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Short Communication

# Association between thyroid hormones and TRAIL

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## ABSTRACT

*Introduction:* Recent studies suggest that a circulating protein called TRAIL (TNF-related apoptosis-inducing ligand) might have a role in the regulation of body weight and metabolism. Interestingly, thyroid hormones seem to increase TRAIL tissue expression. This study aimed at evaluating whether overt thyroid disorders affected circulating TRAIL levels.

*Methods:* TRAIL circulating levels were measured in euthyroid, hyperthyroid, and hypothyroid patients before and after thyroid function normalization. Univariate and multivariate analyses were performed to evaluate the correlation between thyroid hormones and TRAIL. Then, the stimulatory effect of both triiodothyronine (T3) and thyroxine (T4) on TRAIL was evaluated *in vitro* on peripheral blood mononuclear cells.

*Results:* Circulating levels of TRAIL significantly increased in hyperthyroid and decreased in hypothyroid patients as compared to controls. Once thyroid function was restored, TRAIL levels normalized. There was an independent association between TRAIL and both fT3 and fT4. Consistent with these findings, T3 and T4 stimulated TRAIL release *in vitro*.

*Conclusion:* Here we show that thyroid hormones are associated with TRAIL expression *in vivo* and stimulate TRAIL expression *in vitro*. Given the overlap between the metabolic effects of thyroid hormones and TRAIL, this work sheds light on the possibility that TRAIL might be one of the molecules mediating thyroid hormones peripheral effects.

## 1. Introduction

TRAIL, which is an acronym for TNF-related apoptosis inducing ligand, is the name of a protein belonging to the TNF superfamily, which was discovered in 1995 on the basis of its high homology to other TNF family members, such as FasL/CD95L and TNF-α [1]. TRAIL is widely expressed in different cells and tissues [1,2] as a type 2 transmembrane protein, which is usually cleaved to form a circulating protein. Be it in the native or processed form, TRAIL's main function is to activate the extrinsic apoptotic pathway upon binding to its specific receptors (TRAIL-R1 and TRAIL-R2), and to cause cell death [3]. Interestingly, as compared to the other pro-apoptotic TNF family members, TRAIL has the unique ability to induce apoptosis preferentially in transformed cells, such as tumor cells, while it spares the normal ones [3]. The discovery of this property has led to the current study of TRAIL as an anticancer therapy [4,5].

Beside its anticancer potential, recent studies suggest that TRAIL has also significant metabolic effects, such that it could be a potential candidate for the treatment of obesity and its associated diseases [6–9]. In particular, *in vivo* studies have shown that high-fat diet-fed mice put on more weight if they were TRAIL deficient [6], and that TRAIL delivery significantly reduced the amount of fat mass in high-fat diet-fed mice [7]. In addition, *in vitro* studies have shown that TRAIL-R2 activation blocked *de novo* lipogenesis in human adipocytes [10] and that TRAIL treatment inhibited adipocyte differentiation [9].

Interestingly, experimental evidence suggests that thyroid hor-

*Abbreviation:* BMI, body mass index; CLEIA, chemiluminescence enzyme immunoassay; CNT, controls; CRP, C-reactive protein; CV, coefficient of variation; ELISA, enzyme-linked immunosorbent assay; FFA, free fatty acids; fT3, free triiodothyronine; fT4, free thyroxine; HDL-C, high-density lipoprotein cholesterol; HYPER, hyperthyroid patients; HYPO, hypothyroid patients; OPG, osteoprotegerin; PBMC, peripheral blood mononuclear cells; PBS, phosphate buffered saline; TC, total cholesterol; TG, triglycerides; TG-Ab, anti-thyroglobulin antibodies; TPO-Ab, anti-thyroperoxidase antibodies; TRAIL, TNF-related apoptosis-inducing ligand; TSH, thyroid-stimulating hormone; TSHr-Ab, anti-TSH receptor antibodies

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mones [11,12] influence TRAIL tissue expression. The group of Chi and colleagues has demonstrated that thyroid hormones induced TRAIL transcription in hepatoma cell lines [11]. In addition, it has also been reported that TRAIL gene expression was upregulated in the skeletal muscle by levothyroxine replacement therapy [12]. Nevertheless, it remains to be established whether thyroid hormones can affect circulating TRAIL levels. The aim of the present study was to evaluate the effect of overt thyroid disorders (hyperthyroidism and hypothyroidism) on circulating TRAIL levels.

