IJC
International Journal of Cancer

Cancer Epidemiology

Adipokines and inflammation markers and risk of differentiated thyroid carcinoma: The EPIC study

Laure Dossus[1], Silvia Franceschi[2], Carine Biessy[1], Anne-Sophie Navionis[1], Ruth C. Travis[3], Elisabete Weiderpass[4,5,6,7], Augustin Scalbert[1], Isabelle Romieu[1,8,9], Anne Tjønneland[10], Anja Olsen[10], Kim Overvad[11], Marie-Christine Boutron-Ruault[12,13], Fabrice Bonnet[12,13,14,15], Agnès Fournier[12,13], Renee T. Fortner[16], Rudolf Kaaks[16], Krasimira Aleksandrova[17], Antonia Trichopoulou[18], Carlo La Vecchia[18,19], Eleni Peppa[18], Rosario Tumino[20], Salvatore Panico[21], Domenico Palli[22], Claudia Agnoli[23], Paolo Vineis[24,25], H. B(as) Bueno-de-Mesquita[24,26,27,28], Petra H. Peeters[24,29], Guri Skeie[4], Raul Zamora-Ros[30], María-Dolores Chirlaque[31,32,33], Eva Ardanaz[31,34,35], Maria-Jose Sánchez[31,36], Jose Ramón Quirós[37], Miren Dorronsoro[38], Maria Sandström[39], Lena Maria Nilsson[40], Julie A. Schmidt[3], Kay-Tee Khaw[41], Konstantinos K. Tsilidis[24,42], Dagfinn Aune[24,43], Elio Riboli[24] and Sabina Rinaldi[1]

[1] Nutrition and Metabolism Section, International Agency for Research on Cancer, Lyon, France
[2] Infections Section, International Agency for Research on Cancer, Lyon, France
[3] Cancer Epidemiology Unit, Nuffield Department of Population Health, University of Oxford, Oxford, United Kingdom
[4] Department of Community Medicine, Faculty of Health Sciences, University of Tromsø, The Arctic University of Norway, Tromsø, Norway
[5] Cancer Registry of Norway, Institute of Population-Based Cancer Research, Oslo, Norway
[6] Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden
[7] Genetic Epidemiology Group, Folkhälsan Research Center, Helsinki, Finland
[8] Center for Research on Population Health, National Institute of Public Health, Cuernavaca, Morelos, Mexico
[9] Hubert Department of Global Health, Emory University, Atlanta, GA
[10] Danish Cancer Society Research Center, Copenhagen, Denmark
[11] Department of Public Health, Section for Epidemiology, Aarhus University, Aarhus, Denmark
[12] CESP, INSERM U1018, Univ. Paris-Sud, UVSQ, Université Paris-Saclay, Villejuif Cedex, France
[13] Gustave Roussy, Villejuif, France
[14] Department of Endocrinology, Rennes University Hospital (CHU), Rennes, France
[15] Rennes 1 University, Rennes, France
[16] Division of Cancer Epidemiology, German Cancer Research Center, Heidelberg, Germany
[17] German Institute of Human Nutrition Potsdam-Rehbruecke, Nuthetal, Germany
[18] Hellenic Health Foundation, Athens, Greece
[19] Department of Clinical Sciences and Community Health, Università degli Studi di Milano, Milan, Italy

Key words: thyroid cancer, inflammation, cytokine, adipokine, prospective cohort
Additional Supporting Information may be found in the online version of this article.

