The relationship between diabetes mellitus (DM) and thyroid hormones (TH) is a complex one. It is known that TH levels affect not only glycaemic control but also hepatic and muscular insulin resistance and play a major role in energy homeostasis [1]. Both hypothyroidism and thyrotoxicosis have been shown to increase insulin resistance [2]. Decompensated hyperthyroidism can worsen glycaemic control and predispose patients to diabetic ketoacidosis [3].

On the other hand, patients with DM may show abnormalities in serum TH levels in the absence of intrinsic disease of the thyroid axis. These are similar to those present in critically ill patients with the non-thyroidal illness syndrome (NTIS), such as low serum triiodothyronine (T3) and thyroxine (T4) levels and elevated reverse T3 (rT3), with concurrent normal serum TSH levels [4, 5]. This finding is more frequent in those with poor glycaemic control, especially when HbA1c levels are above 10.5-11% [6] and are reversible upon restoration of adequate metabolic control [7]. Although classically related to glycaemic control, the pathophysiology of TH abnormalities in patients with DM has never been fully investigated.

Previous reports highlight the role of systemic inflammation in the genesis of both NTIS in the critically ill [8, 9] and type 2 diabetes mellitus (T2DM) [10, 11]. Therefore, we hypothesized that: 1) thyroid hormone
levels would be significantly different in non-critically ill patients with T2DM, compared to control subjects; 2) body mass index, central adiposity and low grade systemic inflammation in patients with T2DM would be associated with changes in thyroid hormone levels and metabolism, similar to those present in NTIS. The mechanisms underlying the non-thyroidal illness syndrome are poorly understood, and providing information on this topic would help fill a gap in the literature.

**Subjects and Methods**

To test our hypothesis we set up a case-control study involving 104 euthyroid subjects, of which 52 were patients with T2DM followed in the Diabetes Clinic of our University Hospital. The other 52 subjects were individuals without DM who constituted a control group paired by age, gender and body mass index (BMI), selected among the same social-economical strata. All patients and individuals without DM were from an iodine sufficient area. Subjects between 40 and 75 years of age fitting the exclusion criteria were selected if willing to participate in the study during routine appointments at the outpatient clinic.

Exclusion criteria were previous history of thyroid disease, thyroid nodules, use of levothyroxine, acute illnesses, history of illnesses associated with a pro-inflammatory state (i.e. inflammatory and/or auto-immune conditions) and diseases associated with NTIS such as renal impairment (creatinine clearance < 60mL/min/m²), hepatic failure (abnormal coagulation tests or low albumin levels), heart failure (stage 3 and 4 according to NYHA classification), chronic obstructive pulmonary disease, cancer and history of acute myocardial infarction and stroke less than 6 months previously. Patients using medications known to alter thyroid hormone levels (amiodarone, corticosteroids, methimazole and propilthiouracil) and those who were exposed to iodine containing contrasts less than a year previously were also excluded.

Clinical data were recorded by chart review, and consisted of: age, gender, time since diabetes diagnosis, therapy (oral drugs, insulin alone or combination), waist circumference, BMI, presence of hypertension (defined by treatment of this condition or a previous record of blood pressure > 130/80mmHg) smoking habit, and microvascular complications (nephropathy, neuropathy, retinopathy).

Laboratory measurements consisted of fasting glycaemia, HbA1c, serum TSH, total (T) and free (F) T4, total and free T3, rT3 and high sensitivity C-reactive protein (hs-CRP). A morning blood sample was collected from an antecubital vein, immediately processed and the serum stored at -80°C for subsequent analysis.

TSH and FT4 were measured by electro-chemiluminescence (Roche Hitachi-Elecsys Cobas, USA - reference values (RV): TSH: 0.45-4.5mIU/L; FT4: 0.9-1.8ng/dL). Total T4 (RV: 86-187ng/dL), TT3 (RV: 1.4-4.4pg/mL) and rT3 (RV: 0.09-0.350ng/mL) were measured by radioimmunoassay (Siemens Medical Solutions Diagnostics, Los Angeles, USA). All intra and inter-assay coefficient of variation were < 10%. Conversion from mass to SI units: TT4: µg/dL x 12.87 = nmol/L; FT4: ng/dL x 12.87 = pmol/L; TT3: ng/dL x 0.01536 = nmol/L; FT3: pg/mL x 1.536 = pmol/L; glucose: mg/dL ÷ 18 = mmol/L. The ratios FT3/FT4, FT3/rT3 and FT4/rT3 were analysed by simple division of the hormonal results.

