RESEARCH

Circulating adipokines and metabolic setting in differentiated thyroid cancer

Chiara Mele^{1,2}, Maria Teresa Samà³, Alessandro Angelo Bisoffi¹, Marina Caputo¹, Valentina Bullara³, Stefania Mai⁴, Gillian Elisabeth Walker⁵, Flavia Prodam⁵, Paolo Marzullo^{1,2}, Gianluca Aimaretti¹ and Loredana Pagano^{1,6}

¹Division of Endocrinology, Department of Translational Medicine, University of Piemonte Orientale, Novara, Italy

²Division of General Medicine, Istituto Auxologico Italiano, IRCCS, S. Giuseppe Hospital, Piancavallo di Oggebbio (VB), Italy

³Division of Endocrinology, University Hospital 'Maggiore della Carità', Novara, Italy

⁴Laboratory of Metabolic Research, Istituto Auxologico Italiano, IRCCS, S. Giuseppe Hospital, Piancavallo di Oggebbio (VB), Italy

⁵Department of Health Sciences, University of Piemonte Orientale, Novara, Italy

⁶Division of Endocrinology, Diabetology and Metabolism, Department of Medical Sciences, University of Turin, Turin, Italy

Correspondence should be addressed to C Mele: chiara.mele1989@gmail.com

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 BTDC 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
- BTDC 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)². Overweight was defined for any BMI between 25 and 29.9 kg/m² and obesity for any BMI over 30 kg/m². 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, centrifuged 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. β 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 ($\text{BMI} > 30 \text{ kg/m}^2$) 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 (IQR))	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 ²)	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)	43.3 (15.7–220.9)^b	11.9 (3.6–24.7)^a
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)	41.2 (32.9–134.9)^a	37.3 (13.5–81.5)^a
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)	11.3 (8.4–18.5)^{b,e}
HOMA-IR	1.6 (1.4–2.2)	1.6 (1.3–2.6)	2.5 (1.8–4.1)^{b,d}
UAG (pg/mL)	21.7 (11.8–30.8)	79.2 (39.0–129.8)^b	57.8 (28.9–101.2)^b
AG (pg/mL)	4.3 (2.4–6.2)	9.0 (5.3–16.5)^a	5.7 (2.1–14.4)^a
Obestatin (ng/mL)	2.5 (2.2–2.6)	4.6 (4.2–5.0)^c	5.0 (4.4–6.1)^{c,d}
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)	10.8 (5.7–20.4)^{b,f}

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

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	0.312^b	−0.042	−0.150
Age	−0.154	−0.125	−0.151	0.363^b	0.194	0.273^a
BMI (kg/m ²)	−0.160	−0.079	−0.098	0.606^c	−0.026	0.561^c
WC (cm)	−0.037	−0.024	−0.140	0.585^c	−0.154	0.598^c
Glucose (mg/dL)	−0.017	−0.075	0.079	0.304^b	0.210	0.509^c
Insulin (mIU/mL)	−0.236	−0.213	−0.178	0.453^c	−0.279 ^a	0.953^c
HOMA-IR	−0.202	−0.208	−0.138	0.453^c	−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	0.263^a	0.374^b	0.182
TPOAb (IU/mL)	0.258	0.241	0.072	0.161	0.289^a	0.043

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

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, BTDC=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, oncocyctic, 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	0.497^a	−0.250	0.303	−0.149
Histotype ^b	−0.028	0.000	−0.094	−0.175	0.323	−0.109
PTC variants ^c	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	−0.779^d	−0.439^a	0.187	0.073
Histological associated thyroiditis	−0.292	−0.280	−0.227	0.501^d	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	0.443^a	0.281	0.307
TPOAb	0.279	0.300	−0.256	0.333	0.250	−0.082

^a $P < 0.05$, ^d $P < 0.01$. ^bPTC = 0, FTC = 1; ^cclassical = 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 BTDC 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 Roccio (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.

