15	−0.07	0.12	0.27	0.41[a]	−0.16	−0.16	0.42[a]	0.07	0.29	−0.46[b]	0.19
	OH	r	−0.22	−0.11	0.15	0.14	−0.02	0.14	−0.04	0.30	−0.21	−0.11	0.20	0.21	−0.5[b]	−0.07	0.03	0.17	−0.03
LT4 patients				**↓ΔBMI** (kg/m^2)	**↓ΔWC** (cm)	**↓ΔSBP** (mmHg)	**↓ΔDBP** (mmHg)	**↓ΔTSH** (IU/ml)	**↑ΔFT3** (pg/ml)	**↑ΔFT4** (ng/dl)	**↓Δ TPO** (IU/ml)	**↓ΔFBS** (mg/dl)	**↓ΔInsulin** (mU/mL)	**↓ΔHoma-IR**	**↑ΔHDLc** (mg/dl)	**↓ΔLDL-c** (mg/dl)	**↓ΔTotal C** (mg/dl)	**↓ΔTG** (mg/dl)	**↓ΔhsCRP** (mg/L)
↓ΔANGPTL3 (ng/mL)	SCH	r		0.01	0.01	0.26	0.33[a]	0.14	0.36[a]	0.52[a]	0.09	−0.04	−0.04	−0.04	0.1	−0.12	−0.23	−0.09	0.03
	OH	r		−0.22	−0.45[b]	0.09	0.41[a]	0.44[a]	0.12	0.13	0.26	−0.16	0.20	0.18	−0.13	0.16	−0.05	−0.35[a]	−0.29
↓ΔANGPTL4 (ng/mL)	SCH	r		0.07	−0.11	0.26	0.19	0.18	0.04	0.17	−0.53[b]	0.38[a]	−0.09	−0.09	−0.1	−0.03	0.02	−0.41[a]	0.19
	OH	r		0.18	−0.22	0.14	0.241	−0.19	−0.5[b]	0.23	−0.06	−0.05	−0.54[b]	−0.5[b]	−0.01	0.14	0.12	−0.05	−0.12
↓ΔANGPTL8 (pg/mL)	SCH	r		−0.34[a]	−0.27	0.21	0.26	−0.4[a]	0.12	0.27	0.13	0.06	−0.16	−0.19	0.29	0.03	−0.14	0.22	−0.16
	OH	r		−0.33[a]	−0.54[b]	0.37[a]	0.01	0.05	−0.17	0.05	0.22	−0.10	0.21	0.23	−0.30	0.28	0.09	−0.32	−0.11

Data given as r- correlation; significant *P* values: [a]$P < 0.05$ and [b]$P < 0.01$.
all, entire study population; ANGPTL, angiopoietin-like protein; C, cholesterol; DBP, diastolic blood pressure; FPG, fasting plasma glucose; HOMA- IR, homeostatic model assessment of insulin resistance; HDL, high density lipoprotein; hsCRP, high sensitive c reactive protein; LDL, low density lipoprotein; OH, overt hypothyroidism; SBP, systolic blood pressure; SCH, subclinical hypothyroidism; TG, triglyceride; TPO, thyroid peroxidase antibody; WC, waist circumference; ↓Δ, delta decreased level during follow-up; ↑Δ, delta increased level during follow-up.

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Table 4 Correlation of both baseline and delta changes of ANGPTL3, 4 and 8 with corresponding studied cardiovascular function parameters among entire baseline study groups and naïve and treated hypothyroid patients. Spearman's or Pearson's correlation determines the relation between ANGPTL and cardiovascular function parameter.

Naïve patient			**EF** (%)	**FS** (%)	**IVCT** (ms)	**ET** (ms)	**é septal** (cm/s)	**é lateral** (cm/s)	**DT** (ms)	**IVRT** (ms)	**MPI**	**LV mass** (g)	**FMD** (%)	**CIMT** (mm)	**ASI**
ANGPTL3	All	r	−0.39[b]	−0.47[b]	0.33[b]	−0.62[b]	−0.3[b]	−0.28[b]	0.26[b]	0.46[b]	0.68[b]	0.38[b]	−0.74[b]	0.57[b]	0.72[b]
(ng/mL)	SCH	r	−0.04	0.02	−0.02	0.22	0.45[b]	0.43[b]	−0.6[b]	−0.43[b]	−0.09	0.04	−0.41[a]	0.15	−0.01
	OH	r	−0.44[b]	−0.4[b]	0.01	−0.23	−0.23	−0.07	−0.10	−0.11	0.01	0.08	0.09	0.03	−0.06
ANGPTL4	All	r	0.37[b]	−0.52[b]	0.43[b]	−0.78[b]	−0.42[b]	−0.39	0.49[b]	0.64[b]	0.82[b]	0.36[b]	−0.78[b]	0.62[b]	0.74[b]
(ng/mL)	SCH	r	0.01	0.35[a]	0.35[a]	0.51[b]	−0.24	−0.25	0.22	0.12	−0.15	0.07	−0.34[a]	−0.02	−0.23
	OH	r	−0.08	−0.13	−0.02	0.06	0.13	0.18	0.35[a]	0.30	−0.03	−0.26	0.19	0.06	0.016
ANGPTL8	All	r	−0.40[b]	−0.45[b]	0.41[b]	−0.75[b]	−0.42[b]	−0.37[b]	0.45[b]	0.63[b]	0.83[b]	0.42[b]	−0.78[b]	0.65[b]	0.82[b]
(pg/mL)	SCH	r	−0.29	0.13	0.21	0.15	−0.12	0.08	0.07	−0.42[b]	−0.09	0.57[b]	−0.03	0.16	0.09
	OH	r	0.03	0.23	−0.38[a]	−0.15	0.20	0.06	−0.06	0.25	0.28	−0.26	0.14	−0.49[b]	0.21
LT4 patients			↑Δ**EF** (%)	↑Δ**FS** (%)	↓Δ**IVCT** (ms)	↑Δ**ET** (ms)	↑Δ**é septal** (cm/s)	↑Δ**é lateral** (cm/s)	↓Δ**DT** (ms)	↓Δ**IVRT** (ms)	↓Δ**MPI**	↓Δ**LV mass** (g)	↑Δ**FMD** (%)	↓Δ**CIMT** (mm)	↓Δ**ASI**
↓ΔANGPTL 3	SCH	r	−0.3	−0.23	0.02	0.17	0.33[a]	0.36[a]	−0.17	0.31	0.14	0.13	0.14	0.15	0.36[a]
(ng/mL)	OH	r	0.01	−0.02	0.04	0.21	−0.10	−0.11	−0.37[a]	−0.29	−0.15	−0.13	−0.12	0.13	−0.09
↓ΔANGPTL4	SCH	r	−0.03	−0.03	0.2	0.07	0.02	−0.04	−0.14	0.03	−0.17	0.21	−0.27	0.02	−0.2
(ng/mL)	OH	r	−0.15	−0.29	−0.11	−0.56[b]	−0.03	0.07	0.12	0.16	0.39[a]	0.47[b]	0.07	0.01	−0.11
↓ΔANGPTL8	SCH	r	0.09	−0.16	0.03	−0.33[a]	0.23	0.16	−0.11	−0.39[a]	0.21	0.31	0.01	0.08	0.14
(pg/mL)	OH	r	0.11	−0.07	0.01	−0.15	0.06	0.05	−0.25	0.06	0.18	−0.12	−0.08	0.08	0.03