Grant sponsors: French National Cancer Institute (INCa), European Commission (DG-SANCO), International Agency for Research on Cancer, Danish Cancer Society (Denmark), Ligue Contre le Cancer, Institut Gustave Roussy, Mutuelle Générale de l'Education Nationale, Institut National de la Santé et de la Recherche Médicale (INSERM) (France), German Cancer Aid, German Cancer Research Center (DKFZ), Federal Ministry of Education and Research (BMBF), Deutsche Krebshilfe, Deutsches Krebsforschungszentrum, Federal Ministry of Education and Research (Germany), Hellenic Health Foundation (Greece), Associazione Italiana per la Ricerca sul Cancro-AIRC-Italy, National Research Council (Italy), Dutch Ministry of Public Health, Welfare and Sports (VWS), Netherlands Cancer Registry (NKR), LK Research Funds, Dutch Prevention Funds, Dutch ZON (Zorg Onderzoek Nederland), World Cancer Research Fund (WCRF), Statistics Netherlands (the Netherlands), Health Research Fund (FIS); **Grant numbers:** PI13/00061 (EPIC-Granada), PIE14/00045 (EPIC-Granada), PI13/01162 (EPIC-Murcia); **Grant sponsors:** Regional Governments of Andalucıa, Asturias, Basque Country, Murcia and Navarra, ISCIII RETIC (Spain); **Grant number:** RD06/0020; **Grant sponsors:** Swedish Cancer Society, Swedish Research Council and County Councils of Skane and Västerbotten (Sweden), Cancer Research UK; **Grant numbers:** 14136 (EPIC-Norfolk), C570/A16491 (EPIC-Oxford), C8221/A19170 (EPIC-Oxford); **Grant sponsor:** Medical Research Council; **Grant numbers:** 1000143 (EPIC-Norfolk), MR/M012190/1 (EPIC-Oxford)
DOI: 10.1002/ijc.31172
History: Received 18 Sep 2017; Accepted 3 Nov 2017; Online 23 Nov 2017
Correspondence to: Sabina Rinaldi, International Agency for Research on Cancer, 150 Cours Albert Thomas, 69372 Lyon Cedex 08, France, E-mail: rinaldis@iarc.fr; Tel: +33 4 72 73 83 28

[20] Cancer Registry and Histopathology Department, "Civic-M.P. Arezzo" Hospital, ASP Ragusa, Italy
[21] Dipartimento di Medicina Clinica e Chirurgia, Federico II University, Naples, Italy
[22] Cancer Risk Factors and Life-Style Epidemiology Unit, Cancer Research and Prevention Institute – ISPO, Florence, Italy
[23] Epidemiology and Prevention Unit, Fondazione IRCCS Istituto Nazionale dei Tumori, Milano, Italy
[24] Department of Epidemiology and Biostatistics, School of Public Health, Imperial College London, London, United Kingdom
[25] Italian Institute for Genomic Medicine (IIGM), Torino, Italy
[26] Department for Determinants of Chronic Diseases (DCD), National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands
[27] Department of Gastroenterology and Hepatology, University Medical Centre, Utrecht, The Netherlands
[28] Department of Social & Preventive Medicine, Faculty of Medicine, University of Malaya, Pantai Valley, Kuala Lumpur, Malaysia
[29] Department of Epidemiology, Julius Center for Health Sciences and Primary Care, University Medical Center, Utrecht, The Netherlands
[30] Unit of Nutrition and Cancer, Cancer Epidemiology Research Programme, Catalan Institute of Oncology, Bellvitge Biomedical Research Institute (IDIBELL), Barcelona, Spain
[31] CIBER de Epidemiología y Salud Pública (CIBERESP), Madrid, Spain
[32] Department of Epidemiology, Murcia Regional Health Council, Murcia, Spain
[33] Department of Health and Social Sciences, Universidad de Murcia, Murcia, Spain
[34] Navarra Public Health Institute, Pamplona, Spain
[35] IdiSNA, Navarra Institute for Health Research, Pamplona, Spain
[36] Escuela Andaluza de Salud Pública, Instituto de Investigación Biosanitaria (ibs.GRANADA), Hospitales Universitarios de Granada/Universidad de Granada, Granada, Spain
[37] Public Health Directorate, Asturias, Spain
[38] Basque Regional Health Department, Public Health Direction and Biodonostia Research Institute CIBERESP, San Sebastian, Spain
[39] Department of Radiation Sciences, Oncology, Umeå University, Umeå, Sweden
[40] Department of Public Health and Clinical Medicine, Nutritional Research, Umeå University, Umeå, Sweden
[41] Cancer Epidemiology Unit, University of Cambridge, Cambridge, United Kingdom
[42] Department of Hygiene and Epidemiology, University of Ioannina School of Medicine, Ioannina, Greece
[43] Bjørknes University College, Oslo, Norway