References

- Pazaitou-Panayiotou K, Iliadou PK, Chrisoulidou A, Mitsakis P, Doumala E, Fotareli A, Boudina M, Mathiopoulou L, Patakiaouta F & Tziomalos K. The increase in thyroid cancer incidence is not only due to papillary microcarcinomas: a 40-year study in 1778 patients. *Experimental and Clinical Endocrinology and Diabetes* 2013 **121** 397–401. (<https://doi.org/10.1055/s-0033-1345125>)
- Pagano L, Mele C, Arpaia D, Samà MT, Caputo M, Ippolito S, Peirce C, Prodam F, Valente G, Ciancia G, *et al.* How do etiological factors can explain the different clinical features of patients with differentiated thyroid cancer and their histopathological findings? *Endocrine* 2017 **56** 129–137. (<https://doi.org/10.1007/s12020-016-0992-8>)
- Pazaitou-Panayiotou K, Polyzos SA & Mantzoros CS. Obesity and thyroid cancer: epidemiologic associations and underlying mechanisms. *Obesity Reviews* 2013 **14** 1006–1022. (<https://doi.org/10.1111/obr.12070>)
- Elisei R, Molinaro E, Agate L, Bottici V, Masserini L, Ceccarelli C, Lippi F, Grasso L, Basolo F, Bevilacqua G, *et al.* Are the clinical and pathological features of differentiated thyroid carcinoma really changed over the last 35 years? Study on 4187 patients from a single Italian institution to answer this question. *Journal of Clinical Endocrinology and Metabolism* 2010 **95** 1516–1527. (<https://doi.org/10.1210/jc.2009-1536>)
- Lee TJ, Kim S, Cho HJ & Lee JH. The incidence of thyroid cancer is affected by the characteristics of a healthcare system. *Journal of Korean Medical Science* 2012 **27** 1491–1498. (<https://doi.org/10.3346/jkms.2012.27.12.1491>)
- Grodski S, Brown T, Sidhu S, Gill A, Robinson B, Learoyd D, Sywak M, Reeve T & Delbridge L. Increasing incidence of thyroid cancer is due to increased pathologic detection. *Surgery* 2008 **144** 1038–1043; discussion 1043. (<https://doi.org/10.1016/j.surg.2008.08.023>)
- Gomez Segovia I, Gallowitsch HJ, Kresnik E, Kumnig G, Igerc I, Matschnig S, Stronegger WJ & Lind P. Descriptive epidemiology of thyroid carcinoma in Carinthia, Austria: 1984–2001. Histopathologic features and tumor classification of 734 cases under elevated general iodination of table salt since 1990: population-based age-stratified analysis on thyroid carcinoma incidence. *Thyroid* 2004 **14** 277–286. (<https://doi.org/10.1089/105072504323030933>)
- Enewold L, Zhu K, Ron E, Marrogi AJ, Stojadinovic A, Peoples GE & Devesa SS. Rising thyroid cancer incidence in the United States by demographic and tumor characteristics, 1980–2005. *Cancer Epidemiology, Biomarkers and Prevention* 2009 **18** 784–791. (<https://doi.org/10.1158/1055-9965.EPI-08-0960>)
- Marcello MA, Cunha LL, Batista FA & Ward LS. Obesity and thyroid cancer. *Endocrine-Related Cancer* 2014 **21** T255–T271. (<https://doi.org/10.1530/ERC-14-0070>)
- Santini F, Marzullo P, Rotondi M, Ceccarini G, Pagano L, Ippolito S, Chiovato L & Biondi B. Mechanisms in endocrinology: the crosstalk between thyroid gland and adipose tissue: signal integration in health and disease. *European Journal of Endocrinology* 2014 **171** R137–R152. (<https://doi.org/10.1530/EJE-14-0067>)
- Almquist M, Johansen D, Björge T, Ulmer H, Lindkvist B, Stocks T, Hallmans G, Engeland A, Rapp K, Jonsson H, *et al.* Metabolic factors and risk of thyroid cancer in the Metabolic syndrome and Cancer project (Me-Can). *Cancer Causes and Control* 2011 **22** 743–751. (<https://doi.org/10.1007/s10552-011-9747-2>)
- Karaoglu A, Aydin S, Dagli AF, Cummings DE, Ozercan IH, Canatan H & Ozkan Y. Expression of obestatin and ghrelin in papillary thyroid carcinoma. *Molecular and Cellular Biochemistry* 2009 **323** 113–118. (<https://doi.org/10.1007/s11010-008-9969-0>)
- Piya MK, McTernan PG & Kumar S. Adipokine inflammation and insulin resistance: the role of glucose, lipids and endotoxin. *Journal of Endocrinology* 2013 **216** T1–T15. (<https://doi.org/10.1530/JOE-12-0498>)
- Kwon H & Pessin JE. Adipokines mediate inflammation and insulin resistance. *Frontiers in Endocrinology* 2013 **4** 71. (<https://doi.org/10.3389/fendo.2013.00071>)
- Cheng SP, Yin PH, Chang YC, Lee CH, Huang SY & Chi CW. Differential roles of leptin in regulating cell migration in thyroid cancer cells. *Oncology Reports* 2010 **23** 1721–1727. (https://doi.org/10.3892/or_00000817)
- Uddin S, Bavi P, Siraj AK, Ahmed M, Al-Rasheed M, Hussain AR, Ahmed M, Amin T, Alzahrani A, Al-Dayel F, *et al.* Leptin-R and its