Data given as r correlation; significant *P* values: [a]$P < 0.05$ and [b]$P < 0.01$.

ANGPTL, angiopoietin-like protein; ASI, aortic stiffness index; CIMT, carotid media intimal thickness; DT, deceleration time; é, early diastolic pulsed-wave tissue Doppler velocities at the mitral annulus; EF, ejection fraction; ET, ejection time; FS, fractional shortening; FMD, flow-mediated dilation; IVCT, isovolumetric contraction time; IVRT, isovolumetric relaxation time; LT4, thyroxin replacement therapy; MPI, myocardial performance index; OH, overt hypothyroidism; SCH, subclinical hypothyroidism; ↓Δ, delta decreased level during follow-up; ↑Δ, delta increased level during follow-up.

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Table 5 Variables significantly correlated with ANGPTLs related cardiovascular function parameters.

Flow-mediated dilation % among naïve SCH												
	Age (years)	BMI (kg/m2)	WC (cm)	FT3 (pg/ml)	TPO (IU/mL)	FPG (mg/dL)	Insulin (mU/mL)	HOMA-IR	HDL (mg/dL)	Ch (mg/dL)	TG (mg/dL)	Angptl3 (ng/mL)	Angptl4 (ng/mL)
r	*0.42*	−0.34	−0.42	−0.39	*0.38*	−0.41	*−0.42*	*0.47*	*0.44*	*−0.36*	*0.35*	0.45	−0.44
P	*0.009*	0.04	0.01	0.01	*0.02*	0.01	*0.02*	*0.004*	*0.008*	*0.03*	*0.001*	0.006	0.007
Fractional shortening% in naive SCH					**LVM in naïve SCH** (g)		**Ejection fraction in naïve OH** (%)			**Carotid intima-media thickness in naïve OH** (mm)			
	Age (years)	HOMA- IR	hsCRP (mg/L)	Angptl4 (ng/mL)	HDL (mg/dL)	Angptl8 (pg/mL)	FPG	TG (mg/dL)	Angptl3 (ng/mL)	BMI (kg/m^2)	SBP (mmHg)	FPG (mg/dL)	Angptl8 (pg/mL)
r	−0.35	0.34	*0.44*	−0.34	0.33	0.57	0.37	0.39	−0.44	0.41	0.33	0.38	−0.49
P	*0.005*	0.03	*0.007*	0.03	0.04	0.001	0.02	0.01	0.007	0.01	0.04	0.01	
Δ↓Aortic stiffness index in LT4-SCH							**Δ↓MP in LT4-OH**			**Δ↓LVM in LT4-OH** (g)			
	Δ↑FT4 (ng/dL)Δ↑FT4 (ng/dL)		Δ↑HDL (mg/dL)		Δ↓Angptl3 (ng/mL)		Δ↓DBP (mmHg)		Δ↓TPO (IU/mL)	Δ↓Angptl4 (ng/mL)			
r	*0.58*		−0.35		0.36		−0.39		−0.34	0.47			
P	*<0.001*		0.03		0.02		0.01		0.04	0.004			

Spearman's or Pearson's correlation with $P < 0.05$ is considered significant. Variables which were collinear with ANGPTL and cardiovascular function in the same category are italic and underlined and were not entered into the multivariate regression.

ANGPTL, angiopoietin-like protein; Ch, cholesterol; DBP, diastolic blood pressure; FPG, fasting plasma glucose; HDL, high density lipoprotein; hsCRP, high sensitive C-reactive protein; HOMA- IR, homeostatic model for the assessment of insulin resistance; LT4, thyroxin replacement therapy; LVM, left ventricle mass; MPI, myocardial performance index; OH, overt hypothyroidism; SBP, systolic blood pressure; SCH, subclinical hypothyroidism; TG , triglyceride; TPO, thyroid peroxidase antibody; WC, waist circumference; ↓Δ, delta decreased level during follow-up; ↑Δ, delta increased level during follow-up.

sclerosis (28). The present negative correlation between ANGPTL8 and TSH among SCH patients was not confirmed by multivariate analysis. In this context, cellular cell line studies reported enhancing the effect of THs on ANGPTL8 (15).

The novel regulatory roles of ANGPTLs in cardiovascular function were as follows: ANGPTL3 and ANGPTL4 were sponsors for impaired FMD%. Meanwhile, ANGPTL8 was an independent predictor of LV mass among naïve SCH patients. Furthermore, ANGPTL3 and 8 were contributors to EF% and CIMT, respectively, in naïve OH patients. Finally, Δ↓ANGPTL3 was an independent predictor of Δ↓ASI in LT4-SCH patients, whereas Δ↓ANGPTL4 was an independent contributor to Δ↓MPI and Δ↓LV mass among LT4-OH patients.