High-sensitivity CRP was measured by ELISA (Cusabio Biotech Co, Ltd, Wuhan, China). Fasting glycaemia was analysed using the hexokinase method (RV: 70-100mg/dL) and HbA1c by high-performance liquid chromatography (RV: 4.0-5.7%). Anti-thyroperoxidase and anti-thyroglobulin antibodies were measured by electro-chemiluminescence (Roche Hitachi-Elecsys Cobas, USA) and RV were <115IU/mL and <34IU/mL, respectively.

The study was approved by the University Ethics in Research Committee and conducted according to the Declaration of Helsinki. Written informed consent was obtained from each patient or subject after full explanation of the purpose and nature of all procedures.

**Statistical Methods**

We conducted a power analysis in a pilot study involving 10 patients with diabetes to determine the minimum sample size required to detect a 10ng/mL difference in TT3 levels and 0.2pg/mL difference in FT3 levels. The standard deviation found in this pilot study for TT3 and FT3 were 23ng/dL and 0.21pg/mL, respectively. Thus, we estimated that at least 45 patients for TT3 and 40 patients for FT3 would be required in each group.

Descriptive analysis of clinical and laboratorial parameters was done by measurement of position and dispersion for continuous variables and by frequency tables for categorical variables.

Comparison of serum thyroid hormone concentra-
Thyroid hormone abnormalities in T2DM were on oral therapy alone. Participants’ characteristics are shown in Table 1.

Over 70% of patients with T2DM presented low TT3, 12% showed low FT3 and almost 10% elevated rT3 levels. In the control group, these prevalences were 40%, 2% and 0, respectively. Frequencies of impaired TH levels are shown in Table 2.

Comparative analysis between individuals with type 2 diabetes and control subjects
Both groups were paired by age, gender and BMI (Table 1). Patients with T2DM presented larger waist circumference than subjects without DM (106 vs 98cm; \(p=0.04\)). Prevalence of hypertension (78.8% vs 17.3%; \(p<0.001\)) and smoking (11.5% vs 0%; \(p=0.027\)) were higher in patients with T2DM than in the control group. Additionally, patients with diabetes had higher serum levels of hs-CRP (1.53 vs 0.73 mg/dL; \(p<0.001\)).

Patients with diabetes had significantly lower levels of TT3 (73.28 vs 89.97ng/dL; \(p<0.001\)), FT3 (1.85 vs 2.45pg/mL; \(p<0.001\)) and TT4 (6.35 vs 7.11μg/dL; \(p=0.006\)). Furthermore, patients with T2DM had higher levels of FT4 (1.30 vs 1.11ng/dL; \(p<0.001\)). There were no differences in serum TSH (\(p=0.341\)) and rT3 levels (\(p=0.871\)).

There were significant differences between patients with T2DM and the control group for the ratios FT3/rT3 (8.66 vs 11.5; \(p=0.001\)) and TT3/FT4 (1.36 vs 2.03; \(p<0.001\)). The ratio FT4/rT3 was similar in both groups (\(p=0.172\)) as shown in Table 2.

Associations between obesity, low-grade systemic inflammation and thyroid hormones in patients with type 2 diabetes
In the group of patients with diabetes, there was an inverse correlation between BMI and FT4 (\(r = -0.291; p=0.036\)) and BMI and TT3 (\(r = -0.365; p=0.008\)). There was no correlation between TSH and BMI. TT3

<table>
<thead>
<tr>
<th>Table 1 Participants’ characteristics</th>
<th>Patients with T2DM (n=52)</th>
<th>Control group (n=52)</th>
<th>(p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>59.5 (54-64)</td>
<td>56 (51-62)</td>
<td>0.22</td>
</tr>
<tr>
<td>Gender (women/men)</td>
<td>34/18 (65.4/34.6)</td>
<td>34/18 (65.4/34.6)</td>
<td>1</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>30.9 (26.3-35.1)</td>
<td>29.6 (26.4-33.1)</td>
<td>0.51</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>104 (91.3-112)</td>
<td>98 (88-106.8)</td>
<td>0.047</td>
</tr>
<tr>
<td>Smoking</td>
<td>6 (11.5)</td>
<td>0</td>
<td>0.027</td>
</tr>
<tr>
<td>Hypertension</td>
<td>41 (78.8)</td>
<td>9 (17.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>hs-CRP (mg/dL)</td>
<td>1.53 (0.72-2.90)</td>
<td>0.73 (0.40-1.56)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data is shown as median (interquartile range) or number (percentage).
T2DM, type 2 diabetes mellitus; hs-CRP, high sensitivity C-reactive protein
was inversely correlated to waist circumference ($r = -0.285; p=0.04$).

