

# Thyroid Hormone Levels, even within the Euthyroid Range, are Associated with Lipid Levels



RS Sudharshini<sup>1\*</sup>, A Velayutharaj<sup>2</sup>, Monisha Mohan<sup>3</sup>, Praveen Kumar<sup>4</sup>

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## ABSTRACT

**Background:** Thyroid dysfunction of any magnitude is associated with dyslipidemia. But the relationship of thyroid hormones with lipid parameters among euthyroid population is still unclear.

**Materials and methods:** This is a cross-sectional study to assess relationship between measured [free T3 (fT3), free T4 (fT4), and TSH] and derived (free T3/free T4 ratio, and TSH index) parameters of thyroid profile with different components of lipid profile among euthyroid population.

**Results:** We included 100 patients (60 men and 40 women) in this study. The mean free T3, free T4, and TSH levels of our study population were  $2.4 \pm 0.5$  pg/mL,  $1.2 \pm 0.2$  ng/dL, and  $3.0 \pm 1.6$   $\mu$ IU/mL, respectively. Overall, fT3 had a significant positive correlation with HDL cholesterol ( $r = 0.4, p = 0.01$ ) and a negative correlation with total cholesterol levels ( $r = -0.3, p = 0.04$ ). While fT4 ( $r = 0.3, p = 0.04$ ) and fT3/fT4 ratio ( $r = 0.5, p = 0.001$ ) showed positive correlation only with HDL levels. In subgroup analysis, positive association of fT3 ( $r = 0.6, p = 0.008$ ), fT4 ( $r = 0.4, p = 0.04$ ), and fT3/fT4 ratio ( $r = 0.8, p = 0.001$ ) with HDL cholesterol was significant only in men. And only in the subgroup with TSH  $\geq 3$   $\mu$ IU/mL ( $N = 48$ ), we found a significant negative correlation of fT3 with total cholesterol ( $r = -0.5, p = 0.01$ ) and LDL levels ( $r = -0.7, p = 0.001$ ) and a positive correlation with HDL levels ( $r = 0.5, p = 0.02$ ).

**Conclusion:** Among euthyroid subjects, fT3 seems to have a significant and consistent favorable association with lipid levels, especially with HDL cholesterol. This positive association of fT3 with HDL is more marked in men and in subjects with TSH  $\geq 3$   $\mu$ IU/mL.

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## INTRODUCTION

Thyroid dysfunctions have a profound impact on lipid levels.<sup>1</sup> Thyroid hormones (TH) promote synthesis, mobilization, and degradation of lipids. But their effect on degradation is slightly more pronounced than their effect on synthesis and mobilization.<sup>2</sup> These effects are more pronounced in cases of thyroid dysfunction with higher lipid levels in hypothyroidism and lower lipid levels in hyperthyroidism.<sup>3</sup> These changes are also noticeable in cases of subclinical thyroid dysfunction as well.<sup>4</sup> The impact of thyroid dysfunction on lipid levels is usually reversible with treatment.<sup>5</sup> While the effect of TH and TSH on lipid levels in thyroid dysfunction is well established, their impact on lipid levels among euthyroid population remains controversial.<sup>6-10</sup>

Recently, acquired mild resistance to TH has been postulated as a contributor to metabolic syndrome, including dyslipidemia, in the general population.<sup>11</sup> The resistance to TH can be central (at the level of pituitary) or peripheral (at tissue level). The sensitivity to thyroid hormones can be indirectly assessed through composite indices derived from routine thyroid function tests. The central sensitivity to thyroid hormones can be assessed with the help of TSH index

(TSHI), the Thyroid Feedback Quantile-based Index (TFQI) index, and the thyrotrophic T4 resistance index (TT4RI), while peripheral sensitivity to thyroid hormones can be inferred from the free T3/free T4 ratio (fT3/fT4 ratio).<sup>11,12</sup> Higher values of these indices have been shown to be associated with obesity, diabetes, and hypertension among the euthyroid population in previous studies.<sup>11,13</sup> The possible pathogenic association of thyroid insensitivity with altered lipid metabolism has been highlighted by previous studies showing a positive association of thyroid insensitivity with dyslipidemia and metabolic dysfunction associated with steatotic liver disease (MASLD).<sup>13</sup>

In this study, we tried to assess the potential relationship between TH, TSH, and indices of thyroid sensitivity (fT3/fT4 ratio and TSHI) with different components of lipid profile among the euthyroid population.