Our study suggested an atherogenic role of ANGPTLs. We demonstrated that ANGPTL4 is an endothelial function biomarker of the both muscular peripheral and the central elastic arteries meanwhile ANGPTL3 is an endothelial function biomarker of only peripheral arteries. Beyond lipid metabolism regulation, inactivated mutations of ANGPTL3 and ANGPTL4 reduce atherosclerosis and CAD risk (4, 5). Their deficiency inhibits foam cell formation and atherosclerosis in animal studies (7, 8). ANGPTL3 also exerts pro-inflammatory and pro-angiogenic effects and prevents cholesterol efflux; the anti-atherosclerotic effect of anti-ANGPTL3 is well recognized (5, 7). ANGPTL3 was associated with increased CIMT and aortic stiffness in healthy and CAD subjects, respectively, whereas ANGPTL4 was related to ankle–brachial index among COPD patients (10, 12, 29). However, the association between CAD and the ANGPTL4 level is still under debate (30, 31). Moreover, macrophage ANGPTL4 inhibits foam cell formation. Thus, macrophage and systemic ANGPTL4 may have a diverse effect on atherosclerosis (11).

We found a positive association between ANGPTL8 and CIMT among all the study subjects and the hypothyroid group. In contrary, we found an inverse association among OH patients. The atherogenic role of ANGPTL8 is still unclear. ANGPTL8 was correlated with increased CIMT in type 2 diabetes mellitus (T2DM) patients but not among metabolic syndrome patients in the multivariate analysis (32, 33). ANGPTL8 is related to advanced peripheral arterial atherosclerosis and plaque presence and severity but is not a risk factor for CAD (34, 35). ANGPTL8 as an independent predictor of the presence and severity of CAD is still unclear (36, 37). ANGPTL8 promotes macrophage foam cell formation via controlled cholesterol efflux and uptake. ANGPTL8 also alters the expression of the scavenger receptors that favor atherosclerosis. However, its

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enriched effect on foam cell formation was not confirmed using ANGPTL8 overexpression and knockdown mice (9) Contrarily, ANGPTL8 may be a 'brake' for certain inflammatory pathways as intracellular ANGPTL8 exerts an anti-inflammatory effect. However, extracellular ANGPTL8 may cause inflammation (6). The anti-atherogenic role of ANGPTL8 among OH patients was consistent with its association with reduced incidence of cardiovascular events among CAD patients (38). Different study population and low THs may cause this diversity.

Increased ANGPTL3 in naïve SCH and Δ↓ANGPTL3 in LT4-SCH patients were associated with better diastolic function as estimated by enhanced é lateral and é septal mitral annulus velocity in addition to shortened IVRT and DT among naïve SCH patients. Our explanation for this paradoxical finding was presence of two antagonizing effects of ANGPTL3. The direct effect of ANGPTL3 is attenuation of myocardial ischemia and improvement of heart function by promoting angiogenesis. The indirect role Δ↓ANGPTL3 in LT4-SCH patients via reduced ASI with subsequent improved cardiac diastolic function (39, 40).

Among the naïve OH group, elevated ANGPTL3 level was found to be a contributor to reduced cardiac systolic function. ANGPTL3 has dual antagonizing independent effects to regulate the FFA level as the main cardiomyocyte fuel in fasting. It enhances adipose tissue lipolysis with subsequently increased circulating FFA and probably cardiac toxicity if excess. The second effect of ANGPTL3 is inhibition of the systemic LPL with subsequently decreased circulating FFA and hypertriglyceridemia (41). We hypothesized that the inhibitory effect of excess ANGPTL3 on myocardial contractility and systolic function via cardiac lipotoxicity or decreased FFA supply was further suppressed with low THs in OH patients. Thus, ANGPTL3 may have dual effect on cardiac function, which may be modified by its level or associated cofactors.

We reported a negative effect of ANGPTL4 on global cardiac function as reduced ANGPTL4 level in LT4-OH patients was associated with the improvement of MPI and reduction of LVM. We also reported its negative effect on systolic function (FS%) in bivariate, not multivariate, analysis in naïve SCH patients. Consistent with our findings, ANGPTL4 was positively correlated with cardiac dysfunction biomarker and LV mass in COPD (12). ANGPTL4 is widely expressed with tissue specificity with regard to re gulatory factors and activated fragment component. The liver nANGPTL4-fragment has only endocrine role by inhibiting systemic LPL activity. The adipose tissue fANGPTL4-fragment locally inhibits lipolysis, systemically inhibits LPL activity and greatly contributes to circulating ANGPTL4. In addition, macrophage, endothelium, and cardiomyocyte express ANGPTL4. Upregulated cardiac ANGPTL4 physiologically inhibits only local LPL to reduce FFA in order to prevent myocardial lipotoxicity. So, increased circulating ANGPTL4 inhibits lipolysis with a shift of triglyceride and ectopic fat deposition in other tissues (11). ANGPTL4-deficient mice also exhibit enhanced circulating LPL activity as well as unchanged cardiac LPL activity (42). Based on our results and literature, we conclude that more triglyceride and ectopic fat deposition in cardiomyocyte occurs in response to elevated circulating ANGPTL4. This effect may overcome protective role of cardiac ANGPTL4. The excess fat in cardiomyocyte may undergo lipolysis by cardiac LPL. The net effect is production of more fatty acid which may lead to cardiac lipotoxicity and impaired function. We reported the enhancing effect of ANGPTL8 on LV mass in naïve SCH patients. This was matched to restore LV mass via ANGPTL8 gene therapy (43).

Among previous different population studies, cross talk of the studied ANGPTL with traditional cardiovascular risk factors showed much diversity with an unidentified cause-and-effect relationship or presence of vicious circle. This diversity may be attributed to different races, study populations, and technical ELISA kits. We first addressed the correlations and the contributory roles of cardiovascular risk factors with ANGPTL level among hypothyroid patients.