Additionally, hs-CRP was positively correlated with waist circumference ($r = 0.334; p=0.019$) and rT3 ($r = 0.452; p=0.001$); and inversely with FT3/rT3 ($r = -0.338; p=0.014$) and FT4/rT3 ($r = -0.476; p<0.001$) ratios. There was no significant correlation between hs-CRP and BMI or HbA1c. Correlations between hormonal values, hs-CRP and clinical parameters are summarized in Table 3.

When separated by hs-PCR quartiles, patients with T2DM showed differences in rT3 ($p=0.018$), FT3/rT3 ($p=0.49$) and FT4/rT3 ($p=0.009$) as shown in Fig.1.

Glycaemic control, represented by HbA1c, did not present significant correlations with TH levels and no differences were found in TH when separating patients by HbA1c quartiles.

### Linear regression analysis for thyroid hormone levels in patients with type 2 diabetes

The multiple linear regression model included log(hs-CRP) and BMI adjusting for age, diabetes duration, waist circumference and log(HbA1c). BMI was a significant predictor for TT3 ($B = -1.118$, 95%CI = -1.836 – -0.399; $p=0.003$) and FT4 levels ($B = -0.011$, 95%CI = -0.21 – -0.001; $p=0.029$). Systemic inflammation, represented by hs-CRP, was a significant predictor for log(FT4/rT3) ($B = -0.190$, 95%CI = -0.291 -- -0.089; $p<0.001$). For log(rT3), the analysis yielded log(hs-CRP) ($B = 0.235$, 95%CI = 0.123-0.347; $p<0.001$) and BMI ($B = -0.008$, 95%CI= -0.16 – -0.001; $p=0.047$) as significant predictors. Table 4 summarizes regression analysis for BMI and hs-CRP.
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showing low levels of T3 and T4 in patients with DM [5, 6, 12, 13]. Interestingly, 40% of the individuals in the control group and almost three quarters of patients with T2DM presented low TT3 levels. We believe this could be due to the old age and high BMI of the participants. Age and weight gain, especially the central distribution of fat have been associated with higher insulin levels, which could lead to lower levels of thyroid

Discussion

In this study, as was expected, patients with T2DM presented lower serum levels of thyroid hormones than the control group paired by age, gender and BMI. Almost 15% of patients had low FT3 levels simultaneously with normal TSH levels, a finding characteristic of NTIS. This result is in line with previous works showing low levels of T3 and T4 in patients with DM [5, 6, 12, 13]. Interestingly, 40% of the individuals in the control group and almost three quarters of patients with T2DM presented low TT3 levels. We believe this could be due to the old age and high BMI of the participants. Age and weight gain, especially the central distribution of fat have been associated with higher insulin levels, which could lead to lower levels of thyroid
hormone binding globulin [14]. In contrast, FT3 was low in 23% of patients with T2DM compared to only 2% in the control group. Therefore, we believe TT3 alone could overestimate the prevalence of NTIS in these patients, and free hormone levels would better reflect the low T3 state.

Although patients with T2DM did not present differences in rT3 levels compared to the control group, 5 patients in the T2DM group and none in the control group had high rT3. More importantly, the ratios of FT3/rT3 and FT4/rT3 were lower, suggesting that inactivation of FT3 to rT3 was higher and conversion from T4 to biologically active T3 was lower. This could be an explanation of why patients with T2DM showed higher FT4 levels. Abnormalities in deiodinase activity could be implicated in the abovementioned differences. Lower type 2 deiodinase (D2) activity could partially explain the lower serum FT3 and higher FT4 levels encountered in patients with T2DM. Moreover, increased activity of this enzyme in the pituitary, already reported in critically ill patients and in cases of renal failure, could render the gland euthyroid, thus explaining the lack of rise in TSH levels, as would be expected in cases of diminished serum T3 levels [15, 16].