## MATERIALS AND METHODS

This cross-sectional observational study was carried out in a tertiary care hospital in Southern India over a period of ten months from March 2023 to December 2023. The study subjects were mostly patients attending our medicine outpatient department. All consenting apparently

healthy adults aged more than 19 years of both sexes were included. Subjects with a previous history of any known thyroid illness were excluded from the study. Subjects who were taking medications that directly or indirectly affect TH (such as steroids, interferons, amiodarone, lithium, etc.) or lipid levels (such as anti-lipid therapies) were also excluded from this study.

In a similar previous study from South India, Gopalakrishnan et al.<sup>14</sup> had shown the correlation coefficient between TSH and triglyceride (TG) to be 0.675 ( $r$ -square = 0.45). Assuming a similar level of correlation coefficient, for a total of eight study variables, the minimum sample size for 80% power at 5% level of significance of the study was calculated to be around 70. We included a total of 100 subjects in our study.

After informed consent from the participants, a detailed case history with demographic data was obtained. A fasting blood sample was collected and centrifuged for serum separation. Separated serum was stored at  $-20^{\circ}\text{C}$ . Serum fT3, fT4, and TSH levels were measured using chemiluminescence immunoassay (CLIA). Fasting lipid profile, including total cholesterol (TC), TG, HDL, LDL, and VLDL cholesterol levels, was measured using a fully automated analyzer (Roche cobas c311-Enzymatic colorimetric method).

The following formulas were employed for calculating indices of TH sensitivity: TSHI =  $\text{Ln TSH } (\mu\text{IU/mL}) + 0.1345 \times \text{fT4 (pmol/L)}$ . TSHI is inversely proportional to central sensitivity to TH (the higher the value of TSHI, the lower the central sensitivity to TH). fT3/fT4 ratio =  $\text{fT3 (pmol/L)/fT4 (pmol/L)}$ . The fT3/fT4

<sup>1</sup>Postgraduate; <sup>2</sup>Head of Department; <sup>3</sup>Associate Professor, Department of Biochemistry; <sup>4</sup>Associate Professor, Department of General Medicine, Trichy SRM Medical College Hospital and Research Centre, Tiruchirappalli, Tamil Nadu, India; \*Corresponding Author

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ratio is directly proportional to peripheral TH sensitivity, indicating a higher ratio corresponds to greater peripheral sensitivity to TH.<sup>15,16</sup>

All statistical analyses were performed using SPSS 25th version software. Mean  $\pm$  standard deviation (SD) was used to express continuous variables. The correlation analysis between lipid levels and TH was done using Pearson's correlation method. All tests were two-tailed, and a *p*-value less than 0.05 was considered statistically significant.

### Ethical Approval

This study was approved by the Ethics Committee of our institution, Trichy SRM Medical College Hospital and Research Centre, vide letter no. LII-IRB-09 dated 05 April 2023.

## RESULTS

We included 100 subjects, with 60 male and 40 female participants, in this study. Table 1 shows the demographic, anthropometric, and biochemical parameters of study participants. We found no significant difference with regard to any of the studied parameters in both sexes. The results of correlation analysis between measured and derived parameters of thyroid function with different parameters of lipids are shown in Table 2. We found a significant positive correlation of HDL cholesterol with fT3 ( $r = 0.4, p = 0.01$ ), fT4 ( $r = 0.3, p = 0.04$ ) and fT3/fT4 ratio ( $r = 0.5, p < 0.001$ ). fT3 also showed a significant negative correlation with TC ( $r = -0.3, p = 0.04$ ). We did not find any correlation between TSH and any of the studied lipid parameters.