The independent predictors of ANGPTL level were age, waist circumference, and cholesterol for ANGPTL3 and SBP and waist circumference for ANGPTL4 and ANGPTL8, respectively, among naïve SCH patients; age and HDL for ANGPTL3 and ANGPTL8, respectively, among naïve OH patients; Δ↓FPG and Δ↓triglyceride for Δ↓ANGPTL4 among LT4-SCH patients; Δ↓triglyceride for Δ↓ANGPTL3, Δ↓HOMA-IR for Δ↓ANGPTL4, and Δ↓SBP and Δ↓waist circumference for Δ↓ANGPTL8 among LT4-OH patients.

Our results suggested that age, obesity parameters and cholesterol were the main independent regulators of circulating ANGPTL3 among naïve SCH patients. Bivariate analysis revealed that ANGPTL3 had positive associations with insulin and IR and negative associations with TG and SBP among naïve SCH patients. Also, ANGPTL3 was positively correlated with Δ↓DBP among the entire treated hypothyroid patients. However, these associations were not confirmed in further multivariate analysis. The negative effect of age on ANGPTL3 in our study was in conflict with the previously described positive association as one study was among wider age range while the other included older age subjects and mainly males (10, 44). The relationship of

ANGPTL3 with the obesity, glycemic, and lipid parameters is less clear in previous studies. In accordance with our results, BMI had inverse correlation with ANGPTL3 across the wide BMI spectrum (45). This may be explained by the stimulatory effect of ANGPTL3 on hypothalamic LPL activity with subsequently decreased appetite and body weight (41) Contrarily, obese Middle Eastern population and overweight Korean children exhibited elevated and normal ANGPTL3 levels, respectively (27, 46). ANGPTL3 enhances lipolysis in adipose tissue, resulting in increased hepatic and peripheral resistance. It also stimulates fatty acid uptake by white adipose tissue with a subsequent decrease in insulin sensitivity (41). Thus, we found positive associations between ANGPTL3 and insulin and HOMA-IR. This finding is consistent with that of other clinical, animal, and molecular studies. The ANGPTL3 level was increased in IR mice. ANGPTL3 gene silencing and inactivating mutations enhance insulin sensitivity and are associated with lower HOMA-IR (46, 47, 48, 49). Reduced or elevated ANGPTL3 levels were reported in diabetic subjects as hyperinsulinemia may suppress ANGPTL3 or ANGPTL3 is increased in IR states respectively, whereas others reported no association irrespective of the body weight. Moreover, no relationship was observed among ANGPTL3 and the glycemic parameters in the population studies (27, 41, 44, 45, 47, 50). Although the precise mechanism of lipid metabolism is not yet fully understood, ANGPTL3 inhibits LPL activity and endothelial lipase, thereby increasing the lipid levels (49). The inactivating mutation of ANGPTL3 induces total hypolipidemia. Moreover, anti-ANGPTL3 therapy reduces cholesterol and TG (5). Similar to us, Cinkajzlová *et al*. (2019) reported an inverse correlation of ANGPTL3 with TG and its positive correlation with cholesterol (45). ANGPTL3 exhibited marked diversity with lipid profile in clinical studies. The population studies demonstrated no association at all or the presence of positive correlation with HDL-c and negative correlation with TG as well as no association with cholesterol and LDL-c (44, 50). Also, the ELISA kit used in our study detected full-length ANGPTL3 but did not recognize the cleavage active form, which regulates the LPL activity. This may explain the unpredictable association of ANGPTL3 with TG in our study. Finally, we reported dual relation of BP with ANGPTL3 that may depend on the ANGPTL3 level. Previous data reported a normal level of ANGPTL3 in hypertensive patients and a positive association with BP in healthy subjects (10, 51). Our findings may be attributed to the effect of ANGPTL3 on endothelial function which was described in our study.

In our study, ANGPTL4 was positively correlated with most traditional cardiovascular risk factors such as blood pressure, BMI, glycemic indices, and cholesterol. SBP was a positive independent regulator of ANGPTL4 in naïve SCH patients. Δ↓FBS was a positive whereas Δ↓TG a negative independent contributor to Δ↓ANGPTL4 among LT-SCH patients. Finally, Δ↓HOMA-IR was a negative regulator of ANGPTL4 in LT4-HT patients. The BMI among naïve OH patients and IR as well as the insulin, HDL-c and cholesterol level among naïve SCH patients were positively correlated but were not predictors of ANGPTL4. Recent study reported elevated ANGPTL4 level in hypertensive diabetic patients (51). This may be mediated by arterial stiffness effect of ANGPTL4 in our study. In animal studies, ANGPTL4-deficient mice demonstrated enhanced insulin sensitivity and LPL activity with decreased TG level. Conversely, ANGPTL4 overexpression exhibited a much diverse effect on glucose tolerance and decreased LPL activity with elevated triglyceride. The inactivating mutation of ANGPTL4 promotes reduced triglyceride, increased HDL-c, low fasting glucose, enhanced insulin sensitivity, and reduced diabetic risk (3, 52, 53). Moreover, the serum ANGPTL4 levels exhibited widely varying glucose metabolic traits in clinical studies. For example, the ANGPTL4 levels were positively correlated with BMI, blood glucose, HbA1c, triglycerides, and insulin and negatively correlated with HDL-c in one study. Others demonstrated an association neither with lipid profile nor with glycemic parameters. Contrarily, ANGPTL4 was positively correlated with serum glucose and HbA1c but not with lipid profile among subjects with impaired glucose tolerance. Moreover, data regarding T2DM and circulating ANGPTL4 are conflicting (44, 45, 50, 54). However, the exact mechanisms responsible for the close relationship betweenANGPTL4 and metabolic parameters in our study remain largely unidentified. Contrary to the original hypothesis of free fatty acids that the main PPAR ligands are the primary regulators of ANGPTL4 synthesis, others suggested low-grade inflammation as a more important contributor to increased ANGPTL4 levels in IR state. Here, we have demonstrated a strong positive correlation between serum ANGPTL4 and hsCRP in all the study subjects(not in hypothyroid patients) as a marker of low-grade inflammation Our finding of negative association of ANGPTL4 with HOMA-IR among treated hypothyroid patients may be explained by the physiological inhibitory effect of insulin on the ANGPTL4 levels. The ANGPTL4 level is reduced during hyperinsulinemic–euglycemic clamp in healthy subjects (55). The lack of a positive correlation between serum ANGPTL4 and TGs could have resulted from either the limitations of the ELISA kits in our study and most of these studies, as this kit detects

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full-length ANGPTL4 but fails to recognize the N-terminal domain of ANGPTL4, which is responsible for the LPL inhibition, or the modulating role of other factors as THs on the ANGPTL4–triglyceride relationship (52).

