

## RESEARCH

# Circulating adipokines and metabolic setting in differentiated thyroid cancer

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## Abstract

The associative link relating insulin resistance (IR) and adipokines to the occurrence and phenotype of differentiated thyroid cancer (DTC) is unknown. The aim of this study was to evaluate the relationship between IR and adipokines in DTC patients, as compared with carriers of benign thyroid diseases (BTD) and healthy controls. This observational study enrolled 77 subjects phenotyped as DTC ( $N = 30$ ), BTD ( $N = 27$ ) and healthy subjects ( $N = 20$ ). Each subject underwent preoperative analysis of anthropometric parameters, thyroid function and autoimmunity, insulin resistance (HOMA-IR) and levels of unacylated (UAG) and acylated ghrelin (AG), obestatin, leptin and adiponectin. Multivariate regression models were used to test the predictive role of metabolic correlates on thyroid phenotypes and DTC extension. The three groups showed similar age, gender distribution, smoking habit, BMI and thyroid parameters. Obestatin was significantly higher in DTC group compared to BTD ( $P < 0.05$ ) and control subjects ( $P < 0.0001$ ). DTC and BTD groups showed higher levels of UAG ( $P < 0.01$ ) and AG ( $P < 0.05$ ). Leptin levels were comparable between groups, whereas adiponectin levels were lower in DTC compared to BTD group ( $P < 0.0001$ ) and controls ( $P < 0.01$ ). In parallel, HOMA-IR was higher in DTC than BTD ( $P < 0.05$ ) and control group ( $P < 0.01$ ). Stepwise multivariable regression analysis showed that obestatin and UAG were independent predictors of DTC ( $P = 0.01$  for both). In an analysis restricted to the DTC group, obestatin levels were associated with the absence of lymph node metastases ( $P < 0.05$ ). Our results highlight a potential association between metabolic setting, circulating adipokines and thyroid cancer phenotype.

## Key Words

- ▶ adipokines
- ▶ thyroid cancer
- ▶ insulin resistance
- ▶ histological phenotype

*Endocrine Connections*  
(2019) **8**, 997–1006

## Introduction

The incidence rates of differentiated thyroid cancer (DTC) have been rising in many countries over the last few decades (1, 2) along with a parallel increase in obesity and metabolic disorders (3).

Even if the growing incidence of DTC is at least in part dependent on improved accuracy of diagnostic tools, which likely results in 'over-diagnosis' of

microcarcinomas (1, 4, 5, 6), an increasing prevalence of more aggressive forms of DTCs has been highlighted in several studies (1, 7, 8), suggesting that changes in incidence pattern of DTCs are likely real. An epidemiological link between metabolic derangement associated with excess body weight and thyroid cancers has been highlighted in cross-sectional studies and

confirmed in meta-analyses of cross-sectional and prospective studies. Many advocate that environmental and lifestyle factors, such as obesity and insulin resistance (IR), could be implicated in the pathogenesis of thyroid cancer (2, 9, 10). Although the pathways linking obesity and IR to thyroid cancer remain largely unknown, a potential role for insulin, growth hormone (GH) secretagogues and adipokines has been postulated (10). Insulin not only regulates cell metabolism but also stimulates cell growth via its own receptor (IR), and many cancer cells overexpress the A isoform of the IR, which has a predominant mitogenic effect (10). Insulin and hyperinsulinaemia could thus exert oncogenic potential via abnormal stimulation of multiple cellular signalling cascades, enhancing growth factor-dependent cell proliferation and/or by directly affecting cell metabolism (11). Other factors potentially implicated in the loop linking obesity and IR to DTC could be represented by ghrelin, an orexigenic hormone capable of GH secretagogue activity and obestatin, which is encoded by the same gene-encoding ghrelin but shows opposing effects on GH secretion and appetite (10, 12). Both are expressed in papillary thyroid cancer tissues, albeit with inhomogeneous patterns (12). Further, there is evidence that the adipocytokine network could play a role in thyroid tumorigenesis. Leptin, food intake and resting energy expenditure also plays a role in activating monocytes and macrophages and can stimulate angiogenesis and cell proliferation (13, 14). The effect of leptin has been investigated in thyroid cancer cells (10), and it has been shown to promote cell migration in PTC cells (15) while stimulating a more aggressive cancer phenotype (16) and promoting the de-differentiation of thyroid cancer cells (17). Another potentially relevant adipokine is adiponectin known for its ability to improve insulin sensitivity, which influences cell proliferation and regulates the balance of anti- and pro-inflammatory molecules at the cellular level (9, 18). Serum adiponectin levels have been found to be inversely correlated with the presence of DTC (19), while the absence of adiponectin receptors on DTC cells has been associated with extrathyroidal extension, multicentricity and higher tumour-node-metastases (TNM) stage of DTCs (20).