We also performed subgroup analysis based on gender and TSH levels. We arbitrarily chose 3  $\mu$ IU/mL as the TSH cut-point for subgroup analysis, as it led to even distribution of subjects in both groups (less than 3  $\mu$ IU/mL = 48 subjects;  $\geq 3$   $\mu$ IU/mL = 52 subjects). Table 3 shows the results of correlation analysis between thyroid and lipid parameters among men versus women. The statistically significant positive correlation of fT3 ( $r = 0.6, p = 0.008$ ), fT4 ( $r = 0.4, p = 0.04$ ), and fT3/fT4 ratio ( $r = 0.8, p < 0.001$ ) with HDL cholesterol was seen only in men, while the positive association did not reach statistical significance among women. We found a significant positive correlation of TSH with TC in men ( $r = 0.7, p < 0.001$ ) and LDL cholesterol in both men ( $r = 0.6, p = 0.001$ ) and women ( $r = 0.5, p = 0.04$ ).

**Table 1:** Demographic, anthropometric, and biochemical parameters of the study population

Parameters	Men (n = 60)	Women (n = 40)	Overall	<i>p</i> -value
Age (years)	48.9 $\pm$ 16.1	51.9 $\pm$ 10.3	50.4 $\pm$ 13.2	0.6
BMI (kg/m <sup>2</sup> )	28.0 $\pm$ 1.0	28.8 $\pm$ 0.8	28.4 $\pm$ 0.9	0.8
Total cholesterol (mg/dL)	158.8 $\pm$ 46.3	176.7 $\pm$ 45.6	166.9 $\pm$ 46.4	0.2
Triglycerides (mg/dL)	152.1 $\pm$ 81.2	162.8 $\pm$ 93.4	157 $\pm$ 86.2	0.7
HDL (mg/dL)	39.6 $\pm$ 17.6	38.8 $\pm$ 12.4	39.2 $\pm$ 15.3	0.9
LDL (mg/dL)	91.3 $\pm$ 43.6	108.7 $\pm$ 36.9	99.2 $\pm$ 41.2	0.2
VLDL (mg/dL)	31.7 $\pm$ 15.8	29.9 $\pm$ 14.7	30.9 $\pm$ 15.2	0.7
Free T3 (pg/mL)	2.5 $\pm$ 0.6	2.4 $\pm$ 0.5	2.4 $\pm$ 0.5	0.4
Free T4 (ng/dL)	1.3 $\pm$ 0.2	1.2 $\pm$ 0.2	1.2 $\pm$ 0.2	0.4
TSH ( $\mu$ IU/mL)	2.9 $\pm$ 1.7	3.2 $\pm$ 1.5	3.0 $\pm$ 1.6	0.6
Free T3/free T4 ratio	2.0 $\pm$ 0.6	2.0 $\pm$ 0.6	2.0 $\pm$ 0.6	0.9
TSH index	1.1 $\pm$ 0.5	1.3 $\pm$ 0.4	1.1 $\pm$ 0.7	0.2

**Table 2:** Results of correlation analysis between measured and derived parameters of thyroid function with different parameters of lipids

Thyroid parameters	Correlation coefficient ( <i>p</i> -value in parentheses)				
	Total cholesterol	Triglyceride	HDL	LDL	VLDL
Free T3 (pg/mL)	-0.3 (0.04)*	-0.1 (0.2)	0.4 (0.01)*	-0.3 (0.1)	-0.2 (0.2)
Free T4 (ng/dL)	-0.2 (0.2)	0.1 (0.7)	0.3 (0.04)*	-0.2 (0.3)	-0.1 (0.5)
TSH ( $\mu$ IU/mL)	0.2 (0.1)	0.04 (0.8)	0.2 (0.1)	0.2 (0.2)	0.1 (0.9)
Free T3/T4 ratio	-0.1 (0.5)	-0.1 (0.4)	0.5 (0.001)*	-0.2 (0.3)	-0.1 (0.7)
TSH index	0.1 (0.7)	0.1 (0.4)	0.1 (0.5)	0.1 (0.6)	0.1 (0.4)