ANGPTL8 influence the different pathophysiological conditions including inflammation, IR, dyslipidemia, and non-alcoholic fatty liver disease. However, the precise underlying molecular mechanisms still need to be delineated. It regulates both intracellular and extracellular lipid metabolism, LPL activity, inflammatory pathways (11).

Our study demonstrated that obesity and HDL were negative regulators whereas SBP was a positive regulator of ANGPTL8. Among naïve and treated OH patients, waist circumference and its Δ reduction during the follow-up period were independent predictors of ANGPTL8. HDL-c was an independent negative predictor among naïve OH patients, whereas Δreduced SBP was a positive contributor among LT4-OH patients. Our bivariate (not multivariate) analyses revealed a positive association between FPG and HDL and a negative association between TG and ANGPTL8 among naïve SCH patients. Our results were matched to previous studies, the reduced ANGPTL8 level in obese subjects (with or without diabetes) significantly increased 1-year after bariatric surgery (56, 57). However, one study reported nil association of ANGPTL8 and obesity and others recorded entirely contradictory results among obese diabetic patients who had elevated ANGPTL8 levels and showed a long-term ANGPTL8 reduction after bariatric surgery (46, 58). Previous data about blood pressure is scarce. ANGPTL8 level was found to be positively associated with blood pressure in preeclampsia and elevated in hypertensive diabetic, but not in non-diabetic, subjects (51, 59). Unfortunately, the underlying mechanism is still unknown. The negative associations between ANGPTL8 and triglyceride and HDL-c in our study were similar to previously described among other population, CAD patients and those with obesity and glucose intolerance (36, 57). On the other hand, nil and positive associations of ANGPTL8 with atherogenic lipid profile were described (37, 44). Furthermore, inactivating mutations of ANGPTL8 decrease LDL-c and HDL-c levels, and exert an insignificant effect on TG in human genetic studies (6). We only measured full-length ANGPTL8 in the fasting state, but the ANGPTL8 activity was regulated by nutritional status. The effect of ANGPTL8 on triglyceride metabolism might be apo-CIII-dependent; unfortunately, the latter was not measured in our study (6, 36).

Our study demonstrated that ANGPTLs are independent risk biomarkers for endothelial and cardiac function, and depend on TH levels and cardiovascular risk factors in hypothyroid patients. The results are quite promising and have led us to consider whether thyroid analogs could be used to reduce ANGPTL levels in patients with atherosclerosis. However, this study has some limitations: the relatively small sample size and the ELISA kit used to detect full-length ANGPTL.

Supplementary materials

This is linked to the online version of the paper at https://doi.org/10.1530/EC-21-0398.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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References

1 Bielecka-Dabrowa A, Godoy B, Suzuki T, Banach M & von Haehling S. Subclinical hypothyroidism and the development of heart failure: an overview of risk and effects on cardiac function. *Clinical Research in Cardiology* 2019 **108** 225–233. (https://doi.org/10.1007/s00392-018-1340-1)

2 Abdel-Moneim A, Gaber AM, Gouda S, Osama A, Othman SI & Allam G. Relationship of thyroid dysfunction with cardiovascular diseases: updated review on heart failure progression. *Hormones* 2020 **19** 301–309. (https://doi.org/10.1007/s42000-020-00208-8)

3 Zhang R. The ANGPTL3-4-8 model, a molecular mechanism for triglyceride trafficking. *Open Biology* 2016 **6** 150272. (https://doi.org/10.1098/rsob.150272)

4 Dewey FE, Gusarova V, O'Dushlaine C, Gottesman O, Trejos J, Hunt C, Van Hout CV, Habegger L, Buckler D, Lai KM, *et al.* Inactivating variants in ANGPTL4 and risk of coronary artery disease. *New England Journal of Medicine* 2016 **374** 1123–1133. (https://doi.org/10.1056/NEJMoa1510926)

5 Dewey FE, Gusarova V, Dunbar RL, O'Dushlaine C, Schurmann C, Gottesman O, McCarthy S, Van Hout CV, Bruse S, Dansky HM, *et al.* Genetic and pharmacologic inactivation of ANGPTL3 and cardiovascular disease. *New England Journal of Medicine* 2017 **377** 211–221. (https://doi.org/10.1056/NEJMoa1612790)

6 Abu-Farha M, Ghosh A, Al-Khairi I, Madiraju SRM, Abubaker J & Prentki M. The multi-faces of Angptl8 in health and disease: novel functions beyond lipoprotein lipase modulation. *Progress in Lipid Research* 2020 **80** 101067. (https://doi.org/10.1016/j.plipres.2020.101067)

7 Lupo MG & Ferri N. Angiopoietin-Like 3 (ANGPTL3) and atherosclerosis: lipid and non-lipid related effects. *Journal of Cardiovascular Development and Disease* 2018 **5** 39. (https://doi.org/10.3390/jcdd5030039)

8 Adachi H, Fujiwara Y, Kondo T, Nishikawa T, Ogawa R, Matsumura T, Ishii N, Nagai R, Miyata K, Tabata M, *et al.* Angptl 4 deficiency improves lipid metabolism, suppresses foam cell formation and protects against atherosclerosis. *Biochemical and Biophysical Research Communications* 2009 **379** 806–811. (https://doi.org/10.1016/j.bbrc.2008.12.018)

9 Jiao X, Yang Y, Li L, Yu H, Yang Y, Li J, Du Y, Zhang J, Hu C & Qin Y. Angiopoietin-like protein 8 accelerates atherosclerosis in ApoE$^{-/-}$ mice. *Atherosclerosis* 2020 **307** 63–71. (https://doi.org/10.1016/j.atherosclerosis.2020.06.014)