To date, the association relating IR and adipokines to the presence and phenotype of DTC is not completely clear and is still debated. Therefore, the aim of our observational study was to evaluate the relationship between IR and adipokines in DTC patients, as compared with carriers of BTM and subjects without thyroid diseases.

## Materials and methods

### Patients

The study enrolled 77 subjects, consisting of 57 adults undergoing total thyroidectomy because of benign thyroid disease (uninodular or multinodular goitre, follicular adenoma) or suspected DTC on the basis of cytological examination according to Bethesda criteria (21) and classified as TIR3B, TIR4, TIR5. Twenty healthy subjects without thyroid disease served as control group.

Following blood sampling for the purpose of the study, patients who underwent thyroidectomy were divided into two groups, according to the post-surgical histological diagnosis:

- DTC group: 30 patients diagnosed with papillary or follicular thyroid cancer
- BTM group: 27 patients diagnosed with benign thyroid diseases.

Subjects with poorly differentiated, anaplastic, medullary thyroid carcinomas, secondary tumours, history of medical neck irradiation, hormone therapies and treatments interfering with insulin sensitivity were excluded from the analysis.

The experimental procedure was approved by the *ad hoc* Ethical Research Committee of Novara, Italy. A written informed consent was obtained from all patients and controls. The study protocol was conformed to the guidelines of the European Convention on Human Rights and Biomedicine concerning biomedical research.

### Body measurements

All subjects underwent body measurements wearing light underwear, in fasting conditions after voiding. Weight and height were measured in the nearest 0.1 kg and 0.1 cm, respectively, using standard methods. BMI was expressed as body mass (kg)/height (m)<sup>2</sup>. Overweight was defined for any BMI between 25 and 29.9 kg/m<sup>2</sup> and obesity for any BMI over 30 kg/m<sup>2</sup>. Waist circumference (WC) was measured midway between the lowest rib and the top of the iliac crest after gentle expiration.

### Laboratory tests

Blood samples were preoperatively drawn under fasting conditions, centrifugated and stored at –20°C or –80°C, until assay. The protease inhibitor aprotinin was added to plasma aliquots for determination of ghrelin concentrations.

Undiluted serum samples were assayed for thyroid-stimulating hormone (TSH), free thyroxine (fT4) and free triiodothyronine (fT3) using an automated chemiluminescence assay system (ADVIA Centaur Systems TSH3/fT4/fT3 Ultra Ready Pack, Siemens Healthcare Diagnostics).

Serum levels of thyroglobulin (Tg) were determined using an automated chemiluminescence method (LIAISON XL, DiaSorin S.p.A, Saluggia).

Plasma levels of anti-Tg and anti-thyroperoxidase (TPO) antibodies were assessed using an automated chemiluminescence assay system (Anti-Tg, Anti-TPO Ready Pack, Siemens Healthcare Diagnostics).

Plasma glucose levels were determined using an enzymatic method (ADVIA 1800 Chemistry System, Siemens Healthineers). Serum insulin levels were obtained using direct chemiluminescence method (ADVIA Centaur IRI, Siemens). Insulin resistance was calculated by the homeostatic model of insulin resistance (HOMA-IR) index:  $\text{insulin (mIU/mL)} \times (\text{glucose (mmol/L)} / 22.5)$  (22). A HOMA-IR value greater than 2.5 was considered indicative of insulin resistance (23).

For hormone assays, procedures were performed in accordance with the manufacturers' instruction and the samples were analysed in duplicate.