Other than the influence of ionizing radiation and benign thyroid disease, little is known about the risk factors for differentiated thyroid cancer (TC) which is an increasing common cancer worldwide. Consistent evidence shows that body mass is positively associated with TC risk. As excess weight is a state of chronic inflammation, we investigated the relationship between concentrations of leptin, adiponectin, C-reactive protein, interleukin (IL)-6, IL-10 and tumor necrosis factor (TNF)-α and the risk of TC. A case-control study was nested within the European Prospective Investigation into Cancer and Nutrition (EPIC) study and included 475 first primary incident TC cases (399 women and 76 men) and 1,016 matched cancer-free cohort participants. Biomarkers were measured in serum samples using validated and highly sensitive commercially available immunoassays. Odds ratios (ORs) of TC by levels of each biomarker were estimated using conditional logistic regression models, adjusting for BMI and alcohol consumption. Adiponectin was inversely associated with TC risk among women ($OR_{T3vs.T1} = 0.69$, 95% CI: 0.49–0.98, $P_{trend} = 0.04$) but not among men ($OR_{T3vs.T1} = 1.36$, 95% CI: 0.67–2.76, $P_{trend} = 0.37$). Increasing levels of IL-10 were positively associated with TC risk in both genders and significantly so in women ($OR_{T3vs.T1} = 1.59$, 95% CI: 1.13–2.25, $P_{trend} = 0.01$) but not in men ($OR_{T3vs.T1} = 1.78$, 95% CI: 0.80–3.98, $P_{trend} = 0.17$). Leptin, CRP, IL-6 and TNF-α were not associated with TC risk in either gender. These results indicate a positive association of TC risk with IL-10 and a negative association with adiponectin that is probably restricted to women. Inflammation may play a role in TC in combination with or independently of excess weight.

What's new?

How does being overweight lead to thyroid cancer? These authors investigated, using data from the EPIC cohort. Considering obesity as a state of chronic inflammation, they looked at levels of various proteins associated with inflammation, including C-reactive protein, IL-10, adiponectin, and others, and compared these with TC risk. Women with high adiponectin levels were less likely to develop thyroid cancer, while those with high IL-10 levels had increased TC risk. No significant association was seen in men.

Introduction

In Western countries, thyroid cancer incidence has been increasing over the last two decades thereby becoming the second most commonly diagnosed cancer after breast cancer in women younger than 45 years.[1,2] The increase in the search of thyroid nodules mainly through ultrasonography has led to vast increases in differentiated thyroid cancers (TC), the most common form of thyroid cancer that includes papillary and follicular carcinomas.[2] Exposure to ionizing radiations and history of benign thyroid diseases are the only

relatively strong risk factors for TC though unlikely the reason of the upward incidence trends.[2–4] Other possible risk factors are ill-understood. There is consistent evidence that excess body weight and obesity are associated with a modest increase in risk of TC,[5–7] and the mechanisms underlying this association may involve inflammation.[8,9] Indeed, the adipose tissue of overweight individuals is characterized by the infiltration of macrophages resulting in chronic systemic inflammation and in the release of cytokines and adipokines into the circulation.[10] Adipose tissue secretes hundreds of adipokines of which the best studied are leptin and adiponectin, which regulate important biological processes such as appetite, insulin sensitivity, thermogenesis, fatty acid oxidation and immune response.[11] Adipokine secretion is altered in adipose tissue dysfunction and may contribute to a spectrum of obesity-associated diseases.[9]

Both experimental and epidemiological data suggest that local organ-specific inflammation plays a role in TC and benign thyroid conditions. TC is often characterized by the infiltration of inflammation-immune cells, and autoimmune thyroid diseases, such as Hashimoto's thyroiditis and Grave's disease, have been associated, in some studies, with an increased risk of TC.[12,13] Systemic inflammation, characterized by higher circulating levels of cytokines, has also been observed in patients with Graves' disease, hyperthyroidism and toxic nodular goiter.[14,15] Systemic inflammation has also been associated with the development of several cancer types and previous reports have shown that elevated levels of cytokines and low levels of adiponectin were associated with an increased risk of endometrial,[16–18] breast,[19] ovarian,[20] liver[21] and colon[22] cancers.