## 2. Methods

### 2.1. Study population

A total of 39 euthyroid (CNT), 49 hyperthyroid (HYPER), and 28 hypothyroid (HYPO) patients were consecutively selected over a period of 12 months (January 2013–January 2014) from the subjects referring to the Endocrine Service of Cattinara Hospital (Azienda Ospedaliero-Universitaria di Trieste). Euthyroidism, hyperthyroidism, and hypothyroidism were defined biochemically. Euthyroid patients were patients who had undergone thyroid hormone measurement as a part of their medical workup carried out in the Endocrine Service, and who did not result affected by any thyroid dysfunction, such that they were selected as age and sex-matched controls. By contrast, hyperthyroid patients had lower TSH and higher fT3 and fT4, while hypothyroid patients had higher TSH and lower fT3 and fT4 values as compared to reference ranges. Exclusion criteria were: age below 18 or above 80 years, diabetes, dyslipidemia, cardiopulmonary, renal, and hepatic failure. After the first visit, all the subjects were asked to give their written informed consent to participate in this study, whose protocol had been previously approved by the Institutional Ethics Committee of AOUTS.

### 2.2. Study protocol

At presentation, and before starting any specific treatment, euthyroid (CNT), hyperthyroid (HYPER before), and hypothyroid patients (HYPO before) underwent a medical visit and a blood sampling. The clinical and laboratory parameters recorded are reported in Table 1. Then, the appropriate medical treatments were prescribed to the patients with thyroid disorders, and subsequent follow-up visits were scheduled (generally monthly for hyperthyroid and quarterly for hypothyroid patients), to check on their thyroid function. Once euthyroidism was restored, patients underwent a second medical visit and blood sampling (HYPER after and HYPO after). Blood sampling was performed at 08.00 a.m., after an overnight fasting.

### 2.3. Clinical laboratory

TSH, fT3, fT4, TG-Ab, TPO-Ab were measured by chemiluminescence (CLEIA; tracer alkaline phosphatase Lumiphos 530) with a DxI800 analyzer (Beckman Coulter, Fullerton, CA) [13]. TSHr-Ab were measured by ELISA (Alisei, Omnia Diagnostica, CT, Italy). Thyroid hormone assay precision (within-run and between-day) was calculated by daily quality controls (Bio-Rad Immunoassay Plus, Hercules, CA), as summarized in Supplementary material (Table 1). Thyroid hormone reference ranges were 0.4 to 4 μIU/mL for TSH; 7.20 to 15.44 pmol/L for fT4; and 3.0 to 6.9 pmol/L for fT3. These reference ranges have been calculated by our central laboratory on our local population, as recommended by standard published procedures [14]. Glucose, TC, TG, and HDL-C (enzymatic colorimentric method) and CRP (immunoturbidimetric method) were measured with an AU5800 analyzer (Beckman Coulter, Fullerton, CA). All these laboratory data were verified by external proficiency testing (CRB-Centro Ricerca Biomedica-, Padova, Italy and RIQAS, Randox Laboratories LtD). As for TRAIL and OPG (which is the soluble decoy receptor for TRAIL), they were measured by a solid-phase sandwich ELISA (#DTRL00 and #DY805; R & D Systems, Minneapolis, MN) in the sera, as previously reported [15,16]. The CV of TRAIL assay is reported in Supplementary Table 1.