Serum leptin levels were assessed using a commercially available human ELISA kit (Mediagnost, Reutlinger, Germany). Intra-assay CV and inter-assay CV of leptin were less than 10%. Minimum detectable concentration was 0.2 ng/mL.

Serum adiponectin levels were determined using a commercially available human ELISA kit (Mediagnost, Reutlinger, Germany). Intra-assay CV was less than 6.7% and inter-assay CV was less than 4.7%. Minimum detectable concentration was 0.6 ng/mL.

Serum obestatin concentrations were assessed using a commercially available human EIA kit (Yanaihara Institute Inc, Awakura, Japan). Intra-assay CV was 3.5–9.9% and inter-assay CV was 5.6–9.0%. Minimum detectable concentration was 0.231 ng/mL.

Plasma unacylated ghrelin (UAG) levels were determined using a commercially available human ELISA kit (BioVendor Research and Diagnostic Product, Czech Republic). Intra-assay CV was 3.2–11.8% and inter-assay CV was 3.8–13.2%. Minimum detectable concentration was 6 pg/mL.

Plasma acylated ghrelin (AG) levels were assessed using a commercially available human ELISA kit (BioVendor). Intra-assay CV was 2.9–11.8% and inter-assay CV was 3.4–14.4%. Minimum detectable concentration was 5 pg/mL.

## Thyroid cytology and histology

The cytology specimens were evaluated and classified according to the international guidelines (24, 25). Histological slides were reviewed by two independent pathologists for the purpose of this study. For all cases, tumour-associated thyroiditis was assessed. The tumour size, number of foci, focality, extension, presence of loco-regional and/or distant metastases were also reported and classified according to the 2010 TNM system (26).

## Neck ultrasound (US)

Pre-surgical US was routinely performed in all patients. The study was conducted using a 'My Lab 25 Gold' (ESAOTE S.p.A, Genova, Italy) equipped with a linear transducer of 7.5 MHz. Sonographic features predictive of malignancy were considered according to American Thyroid Association and AACE-AME guidelines (24, 27). Cervical lymphadenopathies and their US features were also evaluated.

## Data analysis

Statistical analysis was performed using SPSS version 21 on log transformed data to correct for the non-Gaussian distribution obtained by the Shapiro–Wilk test. Values were expressed as median and interquartile ranges (IQ). For comparative analysis, ANOVA between the three groups was used. Spearman's correlation analysis was used to identify significant associations between variables of interest. ANCOVA multinomial regression analysis was used to evaluate the association of adipokines levels with histological characteristics of DTCs. Stepwise multivariate regression analysis was used to evaluate the independent association of metabolic, anthropometric or biochemical parameters with adipokines and HOMA-IR.  $\beta$  coefficients and related significance values obtained from the models were reported.  $P < 0.05$  was considered as statistically significant.

## Results

### Analysis on all study groups

The anthropometric and biochemical data of our population are reported in Table 1.

Anthropometric parameters were comparable between the groups. Obesity (BMI  $>30 \text{ kg/m}^2$ ) was present in 16 cases (six patients with DTC, eight patients with

BTD and two controls) and overweight in 21 cases (seven patients with DTC, six patients with BTD and eight controls). Before surgery, hypothyroidism under replacement therapy was present in eight patients (six patients with DTC and two patients with BTD), while hyperthyroidism under methimazole treatment was present in three patients with BTD.

Thyroid function parameters were comparable between the groups, whereas TPOAb levels were higher in DTC and BTD group with respect to the control group ( $P < 0.05$ ).

Compared to controls, we observed higher levels of UAG ( $P < 0.01$  for both) and AG ( $P < 0.05$  for both) in DTC and BTD groups. Obestatin concentrations were higher in DTC group as compared both to BTD group ( $P < 0.05$ ) and controls ( $P < 0.0001$ ), as well as in the BTD group with respect to controls ( $P < 0.0001$ ). Leptin levels were comparable between the three groups, whereas adiponectin levels were lower in DTC group compared to

BTD group ( $P < 0.0001$ ) and controls ( $P < 0.01$ ), and in BTD group with respect to controls ( $P < 0.0001$ ).