Furthermore, inflammation has been shown to induce D3 activity [17, 18] and consequently increase the direct inactivation of T4 to rT3, which would account for the lower FT4/rT3 ratio in our patients. Pro-inflammatory cytokines have also been implicated in hypothalamic suppression of thyroid axis in the critically ill and are considered an important contributor to the inappropriately normal TSH levels [19].

Our study has demonstrated an association between TH and low-grade, subclinical inflammation. In patients with T2DM and insulin resistance, the source of pro-inflammatory cytokines is mainly the visceral adipose tissue [20, 21]. Therefore, we hypothesized that the adipose tissue-generated inflammation present in obese patients could be an important etiological factor for abnormal TH levels. In this study, patients with diabetes presented larger waist circumference, which was positively correlated with hs-CRP. Waist circumference was inversely correlated with TT3. At the same time, hs-CRP levels were positively associated with serum rT3, and BMI was inversely correlated with TT3 and FT4 levels. When adjusted for other possible confounders such as age, diabetes duration, waist circumference and HbA1c levels, BMI and hs-CRP were independent predictors for the serum levels of FT4, TT3, rT3 and the ratio of FT4/rT3. The R² analyses from our regression models suggest that BMI and hs-CRP were responsible for 10-25% of the variation in TH levels. Thus, although not representing the major role in these imbalances, the part of obesity and inflammation is significant enough to be clinically meaningful.

In critically ill patients as well as those under outpatient care, NTIS has been associated with increased pro-inflammatory markers [9, 22, 23]. Pro-inflammatory cytokines such as tumour necrosis factor-α [24], interleukin-1 [25] and 6 (IL-6) [26] have a major role in thyroid hormone imbalances. Serum IL-6 levels are often increased in NTIS and have been inversely correlated with T3 levels and TSH suppression [27]. The C-reactive protein is an inflammatory protein used in clinical decision-making produced by the liver in response to IL-6 [20].

Similar results regarding the low T3 state found in this study have been reported in subjects with type 1 diabetes, and also associated to glycaemic control [28]. It is unclear, however, if the same associations found in our study are present in patients with type 1 diabetes, as the pathogenesis of type 1 and type 2 diabetes are different.

Tarcin et al. [29] reported higher FT4 levels and lower FT3/FT4 in obese subjects with metabolic syndrome than in those without. This supports the concept that inflammation and adiposity parameters are more associated with changes in thyroid hormone levels than glycaemic levels, as none of the subjects in this study had T2DM. This is likely due to alterations in peripheral hormone conversion, since FT4 is higher and FT3 lower in these patients. One difference in this study from ours is the positive association of TT3 and waist circumference. This correlation was present in our study, but inverse, suggesting that greater visceral adiposity and T2DM could further impair energy homeostasis and thyroid hormone conversion, and represent a more “severe” state of inflammation and insulin resistance.

This study has some limitations. The case-control design does not allow any definitive conclusions on causality. Longitudinal studies assessing the concurrent changes in TH and inflammation parameters would be more informative on this issue. Also, we did not directly measure deiodinase activity, and results on TH conversion should be interpreted in light of this limitation. Moreover, this study included a relatively small number of subjects. Although our numbers were based on a previous power calculation, this precluded...
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Thyroid hormone abnormalities in T2DM patients, but acts in conjunction with, and perhaps through, obesity and increased inflammatory activity.

Acknowledgments

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Disclosure

None of the authors have any potential conflicts of interest associated with this research.

References


a more extensive regression analysis. However, when interpreting the correlation analysis it is possible to infer that to show associations between TH levels, BMI and hs-CRP that were not statistically significant in this study, a very large cohort of patients would be required (except for the one between FT4 and hs-CRP), since the r values were very low. Therefore, we do not believe that it could bring any additional data of clinical relevance.

In conclusion, this study confirms the finding of lower FT3 and normal TSH levels in patients with T2DM, findings characteristic of NTIS, and further reinforces the caution recommended when diagnosing thyroid disorders in these patients. More importantly, our work has shown that glycaemic control is not the sole responsible for TH imbalances in T2DM patients, but acts in conjunction with, and perhaps through, obesity and increased inflammatory activity.