10 Hatsuda S, Shoji T, Shinohara K, Kimoto E, Mori K, Fukumoto S, Koyama H, Emoto M & Nishizawa Y. Association between plasma angiopoietin-like protein 3 and arterial wall thickness in healthy subjects. *Journal of Vascular Research* 2007 **44** 61–66. (https://doi.org/10.1159/000098153)

11 Yang J, Li X & Xu D. Research progress on the involvement of ANGPTL4 and loss-of-function variants in lipid metabolism and coronary heart disease: is the 'Prime Time' of ANGPTL4-targeted therapy for coronary heart disease approaching? *Cardiovascular Drugs and Therapy* 2021 **35** 467–477. (https://doi.org/10.1007/s10557-020-07001-0)

12 Waschki B, Kirsten AM, Holz O, Meyer T, Lichtinghagen R, Rabe KF, Magnussen H, Welte T, Watz H & Janciauskiene S. Angiopoietin-like protein 4 and cardiovascular function in COPD. *BMJ Open Respiratory Research* 2016 **3** e000161. (https://doi.org/10.1136/bmjresp-2016-000161)

13 Yang L, Yin R, Wang Z, Wang X, Zhang Y & Zhao D. Circulating Angptl3 and Angptl8 are increased in patients with hypothyroidism. *BioMed Research International* 2019 **2019** 3814687. (https://doi.org/10.1155/2019/3814687)

14 Fugier C, Tousaint JJ, Prieur X, Plateroti M, Samarut J & Delerive P. The lipoprotein lipase inhibitor ANGPTL3 is negatively regulated by thyroid hormone. *Journal of Biological Chemistry* 2006 **281** 11553–11559. (https://doi.org/10.1074/jbc.M512554200)

15 Tseng YH, Ke PY, Liao CJ, Wu SM, Chi HC, Tsai CY, Chen CY, Lin YH & Lin KH. Lin KH chromosome 19 open reading frame 80 is up-regulated by thyroid hormone and modulates autophagy and lipid metabolism. *Autophagy* 2014 **10** 20–31. (https://doi.org/10.4161/auto.26126)

16 Garber JR, Cobin RH, Gharib H, Hennessey JV, Klein I, Mechanick JI, Pessah-Pollack R, Singer PA, Woeber KA & American Association of Clinical Endocrinologists and American Thyroid Association Taskforce on Hypothyroidism in Adults. Clinical practice guidelines for hypothyroidism in adults: cosponsored by the American Association of Clinical Endocrinologists and the American Thyroid Association. *Endocrine Practice* 2012 **18** 988–1028. (https://doi.org/10.4158/EP12280.GL)

17 Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF & Turner RC. Homeostasis Model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985 **28** 412–419. (https://doi.org/10.1007/BF00280883)

18 Touboul PJ, Hennerici MG, Meairs S, Adams H, Amarenco P, Bornstein N, Csiba L, Desvarieux M, Ebrahim S, Fatar M, *et al.* Mannheim carotid intima-media thickness consensus (2004-2006). An update on behalf of the Advisory Board of the 3rd and 4th Watching the Risk Symposium, 13th and 15th European Stroke Conferences, Mannheim, Germany, 2004, and Brussels, Belgium, 2006. *Cerebrovascular Diseases* 2007 **23** 75–80. (https://doi.org/10.1159/000097034)

19 Celermajer DS, Sorensen KE, Gooch VM, Spiegelhalter DJ, Miller OI, Sullivan ID, Lloyd JK & Deanfield JE. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. *Lancet* 1992 **340** 1111–1115. (https://doi.org/10.1016/0140-6736(92)93147-f)

20 Stefanadis C, Dernellis J, Tsiamis E, Stratos C, Diamantopoulos L, Michaelides A & Toutouzas P. Aortic stiffness as a risk factor for recurrent acute coronary events in patients with ischaemic heart disease. *European Heart Journal* 2000 **21** 390–396. (https://doi.org/10.1053/euhj.1999.1756)

21 Lang RM, Badano LP, Mor-Avi V, Afilalo J, Armstrong A, Ernande L, Flachskampf FA, Foster E, Goldstein SA, Kuznetsova T, *et al.* Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *Journal of the American Society of Echocardiography* 2015 **28** 39.e14. (https://doi.org/10.1016/j.echo.2014.10.003)

22 Mitter SS, Shah SJ & Thomas JD. A test in ContextE/A and E/e0 to assess diastolic dysfunction and LV filling pressure. *Journal of the American College of Cardiology* 2017 **69** 1451–1464. (https://doi.org/10.1016/j.jacc.2016.12.037)

23 Tei C, Nishimura RA, Seward JB & Tajik AJ. Noninvasive Doppler-derived myocardial performance index: correlation with simultaneous measurements of cardiac catheterization measurements. *Journal of the American Society of Echocardiography* 1997 **10** 169–178. (https://doi.org/10.1016/s0894-7317(97)70090-7)

24 Devereux RB, Alonso DR, Lutas EM, Gottlieb GJ, Campo E, Sachs I & Reichek N. Echocardiographic assessment of left ventricular hypertrophy: comparison to necropsy findings. *American Journal of Cardiology* 1986 **57** 450–458. (https://doi.org/10.1016/0002-9149(86)90771-x)

25 Ali ER. Assessment of functional lung impairment in patients with thyroid disorders. *Egyptian Journal of Bronchology* 2016 **10** 337–347. (https://doi.org/10.4103/1687-8426.193641)

26 Drager LF, Yao Q, Hernandez KL, Shin MK, Bevans-Fonti S, Gay J, Sussan TE, Jun JC, Myers AC, Olivecrona G, *et al.* Chronic intermittent hypoxia induces atherosclerosis via activation of adipose angiopoietin-like 4. *American Journal of Respiratory and Critical Care Medicine* 2013 **188** 240–248. (https://doi.org/10.1164/rccm.201209-1688OC)

27 Abu-Farha M, Al-Khairi I, Cherian P, Chandy B, Sriraman D, Alhubail A, Al-Refaei F, AlTerki A & Abubaker J. Increased ANGPTL3, 4 and ANGPTL8/betatrophin expression levels in obesity and T2D. *Lipids in Health and Disease* 2016 **15** 181. (https://doi.org/10.1186/s12944-016-0337-x)