Analysis of metabolic parameters showed the occurrence of IR in 25 cases of the entire dataset (14 patients with DTC, 8 patients with BTD and 3 controls). Among subjects with IR, 18 were overweight/obese. The DTC group showed higher insulin levels and HOMA-IR than BTD (insulin:  $P < 0.01$ , HOMA-IR:  $P < 0.05$ ) and control group (insulin:  $P < 0.01$ , HOMA-IR:  $P < 0.01$ ).

In bivariate correlation analysis across the three groups (Table 2), expected positive associations were documented between metabolic parameters, HOMA-IR and leptin levels. Both leptin and adiponectin were positively correlated with anti-TG antibodies, while adiponectin was also correlated with AbTPO levels. However, the above mentioned correlations were lost after controlling for age, gender, BMI and thyroid phenotypes.

Stepwise multivariable regression analysis with a model weighted for gender, age, BMI, WC and HOMA-IR

**Table 1** Anthropometric and biochemical data obtained in the two groups and controls.

Parameters (median (IQ))	Controls (n = 20)	BTD group (n = 27)	DTC group (n = 30)
Gender			
No. of males	5	4	9
No. of females	15	23	21
Age (years)	47.0 (37.0–62.5)	56.0 (53.5–65.0)	50.0 (41.0–58.8)
Smokers			
Never	24	17	18
Current	3	5	4
Former	3	5	8
Weight (kg)	69.5 (58.0–73.3)	62.0 (58.0–80.5)	68.0 (57.5–83.8)
BMI (kg/m <sup>2</sup> )	25.1 (22.7–26.4)	25.8 (21.9–30.3)	24.2 (22.1–28.2)
WC (cm)	86.5 (78.8–90.0)	90.0 (76.0–99.5)	93.0 (80.0–102.8)
TSH (μIU/mL)	1.6 (1.2–2.1)	1.3 (1.0–2.0)	1.5 (0.9–2.8)
ft4 (ng/dL)	1.2 (1.1–1.3)	1.1 (1.0–1.3)	1.1 (1.0–1.3)
ft3 (pg/mL)	3.1 (3.0–3.3)	3.4 (2.8–3.5)	3.1 (2.9–3.4)
TG (ng/dL)	17.2 (9.4–30.5)	<b>43.3 (15.7–220.9)<sup>b</sup></b>	<b>11.9 (3.6–24.7)<sup>a</sup></b>
TGAb (IU/mL)	34.5 (21.3–52.0)	32.2 (15.0–54.5)	20.8 (13.5–57.5)
TPOAb (IU/mL)	33.0 (21.0–49.5)	<b>41.2 (32.9–134.9)<sup>a</sup></b>	<b>37.3 (13.5–81.5)<sup>a</sup></b>
Glucose (mg/dL)	86.0 (83.5–94.5)	90.0 (81.5–98.0)	89.0 (80.8–97.8)
Insulin (mIU/mL)	8.1 (6.5–10.1)	7.5 (5.8–11.5)	<b>11.3 (8.4–18.5)<sup>b,e</sup></b>
HOMA-IR	1.6 (1.4–2.2)	1.6 (1.3–2.6)	<b>2.5 (1.8–4.1)<sup>b,d</sup></b>
UAG (pg/mL)	21.7 (11.8–30.8)	<b>79.2 (39.0–129.8)<sup>b</sup></b>	<b>57.8 (28.9–101.2)<sup>b</sup></b>
AG (pg/mL)	4.3 (2.4–6.2)	<b>9.0 (5.3–16.5)<sup>a</sup></b>	<b>5.7 (2.1–14.4)<sup>a</sup></b>
Obestatin (ng/mL)	2.5 (2.2–2.6)	<b>4.6 (4.2–5.0)<sup>c</sup></b>	<b>5.0 (4.4–6.1)<sup>c,d</sup></b>
Leptin (ng/mL)	10.6 (5.7–25.6)	16.8 (13.2–23.1)	11.6 (4.1–19.1)
Adiponectin (μg/mL)	31.4 (15.2–41.0)	22.2 (17.7–31.1)	<b>10.8 (5.7–20.4)<sup>b,f</sup></b>

Data are expressed as median (with interquartile range in parentheses). Comparison between populations was performed by ANOVA test and  $\chi^2$  test. Significant differences are shown in bold characters. Significant differences between controls and BTD or DTC group: <sup>a</sup> $P < 0.05$ , <sup>b</sup> $P < 0.01$ , <sup>c</sup> $P < 0.0001$ . Significant differences between BTD and DTC group: <sup>d</sup> $P < 0.05$ , <sup>e</sup> $P < 0.01$ , <sup>f</sup> $P < 0.0001$ .