### 2.4. In vitro study

PBMC were isolated from 8 blood donors following their informed consent. Briefly, blood samples were collected at fasting and diluted with 2 volumes of cold PBS (Sigma-Aldrich, Milan, Italy), layered on Ficoll (Sigma-Aldrich), and centrifuged for 20 min at 400 × *g* at 4 °C. The mononuclear cell layer obtained after centrifugation was harvested, washed with PBS, and PBMC ($1 \times 10^6$ cells/mL) were cultured in RPMI 1640 (Sigma-Aldrich) supplemented with 10% FCS (Invitrogen) in 24-well plates at 37 °C in 5% $CO_2$. Cells were treated

**Table 1**
Patients' characteristics.

| Parameter | CNT ($n$ = 39) | HYPER before ($n$ = 49) | HYPER after ($n$ = 49) | HYPO before ($n$ = 28) | HYPO after ($n$ = 28) |
|---|---|---|---|---|---|
| Age (years) | 54.8 ± 2.3 | 50.1 ± 2.5 | = | 61.9 ± 3.0[#] | = |
| Sex (F/M) | 29/10 | 39/10 | 39/10 | 23/5 | 23/5 |
| BMI (kg/m$^2$) | 24.6 ± 0.8 | 24.4 ± 0.8 | 26.1 ± 1.0 | 26.8 ± 0.9 | 25.6 ± 1.1 |
| TSH (μIU/mL) | 1.4 ± 0.1 | 0.0 ± 0.0[*] | 2.4 ± 0.3[#] | 68.2 ± 5.7[*,#] | 2.8 ± 0.6[†] |
| fT3 (pmol/L) | 4.6 ± 0.1 | 16.7 ± 13.1[*] | 4.3 ± 0.1[#] | 2.8 ± 0.2[*,#] | 4.2 ± 0.1[†] |
| fT4 (pmol/L) | 11.5 ± 0.5 | 43.6 ± 2.5[*] | 11.1 ± 0.8[#] | 4.1 ± 0.5[*,#] | 13.6 ± 1.0[†] |
| TSHr-Ab (U/L) | 0.7 ± 0.5 | 10.9 ± 3.2 | 4.6 ± 2.0 | 4.2 ± 3.3 | 3.8 ± 3.7 |
| TG-Ab (U/L) | 33.4 ± 14.4 | 237.4 ± 42.1 | 55.2 ± 51.0 | 938.8 ± 240.1[*,#] | 203.7 ± 123.6 |
| TPO-Ab (U/L) | 127.7 ± 69.5 | 216.1 ± 41.9 | 203.4 ± 125.5 | 550 ± 101.1[*,#] | 420.8 ± 195.1 |
| CRP (mg/L) | 4.6 ± 1.6 | 2.9 ± 1.3 | 1.9 ± 0.5 | 2.9 ± 1.3 | 3.1 ± 1.2 |
| Glucose (mM) | 5.2 ± 0.2 | 5.5 ± 0.1 | 5.3 ± 0.2 | 5.3 ± 0.3 | 5.4 ± 0.1 |
| TC (mg/dL) | 5.5 ± 0.1 | 4.3 ± 0.2[*] | 5.5 ± 0.2[#] | 6.4 ± 0.3[*,#] | 5.1 ± 0.2[†] |
| HDL-C (mg/dL) | 1.6 ± 0.1 | 1.3 ± 0.0[*] | 1.6 ± 0.1[#] | 1.6 ± 0.1[#] | 1.7 ± 0.1 |
| TG (mg/dL) | 1.0 ± 0.1 | 1.0 ± 0.1 | 1.2 ± 0.1 | 1.7 ± 0.2[*,#] | 1.3 ± 0.1 |
| OPG (pg/mL) | 541.5 ± 60.1 | 900.4 ± 72[*] | 514.8 ± 69.3[#] | 926.5 ± 133.9[*] | 461.2 ± 41.3[†] |

Data are expressed as mean ± SEM. CNT is for control; HYPER before is for hyperthyroid patients before treatment; HYPER after is for hyperthyroid patients after treatment; HYPO before is for hypothyroid patients before treatment; HYPO after is for hypothyroid patients after treatment; BMI is for body mass index; TSH is for thyroid stimulating hormone; fT3 is for free triiodothyronine; fT4 is for free thyroxine; TSHr-Ab is for anti-TSH receptor antibodies; TG-Ab is for anti-thyroglobulin antibodies; TPO-Ab is for anti-thyroperoxidase antibodies; CRP is for C-reactive protein; TC is for total cholesterol; HDL-C is for high-density lipoprotein cholesterol; TG is for triglycerides.

[*] $p < 0.05$ *vs* CNT.

[#] $p < 0.05$ *vs* HYPER before.

[†] $p < 0.05$ *vs* HYPO before.