Few retrospective case-control studies, mostly with very small sample size (including between 20 and 163 subjects), reported higher circulating levels of some cytokines (including C-reactive protein [CRP], interleukin [IL]-6, IL-10, tumor necrosis factor [TNF]-α) as well as leptin, among TC cases.[23–27] Conversely, circulating levels of adiponectin, an adipokine that is inversely related to adiposity, were found to be significantly lower in 175 TC cases than in 107 controls.[28]

We have set up a study nested within the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort to investigate the relationship between the risk of TC and the prediagnostic concentrations of leptin, adiponectin, CRP, IL-6, IL-10 and TNF-α. To our knowledge, this is the first prospective study investigating the association of prediagnostic levels of adipokines and inflammation markers with TC risk.

Methods

Study population and blood sample collection

The EPIC cohort is a large, multicenter prospective study, designed to investigate the associations between nutritional, lifestyle, metabolic and genetic risk factors and cancer. It was initiated in 1992 in 10 European countries (Denmark, France, Germany, Greece, Italy, the Netherlands, Norway, Spain, Sweden and United Kingdom) and involved about 370,000 women and 150,000 men. Study population and baseline data collection have previously been described in details.[29] In brief, questionnaires included data about diet, reproductive history, use of exogenous hormones, tobacco smoking and alcohol consumption, education level, occupational history, physical activity and history of selected diseases. Anthropometric variables were measured according to standardized protocols.[29]

About 246,000 women and 140,000 men also provided a blood sample, collected according to a standardized protocol in France, Germany, Greece, Italy, the Netherlands, Norway, Spain and the United Kingdom.[29] From each subject, about 30 ml of blood were drawn, and serum (except in Norway), plasma, erythrocytes and buffy coat were aliquoted in plastic straws of 0.5 ml each, which were stored in liquid nitrogen (–196°C) in a centralized biobank. In Denmark, blood fractions were aliquoted into 1 ml tubes, and stored in the vapor phase of liquid nitrogen containers (–150°C). In the Swedish center of Umea, blood samples were divided into 10 aliquots of 1.5 ml each (six plasma, two buffy coat and two erythrocytes), which were rapidly frozen at −80°C in standard freezers.

All participants have given their consent to participate into the EPIC study. The Internal Review Board of IARC and local institutional review boards in participating centers have approved the study.

Follow-up for cancer incidence and vital status

Incident cancer cases were identified through record linkage with regional cancer registries in most countries and through health insurance records, cancer and pathology registries and active follow-up of study subjects in France, Germany and Greece. Data on vital status were obtained from mortality registries at the regional or national level, in combination with data collected by active follow-up (Greece). For each EPIC center, closure dates of the study period were defined as the latest dates of complete follow-up for both cancer incidence and vital status (dates varied between centers, from June 2008 to December 2013).

Selection of cases and controls

Case subjects were selected among participants who were cancer-free (other than nonmelanoma skin cancer), had donated blood at recruitment into the cohort and who were diagnosed with TC between recruitment and the end of follow-up. Cases were coded according to the 10th revision of the WHO International Classification of Disease (code C73). This analysis focused on differentiated thyroid cancer, i.e., papillary (morphologic codes: 8050, 8130, 8260, 8340–8344 and 8350), follicular carcinomas (8290, 8330–8335) and not otherwise specified, which are likely to also be papillary (8000, 8010, 8140). Thyroid cancer cases with rare or missing histological types (37 medullary, 9 anaplastic, 1 lymphoma, 4 other morphologies and 1 missing) were not included. A total

of 475 incident TC cases were included (363 papillary, 84 follicular and 28 not otherwise specified TC). Tumor-node-metastasis (TNM) stage was known for 56.4% of the eligible cases and used to group cancers into localized (T1) and more advanced (≥T2) cancers.

For each case subject with TC, two control subjects for women, and three for men were chosen at random among appropriate risk sets consisting of all cohort members who were alive and without a reported cancer diagnosis (except nonmelanoma skin cancer) at the time of diagnosis of the index case. Matching procedures have been described before.[30] In brief, matching variables included study recruitment center, sex, age, date, time, fasting status at blood collection and duration of follow-up (duration for the control must be greater than for the index case). A total of 1,016 controls were selected for the study.

Laboratory measurements

Serum was used for laboratory assays except for samples from Norway (citrated plasma) and Umea, Sweden (heparin plasma). Leptin was measured by immunoradiometric assay (IDS, Paris, France), while adiponectin and CRP were measured by enzyme-linked immunosorbent assays (R&D, United Kingdom). IL-6, IL-10 and TNF-α were measured by a highly sensitive multiplexing electrochemiluminescent method (V-PLEX^{TM} Custom Human Cytokine Kit, Meso Scale Discovery, Rockville, MD).