- association with PI3K/AKT signaling pathway in papillary thyroid carcinoma. *Endocrine-Related Cancer* 2010 **17** 191–202. (<https://doi.org/10.1677/ERC-09-0153>)
- 17 Kim WG, Park JW, Willingham MC & Cheng SY. Diet-induced obesity increases tumor growth and promotes anaplastic change in thyroid cancer in a mouse model. *Endocrinology* 2013 **154** 2936–2947. (<https://doi.org/10.1210/en.2013-1128>)
 - 18 Li S, Shin HJ, Ding EL & van Dam RM. Adiponectin levels and risk of type 2 diabetes: a systematic review and meta-analysis. *JAMA* 2009 **302** 179–188. (<https://doi.org/10.1001/jama.2009.976>)
 - 19 Mitsiades N, Pazaitou-Panayiotou K, Aronis KN, Moon HS, Chamberland JP, Liu X, Diakopoulos KN, Kyttaris V, Panagiotou V, Mylvaganam G, *et al.* Circulating adiponectin is inversely associated with risk of thyroid cancer: in vivo and in vitro studies. *Journal of Clinical Endocrinology and Metabolism* 2011 **96** E2023–E2028. (<https://doi.org/10.1210/jc.2010-1908>)
 - 20 Cheng SP, Liu CL, Hsu YC, Chang YC, Huang SY & Lee JJ. Expression and biologic significance of adiponectin receptors in papillary thyroid carcinoma. *Cell Biochemistry and Biophysics* 2013 **65** 203–210. (<https://doi.org/10.1007/s12013-012-9419-1>)
 - 21 Cibas ES & Ali SZ. The 2017 Bethesda system for reporting thyroid cytopathology. *Thyroid* 2017 **27** 1341–1346. (<https://doi.org/10.1089/thy.2017.0500>)
 - 22 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>)
 - 23 Muniyappa R, Lee S, Chen H & Quon MJ. Current approaches for assessing insulin sensitivity and resistance in vivo: advantages, limitations, and appropriate usage. *American Journal of Physiology: Endocrinology and Metabolism* 2008 **294** E15–E26. (<https://doi.org/10.1152/ajpendo.00645.2007>)
 - 24 Haugen BR, Alexander EK, Bible KC, Doherty GM, Mandel SJ, Nikiforov YE, Pacini F, Randolph GW, Sawka AM, Schlumberger M, *et al.* 2015 American Thyroid Association management guidelines for adult patients with thyroid nodules and differentiated thyroid cancer: the American Thyroid Association Guidelines Task Force on Thyroid Nodules and Differentiated Thyroid Cancer. *Thyroid* 2016 **26** 1–133. (<https://doi.org/10.1089/thy.2015.0020>)
 - 25 Nardi F, Basolo F, Crescenzi A, Fadda G, Frasoldati A, Orlandi F, Palombini L, Papini E, Zini M, Pontecorvi A, *et al.* Italian consensus for the classification and reporting of thyroid cytology. *Journal of Endocrinological Investigation* 2014 **37** 593–599. (<https://doi.org/10.1007/s40618-014-0062-0>)
 - 26 Edge SB, Byrd DR, Compton CC, Fritz AG, Greene F & Trotti A. *AJCC Cancer Staging Manual*, 7th ed., pp 87–96. New York, NY, USA: Springer, 2010.