28 Kamermans A, Rijnsburger M, Chakraborty A, van der Pol S, de Vries HE, van Horssen J & Angiopoietin-Like R. Reduced angiopoietin-like 4 expression in multiple sclerosis lesions facilitates lipid uptake by phagocytes via modulation of lipoprotein-lipase activity. *Frontiers in Immunology* 2019 **10** 950. (https://doi.org/10.3389/fimmu.2019.00950)

29 Fu YS, Wang JH, Lee CJ & Hsu BG. Positive correlation of the serum angiopoietin-like protein 3 levels with the aortic augmentation index in patients with coronary artery disease. *Therapeutics and Clinical Risk Management* 2018 **14** 231–236. (https://doi.org/10.2147/TCRM.S150476)

30 Smart-Halajko MC, Robciuc MR, Cooper JA, Jauhiainen M, Kumari M, Kivimaki M, Khaw KT, Boekholdt SM, Wareham NJ, Gaunt TR, *et al.* The relationship between plasma angiopoietin-like protein 4 levels, angiopoietin-like protein 4 genotype, and coronary heart disease risk. *Arteriosclerosis, Thrombosis, and Vascular Biology* 2010 **30** 2277–2282. (https://doi.org/10.1161/ATVBAHA.110.212209)

31 Muendlein A, Saely CH, Leiherer A, Fraunberger P, Kinz E, Rein P, Vonbank A, Zanolin D, Malin C & Drexel H. Angiopoietin-like protein 4 significantly predicts future cardiovascular events in coronary patients. *Atherosclerosis* 2014 **237** 632–638. (https://doi.org/10.1016/j.atherosclerosis.2014.10.028)

32 Zheng T, Ge B, Liu H, Chen B, Qin L, Xiao L & Song J. Triglyceride-mediated influence of serum angiopoietin-like protein 8 on subclinical atherosclerosis in type 2 diabetic patients: results from the GDMD study in China. *Cardiovascular Diabetology* 2018 **17** 84. (https://doi.org/10.1186/s12933-018-0687-y)

33 Maurer L, Schwarz F, Fischer-Rosinsky A, Schlueter N, Brachs S, Möhlig M, Pfeiffer A, Mai K, Spranger J & Bobbert T. Renal function is independently associated with circulating betatrophin. *PLoS ONE* 2017 **12** e0173197. (https://doi.org/10.1371/journal.pone.0173197)

34 Niki H, Kishimoto Y, Ibe S, Saita E, Sasaki K, Miura K, Ikegami Y, Ohmori R, Kondo K & Momiyama Y. Associations between plasma betatrophin levels and coronary and peripheral artery disease. *Journal of Atherosclerosis and Thrombosis* 2019 **26** 573–581. (https://doi.org/10.5551/jat.46508)

35 Niki H, Kishimoto Y, Saita E, Ohmori R, Kondo K & Momiyama Y. Plasma betatrophin levels and carotid atherosclerosis. *Disease Markers* 2019 **2019** 4214650. (https://doi.org/10.1155/2019/4214650)

36 Luo M, Su X, Yi Y, Yang Y & Peng D. Apolipoprotein CIII may mediate the impacts of angiopoietin-like protein 8 on triglyceride metabolism. *Lipids in Health and Disease* 2018 **17** 160. (https://doi.org/10.1186/s12944-018-0777-6)

37 Jiao X, He J, Yang Y, Yang S, Li J & Qin Y. Associations between circulating full-length angiopoietin-like protein 8 levels and severity of coronary artery disease in Chinese non-diabetic patients: a case-control study. *Cardiovascular Diabetolology* 2018 **17** 92. (https://doi.org/10.1186/s12933-018-0736-6)

38 Leiherer A, Ebner J, Muendlein A, Brandtner EM, Zach C, Geiger K, Fraunberger P & Drexel H. High betatrophin in coronary patients protects from cardiovascular events. *Atherosclerosis* 2020 **293** 62–68. (https://doi.org/10.1016/j.atherosclerosis.2019.11.011)

39 Weber T, O'Rourke MF, Ammer M, Kvas E, Punzengruber C & Eber B. Arterial stiffness and arterial wave reflections are associated with systolic and diastolic function in patients with normal ejection fraction. *American Journal of Hypertension* 2008 **21** 1194–1202. (https://doi.org/10.1038/ajh.2008.277)

40 Luo F, Wu P, Chen J, Guo Y, Wang J, Li X & Fang Z. ANGPTL3 possibly promotes cardiac angiogenesis through improving proangiogenic ability of endothelial progenitor cells after myocardial infarction. *Lipids in Health and Disease* 2018 **17** 184. (https://doi.org/10.1186/s12944-018-0835-0)

41 Christopoulou E, Elisaf M & Filippatos T. Effects of angiopoietin-like 3 on triglyceride regulation, glucose homeostasis, and diabetes. *Disease Markers* 2019 **2019** Article ID 6578327. (https://doi.org/10.1155/2019/6578327)

42 Cushing EM, Chi X, Sylvers KL, Shetty SK, Potthoff MJ & Davies BSJ. Angiopoietin-like 4 directs uptake of dietary fat away from adipose during fasting. *Molecular Metabolism* 2017 **6** 809–818. (https://doi.org/10.1016/j.molmet.2017.06.007)

43 Chen S, Chen J, Meng XL, Shen JS, Huang J, Huang P, Pu Z, McNeill NH & Grayburn PA. ANGPTL8 reverses established adriamycin cardiomyopathy by stimulating adult cardiac progenitor cells. *Oncotarget* 2016 **7** 80391–80403. (https://doi.org/10.18632/oncotarget.13061)

44 Morinaga J, Zhao J, Endo M, Kadomatsu T, Miyata K, Sugizaki T, Okadome Y, Tian Z, Horiguchi H, Miyashita K, *et al.* Association of circulating ANGPTL 3, 4, and 8 levels with medical status in a population undergoing routine medical checkups: a cross-sectional study. *PLoS ONE* 2018 **13** e0193731. (https://doi.org/10.1371/journal.pone.0193731)