\**p*-value < 0.05

**Table 3:** Results of correlation analysis between thyroid and lipid parameters among men versus women

Thyroid profile	Correlation coefficient ( <i>p</i> -value in parentheses)									
	TC		Triglyceride		HDL		LDL		VLDL	
	Men	women	Men	women	Men	Women	Men	women	Men	women
Free T3	-0.2 (0.3)	-0.3 (0.1)	-0.2 (0.5)	-0.3 (0.3)	0.6 (0.008)*	0.09 (0.7)	-0.2 (0.5)	-0.4 (0.1)	-0.2 (0.5)	-0.3 (0.3)
Free T4	-0.4 (0.1)	0.01 (0.9)	-0.2 (0.3)	0.3 (0.2)	0.4 (0.04)*	-0.2 (0.4)	-0.2 (0.3)	-0.02 (0.9)	-0.2 (0.3)	0.1 (0.7)
TSH	0.7 (<0.01)*	-0.4 (0.07)	0.2 (0.3)	-0.2 (0.4)	0.3 (0.09)	0.1 (0.8)	0.6 (0.01)*	0.5 (0.04)*	0.1 (0.5)	-0.2 (0.4)
fT3/fT4	0.03 (0.9)	-0.3 (0.3)	0.03 (0.9)	-0.3 (0.2)	0.8 (<0.001)*	0.1 (0.6)	-0.1 (0.8)	-0.3 (0.2)	0.03 (0.9)	-0.2 (0.5)
TSH index	0.4 (0.2)	-0.4 (0.2)	-0.2 (0.5)	-0.2 (0.6)	0.4 (0.2)	-0.1 (0.6)	0.4 (0.1)	-0.5 (0.1)	-0.2 (0.5)	-0.1 (0.7)

TC, total cholesterol; fT3/fT4, free T3/free T4 ratio; \**p*-value < 0.05

**Table 4:** Results of correlation analysis between thyroid and lipid parameters between TSH subgroups (< 3 µIU/mL vs ≥ 3 µIU/mL)

Thyroid profile	Correlation coefficient (p-value in parentheses)									
	TC		Triglyceride		HDL		LDL		VLDL	
	< 3	≥ 3	< 3	≥ 3	< 3	≥ 3	< 3	≥ 3	< 3	≥ 3
Free T3	-0.1 (0.6)	-0.5 (0.01)*	-0.3 (0.3)	-0.2 (0.4)	0.3 (0.2)	0.5 (0.02)*	0.04 (0.9)	-0.7 (0.001)*	-0.3 (0.2)	-0.2 (0.4)
Free T4	-0.1 (0.5)	-0.3 (0.2)	0.4 (0.1)	-0.1 (0.5)	-0.3 (0.2)	-0.3 (0.1)	-0.3 (0.3)	-0.1 (0.7)	-0.1 (0.9)	-0.1 (0.5)
TSH	0.2 (0.3)	0.4 (0.1)	0.2 (0.4)	0.4 (0.1)	0.1 (0.6)	0.3 (0.1)	0.2 (0.3)	0.2 (0.5)	-0.1 (0.6)	0.4 (0.1)
ft3/ft4	-0.1 (0.8)	-0.2 (0.5)	-0.4 (0.1)	0.1 (0.8)	0.4 (0.1)	0.6 (0.003)*	0.1 (0.6)	-0.4 (0.1)	-0.3 (0.2)	0.1 (0.8)
TSH index	0.1 (0.8)	0.3 (0.1)	0.3 (0.2)	0.5 (0.03)*	0.01 (0.9)	0.2 (0.3)	0.1 (0.8)	0.1 (0.6)	0.2 (0.5)	0.5 (0.02)*

TC, total cholesterol; ft3/ft4, free T3/free T4 ratio; \*p-value < 0.05

Table 4 shows the results of the correlation analysis between thyroid and lipid parameters across TSH subgroups (< 3 µIU/mL vs ≥ 3 µIU/mL). Again, ft3 showed statistically significant positive correlation with HDL ( $r = 0.5, p = 0.02$ ) and negative correlation with TC ( $r = -0.5, p = 0.01$ ) and LDL ( $r = -0.7, p = 0.001$ ) in TSH ≥ 3 µIU/mL group. While ft3/ft4 ratio showed a significant positive correlation with HDL ( $r = 0.6, p = 0.003$ ) in TSH ≥ 3 µIU/mL group. TSH index showed positive correlation with TG ( $r = 0.5, p = 0.03$ ) and VLDL ( $r = 0.5, p = 0.02$ ) in the subgroup with TSH ≥ 3 µIU/mL. There was no association noted between any of the studied thyroid and lipid parameters in the TSH < 3 µIU/mL group.