AG, acylated ghrelin; BMI, body mass index; ft3, free triiodothyronine; ft4, free thyroxine; HOMA-IR, Homeostatic Model of Insulin Resistance; TG, thyroglobulin; TGAb, Anti-thyroglobulin antibodies; TPOAb, anti-thyroperoxidase antibodies; TSH, thyroid-stimulating hormone; UAG, unacylated ghrelin; WC, waist circumference.

**Table 2** Spearman's correlation analysis between adipokines levels, HOMA-IR and anthropometric-biochemical parameters in the population as a whole.

Variables (correlation coefficient)	UAG (pg/mL)	AG (pg/mL)	Obestatin (ng/mL)	Leptin (ng/mL)	Adiponectin (µg/mL)	HOMA-IR
Gender	0.069	0.255	0.056	<b>0.312<sup>b</sup></b>	-0.042	-0.150
Age	-0.154	-0.125	-0.151	<b>0.363<sup>b</sup></b>	0.194	<b>0.273<sup>a</sup></b>
BMI (kg/m <sup>2</sup> )	-0.160	-0.079	-0.098	<b>0.606<sup>c</sup></b>	-0.026	<b>0.561<sup>c</sup></b>
WC (cm)	-0.037	-0.024	-0.140	<b>0.585<sup>c</sup></b>	-0.154	<b>0.598<sup>c</sup></b>
Glucose (mg/dL)	-0.017	-0.075	0.079	<b>0.304<sup>b</sup></b>	0.210	<b>0.509<sup>c</sup></b>
Insulin (mIU/mL)	-0.236	-0.213	-0.178	<b>0.453<sup>c</sup></b>	<b>-0.279<sup>a</sup></b>	<b>0.953<sup>c</sup></b>
HOMA-IR	-0.202	-0.208	-0.138	<b>0.453<sup>c</sup></b>	-0.195	-
TSH (µIU/mL)	-0.008	-0.090	0.087	-0.014	-0.049	0.068
ft4 (ng/dL)	0.101	0.138	-0.125	0.051	0.083	-0.007
ft3 (pg/mL)	-0.161	-0.099	0.007	0.006	0.028	-0.067
TG (ng/dL)	-0.126	-0.080	-0.022	-0.045	0.234	-0.191
TgAb (IU/mL)	0.032	0.045	-0.031	<b>0.263<sup>a</sup></b>	<b>0.374<sup>b</sup></b>	0.182
TPOAb (IU/mL)	0.258	0.241	0.072	0.161	<b>0.289<sup>a</sup></b>	0.043

Significant correlations are written in bold. For significance: <sup>a</sup> $P < 0.05$ , <sup>b</sup> $P < 0.01$ , <sup>c</sup> $P < 0.0001$ .

BMI, body mass index; ft3, free triiodothyronine; ft4, free thyroxine; HOMA-IR, Homeostatic Model of Insulin Resistance; TG, thyroglobulin; TgAb, anti-thyroglobulin antibodies; TPOAb, anti-thyroperoxidase antibodies; TSH, thyroid-stimulating hormone; WC, waist circumference.

documented that the presence of DTC (DTC=0, BTD=1, controls=2) was independently predicted by obestatin and UAG levels (respectively  $\beta = -0.339$  and  $\beta = -0.357$ ,  $P = 0.01$ ).

### Analysis of patients with DTC

Histopathological characteristics of all patients with DTC are summarised in Table 3.

As expected, the most frequent histotype was PTC (25/30 cases) and the most common variant was the classical one (9/30 cases), followed by other more aggressive variants such as tall cell, oncocytic, solid and Warthin-like variants. Capsular invasion was present in 13/30 cases, while bilaterality and multifocality were described in 5/30 and 8/30 patients, respectively. Overall, 18/30 tumours were classified as stage I, followed by stage IV (5/30). Among patients who underwent lymph node dissection, seven patients had lymph node metastases. Lastly, tumour-associated thyroiditis was found in 7/30 cases.