 - 27 Gharib H, Papini E, Garber JR, Duick DS, Harrell RM, Hegedüs L, Paschke R, Valcavi R, Vitti P & AACE/ACE/AME Task Force on Thyroid Nodules. American Association of Clinical Endocrinologists, American College of Endocrinology, and Associazione Medici Endocrinologi Medical Guidelines for Clinical Practice for the diagnosis and management of thyroid nodules – 2016 update. *Endocrine Practice* 2016 **22** 622–639. (<https://doi.org/10.4158/EP161208.GL>)
 - 28 Leenhardt L & Grosclaude P. Epidemiology of thyroid carcinoma over the world. *Annales d'Endocrinologie* 2011 **72** 136–148. (<https://doi.org/10.1016/j.ando.2011.03.025>)
 - 29 Malaguarnera R, Vella V, Nicolosi ML & Belfiore A. Insulin resistance: any role in the changing epidemiology of thyroid cancer? *Frontiers in Endocrinology* 2017 **8** 314. (<https://doi.org/10.3389/fendo.2017.00314>)
 - 30 Rezzonico JN, Rezzonico M, Pusiol E, Pitoia F & Niepomniszcze H. Increased prevalence of insulin resistance in patients with differentiated thyroid carcinoma. *Metabolic Syndrome and Related Disorders* 2009 **7** 375–380. (<https://doi.org/10.1089/met.2008.0062>)
 - 31 Bae MJ, Kim SS, Kim WJ, Yi YS, Jeon YK, Kim BH, Lee BJ, Lee JC, Kim IJ, Wang SG, *et al.* High prevalence of papillary thyroid cancer in Korean women with insulin resistance. *Head and Neck* 2016 **38** 66–71. (<https://doi.org/10.1002/hed.23848>)
 - 32 Inoue M & Tsugane S. Insulin resistance and cancer: epidemiological evidence. *Endocrine-Related Cancer* 2012 **19** F1–F8. (<https://doi.org/10.1530/ERC-12-0142>)
 - 33 Catalan V, Gomez-Ambrosi J, Rodriguez A & Fruhbeck G. Adipose tissue immunity and cancer. *Frontiers in Physiology* 2013 **4** 275. (<https://doi.org/10.3389/fphys.2013.00275>)
 - 34 Cunha LL, Marcello MA & Ward LS. The role of the inflammatory microenvironment in thyroid carcinogenesis. *Endocrine-Related Cancer* 2014 **21** R85–R103. (<https://doi.org/10.1530/ERC-13-0431>)
 - 35 Dutta D, Ghosh S, Pandit K, Mukhopadhyay P & Chowdhury S. Leptin and cancer: pathogenesis and modulation. *Indian Journal of Endocrinology and Metabolism* 2012 **16** S596–S600. (<https://doi.org/10.4103/2230-8210.105577>)
 - 36 Vansaun MN. Molecular pathways: adiponectin and leptin signalling in cancer. *Clinical Cancer Research* 2013 **19** 1926–1932. (<https://doi.org/10.1158/1078-0432.CCR-12-0930>)
 - 37 Cheng SP, Chi CW, Tzen CY, Yang TL, Lee JJ, Liu TP & Liu CL. Clinicopathologic significance of leptin and leptin receptor expressions in papillary thyroid carcinoma. *Surgery* 2010 **147** 847–853. (<https://doi.org/10.1016/j.surg.2009.11.004>)