## DISCUSSION

Overall, ft3, ft4, and ft3/ft4 ratio show consistent positive association with HDL cholesterol, especially in men and subjects with TSH ≥ 3 µIU/mL. FT3 also showed significant negative correlation with total cholesterol.

TH plays a vital physiological role in regulating the production, transport, and breakdown of lipids. They impact lipid metabolism by binding to TH receptors (THR), mainly THRβ isoforms, which are highly expressed in liver. TH directly enhances lipid synthesis in liver by inducing the expression of rate-limiting enzyme in cholesterol synthesis, HMG-CoA reductase (HMGCR).<sup>17</sup> They can also promote lipogenesis indirectly by enhancing expression of transcription factors involved in lipid synthesis such as carbohydrate response element-binding-protein (ChREBP) and the sterol regulatory element-binding protein 1c (SREBP1c), etc.<sup>18</sup> On the other hand, TH promotes catabolism of TG in white adipose tissue as well as in liver by promoting activity of adipose triglyceride lipase and hepatic lipase, respectively. TH also upregulates expression of key enzymes involved in fatty acid β-oxidation in the liver, such as carnitine O-palmitoyl transferase 1 liver isoform (CPT1-Lα), pyruvate dehydrogenase kinase isoform 4 (PDK4), medium-chain acyl-CoA

dehydrogenase (MCAD), and uncoupling protein 2 (UCP2).<sup>19</sup> TH also plays a vital role in the turnover of serum cholesterol levels via synthesis and export of cholesterol in the form of LDL from liver, reverse transporting cholesterol from peripheral tissues, reuptake of cholesterol via hepatic LDL receptors and conversion of cholesterol into bile acids for excretion into the intestine.<sup>20</sup>

Previous studies have shown contradictory and inconsistent impact of TH and TSH on lipid profile in euthyroid adults. Most studies have focused only on TSH and ft4. Only very few studies have included ft3 in the analysis. TSH has shown a consistent positive association with TC, TG, and LDL cholesterol in previous studies.<sup>9,10</sup> Even TSH levels in the high normal range have been shown to be associated with dyslipidemia and other components of metabolic syndrome among euthyroid subjects.<sup>10,21,22</sup> The causality of these findings has been proven in a few Mendelian randomization studies as well.<sup>23,24</sup> Overall, we found no association of TSH with any of the lipid parameters in this study. But we did find a positive association of TSH with LDL in both sexes and with TC in men alone.

After TSH, ft4 has been shown consistently to impact lipid parameters among euthyroid adults in previous studies. Most studies have found a negative association of ft4 with TC, TG, and LDL.<sup>6-10</sup> Low normal ft4 has been shown to be associated with atherogenic lipid profile,<sup>7,10</sup> insulin resistance<sup>8</sup> and components of metabolic syndrome.<sup>6</sup> Lower ft4 among euthyroid subjects has shown a causal association with increased TC and LDL in a Mendelian randomization study.<sup>24</sup> We did not observe any association of ft4 with TC, TG, and LDL cholesterol overall, as well as in subgroup analysis. But ft4 showed a positive association with HDL cholesterol, especially in men. Only few studies have shown a similar association of ft4 with HDL,<sup>8,10</sup> while others have failed to show any association.

ft3 is the only thyroid parameter in our study that showed a consistent positive association with HDL, more so in men and in euthyroid subjects with TSH ≥ 3 µIU/mL. We

also found a negative correlation of ft3 with TC. These findings contradict results from previous studies, which usually found high ft3 to be associated with an unfavorable lipid profile characterized by lower HDL and higher TC.<sup>9,10</sup> The difference in ethnicity and anthropometric characteristics of our study population could be the reason for this contradictory observation. Our study subjects had higher body mass index (BMI) compared to previous studies, and BMI has been found to be a major confounding factor in assessing the association of thyroid parameters with lipids in euthyroid subjects.<sup>10</sup>