Bivariate correlation analysis restricted to the DTC group is reported in Table 4. After adjusting for age, BMI, WC and gender, only the negative association between obestatin levels and the presence of lymph node metastases ( $r = -0.862$ ,  $P < 0.05$ ) and the positive association between obestatin and TPOAb titer ( $r = 0.856$ ,  $P < 0.05$ ) remained significant.

Logistic regression analysis, performed to study the association between adipokines levels or HOMA-IR and DTC histological characteristics, showed that obestatin levels were significantly associated with the absence of

lymph node metastases (odds ratio (OR)=4.370, CI 95% 1.080–10.964,  $P < 0.05$ ).

### Discussion

The present study analysed the association relating IR and multiple adipokines with DTC phenotypes. Our results showed that the prevalence of IR was higher in patients with DTC and that these subjects harbour higher level of obestatin than patient with benign nodules and controls. However, in the analysis restricted to DTC cases, obestatin levels were inversely associated with the metastatic capacity of tumour. While leptin levels were comparable between groups, adiponectin levels were lower in patients with DTC in respect to subjects with benign nodules and controls. Moreover, in patients with either benign or malignant thyroid nodules, higher concentrations of UAG and AG were observed as compared to controls. Higher obestatin and UAG levels were also associated with the presence of malignancy in patients with thyroid nodules.

In the past few decades, the incidence of DTC has shown a steady increase worldwide (2, 8). Some evidence suggests that environmental and lifestyle factors can play an important role (2, 28). Among the potential risk factors involved in the changing epidemiology of TC, particular attention has been accorded to IR and the metabolic syndrome, which have been also rapidly increasing worldwide due to widespread dietary and lifestyle changes (29). On this basis, it has been suggested that IR is associated with the rising incidence of DTCs. A small

**Table 3** Histopathological characteristics of all patients with DTC.

	N. of patients (30 cases)
Cytology	
Not performed	1
Thy 2	1
Thy 3f	1
Thy 4	5
Thy 5	1
Histotype	
PTC	25
FTC	5
PTC variant	
Classical	9
Follicular	5
Classical and follicular	5
Other	6
FTC variant	
Minimally invasive	4
Invasive	1
Tumour size (cm)	
≤1 cm	6
1.0–2.0 cm	11
2.0–4.0 cm	11
>4 cm	2
Multifocality	
Present	8
Bilaterality	
Present	5
Thyroid capsular invasion	
Present	13
Staging (TNM 2010)	
Stage I	18
Stage II	4
Stage III	3
Stage IV	5
Histological associated thyroiditis	
Present	7
Lymph node metastases	
Present (18 lymphadenectomy)	7

FTC, follicular thyroid cancer; PTC, papillary thyroid cancer.

cross-sectional study detected an increased prevalence of IR in patients with DTC (30), and a recent study by Bae *et al.* (31) showed a clear-cut association relating insulin and HOMA-IR index to the risk of PTC and the multifocality of this disease. Our results reflected these findings. In fact, we found that patients with DTC harbour higher insulin levels and HOMA-IR index with respect to patients with benign nodules and subjects without thyroid diseases. IR is known to be an important risk factor responsible for carcinogenesis (32). However, we failed to demonstrate the direct associations between insulin levels and DTC, suggesting that the metabolic setting rather than insulin levels could play a role in this relationship. This circumstance would agree with the evidence that

IR enhances the pro-inflammatory state by decreasing the production of anti-inflammatory adipokines, such as adiponectin, and increasing the production of pro-inflammatory adipokines, such as leptin, which could interact with molecular pathways involved in tumour development (33).