 - 38 Cheng SP, Yin PH, Hsu YC, Chang YC, Huang SY, Lee JJ & Chi CW. Leptin enhances migration of human papillary thyroid cancer cells through the PI3K/AKT and MEK/ERK signaling pathways. *Oncology Reports* 2011 **26** 1265–1271. (<https://doi.org/10.3892/or.2011.1388>)
 - 39 Cheng SP, Liu CL, Hsu YC, Chang YC, Huang SY & Lee JJ. Regulation of leptin receptor expression in human papillary thyroid cancer cells. *Biomedicine and Pharmacotherapy* 2012 **66** 469–473. (<https://doi.org/10.1016/j.biopha.2012.03.008>)
 - 40 Zhang GA, Hou S, Han S, Zhou J, Wang X & Cui W. Clinicopathological implications of leptin and leptin receptor expression in papillary thyroid cancer. *Oncology Letters* 2013 **5** 797–800. (<https://doi.org/10.3892/ol.2013.1125>)
 - 41 Yokota T, Oritani K, Takahashi I, Ishikawa J, Matsuyama A, Ouchi N, Kihara S, Funahashi T, Tenner AJ, Tomiyama Y, *et al.* Adiponectin, a new member of the family of soluble defense collagens, negatively regulates the growth of myelomonocytic progenitors and the functions of macrophages. *Blood* 2000 **96** 1723–1732.
 - 42 Kumada M, Kihara S, Ouchi N, Kobayashi H, Okamoto Y, Ohashi K, Maeda K, Nagaretani H, Kishida K, Maeda N, *et al.* Adiponectin specifically increased tissue inhibitor of metalloproteinase-1 through interleukin-10 expression in human macrophages. *Circulation* 2004 **109** 2046–2049. (<https://doi.org/10.1161/01.CIR.0000127953.98131.ED>)
 - 43 Takemura Y, Ouchi N, Shibata R, Aprahamian T, Kirber MT, Summer RS, Kihara S & Walsh K. Adiponectin modulates inflammatory reactions via calreticulin receptor-dependent clearance of early apoptotic bodies. *Journal of Clinical Investigation* 2007 **117** 375–386. (<https://doi.org/10.1172/JCI29709>)
 - 44 Yadav A, Kataria MA, Saini V & Yadav A. Role of leptin and adiponectin in insulin resistance. *Clinica Chimica Acta* 2013 **417** 80–84. (<https://doi.org/10.1016/j.cca.2012.12.007>)
 - 45 Yamauchi T, Kamon J, Minokoshi Y, Ito Y, Waki H, Uchida S, Yamashita S, Noda M, Kita S, Ueki K, *et al.* Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. *Nature Medicine* 2002 **8** 1288–1295. (<https://doi.org/10.1038/nm788>)
 - 46 Zakikhani M, Dowling RJ, Sonenberg N & Pollak MN. The effects of adiponectin and metformin on prostate and colon neoplasia involve activation of AMP-activated protein kinase. *Cancer Prevention Research* 2008 **1** 369–375. (<https://doi.org/10.1158/1940-6207.CAPR-08-0081>)
 - 47 Brakenhielm E, Veitonmäki N, Cao R, Kihara S, Matsuzawa Y, Zhivotovsky B, Funahashi T & Cao Y. Adiponectin-induced antiangiogenesis and antitumor activity involve caspase-mediated