45 Cinkajzlová A, Mráz M, Lacinová Z, Kloučková J, Kaválková P, Kratochvílová H, Trachta P, Křížová J, Haluzíková D, Škrha J, *et al.* Angiopoietin-like protein 3 and 4 in obesity, type 2 diabetes mellitus, and malnutrition: the effect of weight reduction and realimentation. *Nutrition and Diabetes* 2018 **8** 21. (https://doi.org/10.1038/s41387-018-0032-2)

46 Chung HS, Lee MJ, Hwang SY, Lee HJ, Yoo HJ, Seo JA, Kim SG, Kim NH, Baik SH, Choi DS, *et al.* Circulating angiopoietin-like protein 8 (ANGPTL8) and ANGPTL3 concentrations in relation to anthropometric and metabolic profiles in Korean children: a prospective cohort study. *Cardiovascular Diabetology* 2016 **15** 1. (https://doi.org/10.1186/s12933-015-0324-y)

47 Nidhina PA, Soronen J, Sädevirta S, Mysore R, Quagliarini F, Pasternack A, Metso J, Perttilä J, Leivonen M, Smas CM, *et al.* Regulation of angiopoietin-like proteins (ANGPTLs) 3 and 8 by insulin. *Journal of Clinical Endocrinology and Metabolism* 2015 **100** E1299–E1307. (https://doi.org/10.1210/jc.2015-1254)

48 Tikka A, Soronen J, Laurila PP, Metso J, Ehnholm C & Jauhiainen M. Silencing of ANGPTL 3 (angiopoietin-like protein 3) in human hepatocytes results in decreased expression of gluconeogenic genes and reduced triacylglycerol-rich VLDL secretion upon insulin stimulation. *Bioscience Reports* 2014 **34** e00160. (https://doi.org/10.1042/BSR20140115)

49 Robciuc MR, Maranghi M, Lahikainen A, Rader D, Bensadoun A, Öörni K, Metso J, Minicocci I, Ciociola E, Ceci F, *et al.* Angptl3 deficiency is associated with increased insulin sensitivity, lipoprotein lipase activity, and decreased serum free fatty acids. *Arteriosclerosis, Thrombosis, and Vascular Biology* 2013 **33** 1706–1713. (https://doi.org/10.1161/ATVBAHA.113.301397)

50 Robciuc MR, Tahvanainen E, Jauhiainen M & Ehnholm C. Quantitation of serum angiopoietin-like proteins 3 and 4 in a Finnish population sample. *Journal of Lipid Research* 2010 **51** 824–831. (https://doi.org/10.1194/jlr.M002618)

51 Abu-Farha M, Cherian P, Qaddoumi MG, AlKhairi I, Sriraman D, Alanbaei M & Abubaker J. Increased plasma and adipose tissue levels of ANGPTL8/betatrophin and ANGPTL4 in people with hypertension. *Lipids in Health and Disease* 2018 **17** 35. (https://doi.org/10.1186/s12944-018-0681-0)

52 Aryal B, Price NL, Suarez Y & Fernández-Hernando C. ANGPTL4 in metabolic and cardiovascular disease. *Trends in Molecular Medicine* 2019 **25** 723–734. (https://doi.org/10.1016/j.molmed.2019.05.010)

53 Gusarova V, O'Dushlaine C, Teslovich TM, Benotti PN, Mirshahi T, Gottesman O, Van Hout CV, Murray MF, Mahajan A, Nielsen JB, *et al.* Genetic inactivation of ANGPTL4 improves glucose homeostasis and is associated with reduced risk of diabetes. *Nature Communications* 2018 **9** 2252. (https://doi.org/10.1038/s41467-018-04611-z)

54 Xu A, Lam MC, Chan KW, Wang Y, Zhang J, Hoo RL, Xu JY, Chen B, Chow WS, Tso AW, *et al.* Angiopoietin-like protein 4 decreases blood glucose and improves glucose tolerance but induces hyperlipidemia and hepatic steatosis in mice. *PNAS* 2005 **102** 6086–6091. (https://doi.org/10.1073/pnas.0408452102)

55 van Raalte DH, Brands M, Serlie MJ, Mudde K, Stienstra R, Sauerwein HP, Kersten S & Diamant M. Angiopoietin-like protein 4 is differentially regulated by glucocorticoids and insulin in vitro and in vivo in healthy humans. *Experimental and Clinical Endocrinology and Diabetes* 2012 **120** 598–603. (https://doi.org/10.1055/s-0032-1321864)

56 Ejarque M, Borlaug M, Vilarrasa N, Martinez-Perez B, Llauradó G, Megía A, Helland T, Gutierrez C, Serena C, Folkestad O, *et al.* Angiopoietin-like protein 8/betatrophin as a new determinant of type 2 diabetes remission after bariatric surgery. *Translational Research* 2017 **184** 35.e4–44.e4. (https://doi.org/10.1016/j.trsl.2017.03.001)

57 Gómez-Ambrosi J, Pascual E, Catalán V, Rodríguez A, Ramírez B, Silva C, Gil MJ, Salvador J & Frühbeck G. Circulating betatrophin concentrations are decreased in human obesity and type 2 diabetes. *Journal of Clinical Endocrinology and Metabolism* 2014 **99** E2004–E2009. (https://doi.org/10.1210/jc.2014-1568)

58 Al-Shawaf E, Al-Ozairi E, Al-Asfar F, Al-Beloushi S, Kumari S, Tuomilehto J & Arefanian H. Biphasic changes in angiopoietin-like 8 level after laparoscopic sleeve gastrectomy and type 2 diabetes remission during a 1-year follow-up. *Surgery for Obesity and Related Diseases* 2018 **14** 1284–1294. (https://doi.org/10.1016/j.soard.2018.05.026)

59 Wang W, Li X & Ji D. Molecular mechanism of the effect of angiopoietin-like protein 8 on the proliferation, invasion and migration of placental trophoblasts in preeclampsia. *Experimental and Therapeutic Medicine* 2020 **19** 3461–3468. (https://doi.org/10.3892/etm.2020.8647)

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