Adipokines are a subset of cytokines produced by the adipose tissue (34). They are involved not only in immune responses, but also in the regulation of appetite and energy balance, insulin sensitivity, angiogenesis, blood pressure regulation and lipid metabolism (14). In particular, leptin is involved in the control of food intake through satiety sensation, regulation of energy expenditure, activation of monocytes and macrophages, stimulation of VEGF, angiogenesis, cell proliferation and suppression of anti-inflammatory cytokines. A relationship of this molecule with DTC and other cancers has been demonstrated (35, 36). With regard to DTC, leptin was previously found to be associated with a high risk of lymph node metastases (37), and it seems to be involved in the clinical phenotype of the tumours as well as in the migration of thyroid cell, promoting metastases formation and diffusion (15, 17, 38, 39, 40). Although our study failed to detect differences in serum leptin levels across the three groups, we observed a significant association between circulating leptin and the presence of lymph node metastases, which was lost after controlling for confounders (age, BMI and gender). However, it is worth considering that the serum concentration of leptin may not reflect its action on thyroid tissue. The higher levels of this adipokine found in subjects with benign nodules, would require future analysis to evaluate its modulatory role in the hyperplastic phase of the thyroid follicle.

Adiponectin is an adipokine with strong anti-inflammatory properties. It is able to improve insulin sensitivity, influence cell proliferation and regulate the balance of anti- and pro-inflammatory molecules and cells (41, 42, 43). Owing to its complex anti-proliferative and inflammation-restraining functions, adiponectin has been suggested to have anti-neoplastic properties in some types of cancers (9). Our results showed lower adiponectin levels in patients with thyroid cancer. Similar findings were shown by Mitsiades *et al.* (19) who demonstrated that serum adiponectin levels are inversely associated with the prevalence of DTCs, suggesting a potential protective effect of adiponectin against the development of this cancer. The mechanisms by which adiponectin may act on thyroid cancer still remain to be identified, but it may include a protection against the development of IR (44), in particular through the activation of the

**Table 4** Spearman's correlation analysis between adipokines levels and tumour characteristics and thyroid biochemical parameters in patients with DTC.

Variables (correlation coefficient)	UAG (pg/mL)	AG (pg/mL)	Obestatin (ng/mL)	Leptin (ng/mL)	Adiponectin (µg/mL)	HOMA-IR
Tumour size	0.110	-0.004	<b>0.497<sup>a</sup></b>	-0.250	0.303	-0.149
Histotype <sup>b</sup>	-0.028	0.000	-0.094	-0.175	0.323	-0.109
PTC variants <sup>c</sup>	0.156	0.101	0.079	-0.011	0.306	-0.142
Bilaterality	-0.254	-0.085	0.158	0.316	-0.022	0.164
Multifocality	-0.089	0.022	0.227	0.194	0.231	0.074
Thyroid capsular invasion	-0.333	-0.210	0.136	0.207	-0.091	0.124
Lymph node metastases	-0.056	-0.280	<b>-0.779<sup>d</sup></b>	<b>-0.439<sup>a</sup></b>	0.187	0.073
Histological associated thyroiditis	-0.292	-0.280	-0.227	<b>0.501<sup>d</sup></b>	0.106	0.308
Staging	-0.314	-0.340	0.272	-0.044	0.180	0.223
TSH	-0.222	-0.105	0.193	0.090	-0.120	0.111
ft4	0.197	0.128	-0.159	0.145	-0.084	-0.168
ft3	-0.189	-0.104	-0.095	-0.213	-0.151	-0.275
Tg	-0.089	-0.230	0.210	-0.229	0.043	-0.046
TgAB	0.017	0.007	-0.202	<b>0.443<sup>a</sup></b>	0.281	0.307
TPOAb	0.279	0.300	-0.256	0.333	0.250	-0.082

<sup>a</sup> $P < 0.05$ , <sup>d</sup> $P < 0.01$ . <sup>b</sup>PTC = 0, FTC = 1; <sup>c</sup>classical = 0, follicular = 1, classical and follicular = 2, other variants = 3.

ft3, free triiodothyronine; ft4, free thyroxine; PTC, papillary thyroid cancer; Tg, thyroglobulin; TGAb, anti-thyroglobulin antibodies; TPOAb, anti-thyroperoxidase antibodies; TSH, thyroid-stimulating hormone.

adenosine monophosphate kinase (AMPK) pathway (20, 45, 46). Adiponectin has also been shown to directly inhibit angiogenesis and promote apoptosis *in vivo*, through the activation of the caspase cascade (47). Low adiponectin has been proposed as a possible mediator in the association between BMI and cancer risk (48).