- endothelial cell apoptosis. *PNAS* 2004 **101** 2476–2481. (<https://doi.org/10.1073/pnas.0308671100>)
- 48 Dalamaga M, Diakopoulos KN & Mantzoros CS. The role of adiponectin in cancer: a review of current evidence. *Endocrine Reviews* 2012 **33** 547–594. (<https://doi.org/10.1210/er.2011-1015>)
- 49 Ucan B, Sahin M, Kizilgul M, Ozbek M, Ozdemir S, Caliskan M & Cakal E. Serum ghrelin levels in papillary thyroid carcinoma. *Archives of Endocrinology and Metabolism* 2017 **61** 464–469. (<https://doi.org/10.1590/2359-39970000000290>)
- 50 Chopin L, Walpole C, Seim I, Cunningham P, Murray R, Whiteside E, Josh P & Herington A. Ghrelin and cancer. *Molecular and Cellular Endocrinology* 2011 **340** 65–69. (<https://doi.org/10.1016/j.mce.2011.04.013>)
- 51 Malendowicz W, Ziolkowska A, Szyszka M & Kwias Z. Elevated blood active ghrelin and unaltered total ghrelin and obestatin concentrations in prostate carcinoma. *Urologia Internationalis* 2009 **83** 471–475. (<https://doi.org/10.1159/000251190>)
- 52 Markowska A, Ziolkowska A, Jaszczyńska-Nowinka K, Madry R & Malendowicz LK. Elevated blood plasma concentrations of active ghrelin and obestatin in benign ovarian neoplasms and ovarian cancers. *European Journal of Gynaecological Oncology* 2009 **30** 518–522.
- 53 Samani AA, Yakar S, LeRoith D & Brodt P. The role of the IGF system in cancer growth and metastasis: overview and recent insights. *Endocrine Reviews* 2007 **28** 20–47. (<https://doi.org/10.1210/er.2006-0001>)
- 54 Delhanty PJ, Neggers SJ & van der Lely AJ. Des-acyl ghrelin: a metabolically active peptide. *Endocrine Development* 2013 **25** 112–121. (<https://doi.org/10.1159/000346059>)
- 55 Cassoni P, Allia E, Marrocco T, Ghè C, Ghigo E, Muccioli G & Papotti M. Ghrelin and cortistatin in lung cancer: expression of peptides and related receptors in human primary tumors and in vitro effect on the H345 small cell carcinoma cell line. *Journal of Endocrinological Investigation* 2006 **29** 781–790. (<https://doi.org/10.1007/BF03347371>)
- 56 Camiña JP, Campos JF, Caminos JE, Dieguez C & Casanueva FF. Obestatin-mediated proliferation of human retinal pigment epithelial cells: regulatory mechanisms. *Journal of Cellular Physiology* 2007 **211** 1–9. (<https://doi.org/10.1002/jcp.20925>)
- 57 Pazos Y, Alvarez CJ, Camiña JP & Casanueva FF. Stimulation of extracellular signal-regulated kinases and proliferation in the human gastric cancer cells Kato-III by obestatin. *Growth Factors* 2007 **25** 373–381. (<https://doi.org/10.1080/08977190801889313>)
- 58 Volante M, Rosas R, Ceppi P, Rapa I, Cassoni P, Wiedenmann B, Settanni F, Granata R & Papotti M. Obestatin in human neuroendocrine tissues and tumours: expression and effect on tumour growth. *Journal of Pathology* 2009 **218** 458–466. (<https://doi.org/10.1002/path.2551>)
- 59 Gurgul E, Kasprzak A, Blaszczyk A, Biczysko M, Surdyk-Zasada J, Seraszek-Jaros A & Ruchala M. Ghrelin and obestatin in thyroid gland – immunohistochemical expression in nodular goiter, papillary and medullary cancer. *Folia Histochemica et Cytobiologica* 2015 **53** 19–25. (<https://doi.org/10.5603/FHC.a2015.0004>)

Received in final form 27 May 2019

Accepted 17 June 2019

Accepted Preprint published online 17 June 2019