Another interesting finding of our study is that HDL seems to be the most affected lipid parameter, showing consistent association with most thyroid parameters, unlike previous study results.<sup>6-10</sup> Though TH significantly impacts HDL metabolism, they show inconsistent association with HDL levels because TH affects both synthesis and catabolism of HDL cholesterol.<sup>25</sup> TH strongly promotes reverse cholesterol transport. TH increases cholesterol efflux from peripheral tissues and macrophages to HDL by inducing the expression of ApoA1 and ATP-binding cassette transporter A1 protein, respectively. On the other hand, TH promotes HDL degradation by stimulating hepatic lipase.<sup>26</sup>

We calculated the ft3/ft4 ratio and TSHI to assess peripheral and central TH sensitivity, respectively. Overall, we observed a significant positive association of the ft3/ft4 ratio with HDL levels. This association was noted only among men and subjects with TSH ≥ 3 µIU/mL in subgroup analysis. Similar findings were noted in some studies where a higher ft3/ft4 ratio was associated with a favorable lipid profile,<sup>16,27</sup> while other studies have shown contradictory results.<sup>9,11-13</sup> Subjects with established dyslipidemia-associated comorbidities, such as morbid obesity, NAFLD, usually have higher ft3 and ft3/ft4 ratio. The increase in ft3 in these conditions is generally regarded as a failed adaptive response. This may explain the association of the ft3/ft4 ratio with unfavorable lipid profiles in studies where the participants had advanced

diseases, such as morbid obesity or NAFLD.<sup>12,13</sup> Unlike the fT3/fT4 ratio, TSHI has shown a consistent association with an unfavorable lipid profile in previous studies.<sup>16</sup> In our study, TSHI showed a positive correlation with TG and VLDL cholesterol in the subgroup with TSH  $\geq 3$   $\mu$ U/mL.

In subgroup analysis, most observed associations between thyroid and lipid levels were seen only in men. One plausible explanation for this gender difference could be sex hormone-related discrepancy in the TSH-fT3 feedback and differential effects of sex hormones on TH metabolism.<sup>28</sup> In our study, the majority (97%) were premenopausal women. All the noted significant associations of thyroid parameters with lipid levels were significant only in subjects with TSH  $\geq 3$   $\mu$ U/mL in our study. We hypothesize that subjects with TSH  $\geq 3$   $\mu$ U/mL may have mild acquired TH resistance and TH may impact lipid levels significantly only in these subjects.

Our study had a few limitations. Thyroid autoantibody levels were not measured, and previous studies have shown that antithyroid peroxidase antibody status could compound association of thyroid parameters with lipid levels among euthyroid subjects.<sup>7</sup> Our findings need validation in further large-scale studies. Association does not imply causation, as is the case with any observational research. We could not perform logistic regression analysis due to small sample size of our study.

## CONCLUSION

To conclude, we found a significant association of thyroid parameters with lipid levels, especially with HDL cholesterol. We observed higher fT3, higher fT4, higher fT3/fT4 ratio, lower TSH, and lower TSHI associated with an unfavorable lipid profile, characterized by higher TC, TG, LDL, VLDL, and lower HDL levels. Resmetirom, an oral, liver-directed, thyroid hormone receptor beta-selective agonist, has recently been approved for treatment of adults with non-alcoholic steatohepatitis (NASH) with moderate to advanced liver scarring (fibrosis).<sup>29</sup> Similarly, in the future, hepato-selective T3 or T4 analogs could be employed for treating dyslipidemia, considering their intimate association with lipid parameters.

## SOURCE OF SUPPORT

Nil.

## CONFLICTS OF INTEREST

None.

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## AUTHORS' CONTRIBUTION

SRS was responsible for patient management, data analysis, manuscript drafting, and revisions. VA handled analysis, manuscript drafting, and revisions. MM and PK managed patients, collected and analyzed data, and drafted and revised the manuscript.

## ORCID

RS Sudharshini  <https://orcid.org/0009-0001-2245-0577>

Monisha Mohan  <https://orcid.org/0000-0003-0235-4163>

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