Ghrelin is implicated in several processes of cancer progression including cell proliferation, cell migration and invasion, angiogenesis and apoptosis, probably via an autocrine/paracrine mechanism (49). Some reports demonstrated that ghrelin may have an inhibitory effect in the proliferation of some cancer types, including thyroid, prostate, breast and small-cell lung carcinoma (50). However, the exact role of ghrelin in thyroid tumorigenesis is still debated. Ucan and colleagues (49) did not found any significant differences in serum ghrelin levels between patients with PTC and healthy controls. Our study demonstrated that AG concentrations were higher in the patients with malignant and benign thyroid nodules with respect to controls. These findings seem to support the hypothesised role of AG in promoting tumorigenesis (51, 52). In particular, the increased expression of this molecule could influence thyroid tumor proliferation through deregulation of GH and IGF-1 secretion – factors involved in cancer growth (53).

With regard to UAG, our data showed that this hormone was higher in patients with DTC and benign thyroid nodules. Moreover, we found that the presence of DTC was independently predicted by UAG.

Recently, UAG has been hypothesised to be involved in various biological activities, including the inhibitory role on AG (54), the anti-proliferative and pro-apoptotic capacity on different cell lines *in vitro* (55). However, these are preliminary results and its role is still debated. Further prospective studies investigating ghrelin expression in DTCs and its association with serum ghrelin levels could be helpful to clarify this issue.

Finally, the role of obestatin in promoting thyroid cancer tumorigenesis is still controversial. However, this molecule appears very interesting for its probable involvement in cell proliferation through AKT-dependent signalling (50, 56, 57). In previous studies, Volante *et al.* (58) found obestatin expression in medullary, papillary, follicular and poorly differentiated thyroid cancer. The authors detected obestatin immunoreactivity mostly in ghrelin-positive areas of DTC, whereas there was no obestatin expression in normal thyroid tissue (58). On the contrary, Karaoglu *et al.* demonstrated obestatin expression in healthy thyroid, Hashimoto thyroiditis and PTC without evident differences in the intensity of reaction (12). In a recent study, obestatin immunoreactivity was observed in benign nodules, as well as cancer cells (59). However, the intensity of immunohistochemical expression was poor and obestatin-positive cells were accompanied by regions without any immunoreactivity (58). In our study, serum obestatin concentrations were, overall, higher in patients with DTC with respect to subjects with BTN and controls, thus representing a predictor of the presence of DTC.

Interestingly, within the DTC group obestatin levels were significantly associated with the absence of lymph node metastases. Together, our data suggest a potential dual role for obestatin, such that its circulating levels could represent a biomarker of the metabolic setting of DTC, but at the same time, this adipokine could reflect lower proneness to the metastatic diffusion of DTC.

Our study has several limitations which have to be pointed out. First, the small patient population and the associative nature of the study do not allow us to draw any conclusion about the mechanisms involved in the association between IR, adipokines and DTC tumorigenesis. Second, we did not perform any analysis on the tissue expression of adipokines.

In conclusion, our study highlighted a potential association between metabolic setting, circulating adipokines and thyroid cancer phenotype. An IR-metabolic phenotype and its related biomarkers could influence the pathogenesis of nodular goitre and thyroid cancer. Further studies are awaited to clarify the role of adipokines and metabolic setting in determining DTC phenotype and tumorigenesis.

#### 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.

#### Funding

This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

#### Author contribution statement

C M, M T S and L P designed the study and wrote the main manuscript text; S M and G W performed biochemical assays; A A B, M C and V B recruited patients and managed the dataset; G A, P M and F P performed data analysis and critical overview. All authors reviewed the manuscript.

#### Acknowledgements

The authors thank Stefania Moia, Marilisa De Feusis, Marta Rocco (Department of Health Sciences, University of Piemonte Orientale, Novara, Italy) and Roberta Vietti (Laboratory of Metabolic Research, Istituto Auxologico Italiano, Piancavallo (VB), Italy) for laboratory assistance.

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Received in final form 27 May 2019

Accepted 17 June 2019

Accepted Preprint published online 17 June 2019