All assays were performed at IARC by technicians who were blind to case-control status of the subjects. Samples from cases and matched controls were analyzed together, within the same analytical batch. For quality control, three plasma samples from nondiseased subjects were analyzed in duplicate within each analytical batch. Mean intrabatch coefficients of variation, calculated on the concentrations from the quality control samples, varied between 2.6% for CRP, to 9.9% for IL-10.

Of a total of 1,491 subjects, 5 had missing values for adiponectin, 6 for CRP, 37 for leptin and 49 for IL-6, IL-10 and TNF-α. These subjects were excluded from the analyses for each particular biomarker. Twenty-seven values (1.8%) were below the limit of quantification (LOQ) for CRP, 75 (5%) for IL-10 and 46 (3%) for IL-6. These values were set to the LOQ: 78 pg/ml for CRP, 0.15 pg/ml for IL-10 and 0.16 pg/ml for IL-6. No value was below the LOQ for leptin, adiponectin or TNF-α.

Statistical analyses

Participant characteristics (means or percentages) were compared between cases and controls within matched sets, for men and women separately, using conditional logistic regression. Concentrations of the biomarkers were transformed logarithmically to approximate the normal distribution in all parametric analyses.

Prediagnostic levels of inflammation markers among controls were compared between men and women, using Mann-Whitney-Wilcoxon test. Spearman's partial correlation coefficients between inflammation markers and lifestyle factors were calculated among controls, adjusting for age at blood donation (continuous) and laboratory batch.

Odds ratios (ORs) and corresponding 95% confidence intervals (CIs) for TC in relation to sex-specific tertiles of inflammation markers (based on the distribution of controls) were estimated using logistic regression conditional on matching factors (basic models), separately for men and women. Tests for trends in ORs by tertiles were computed by assigning consecutive scores to the tertiles.

The effects of potential confounders (additionally to the matching criteria, controlled for by design) were examined by including additional terms into the logistic regression models. The following confounders were tested: smoking status, duration of smoking, physical activity, alcohol consumption, education level, age at menarche, parity, age at first FTP, number of FTP, breast feeding, menopausal status, exogenous hormone use, anthropometric variables (height, BMI, WC, HC and WHR). Only BMI and alcohol consumption affected point estimates by >10% and were therefore retained in the adjusted models.

For analyses of statistical heterogeneity by selected cofactors, *p*-values for heterogeneity were derived by testing an interaction term between the tertile score of inflammation markers and the cofactor.

All statistical tests and corresponding *p*-values were two-sided, and *p*-values <0.05 were considered statistically significant. All analyses were performed using the SAS software package (Version 9.4, SAS Institute, Cary, NC).

Results

Baseline characteristics of the study population are described in Table 1. Cases were diagnosed at a mean age of ∼58 years, after an average follow-up time of about 8 years. Among women, TC cases were significantly taller, had a larger BMI and waist circumference and a lower alcohol intake than controls. The associations with height and alcohol intake but not BMI and waist circumference, were in the same direction in men as in women but no associations were significant, possibly due to the lower number of male than female cases.

Supporting Information Table 1 shows prediagnostic levels of inflammation markers in controls by gender. Levels of leptin and adiponectin were significantly higher while TNF-α levels were significantly lower in women than in men. Levels of CRP and other cytokines were similar in both genders.

Table 2 shows correlations between different inflammation markers, age and anthropometric indexes. The strongest correlations (≥0.49) were found between leptin and BMI and waist circumference in both genders. Adiponectin was moderately inversely correlated with BMI, waist circumference and leptin but positively correlated with age (∼±0.20). CRP, IL-6 and TNF-α were also moderately correlated with BMI, waist circumference and leptin and with each other. None of

Table 1. Selected characteristics of the study population at blood collection by gender

	Women			Men		
	Cases	Controls	*p*-Value[1]	Cases	Controls	*p*-Value[1]
Number	399	791	–	76	225	–
Age at blood collection (years)[2]	50.2 (8.5)	50.2 (8.6)	–	49.9 (8.9)	49.8 (8.7)	–
Age at diagnosis (years)[2]	58.4 (8.6)	–	–	58.5 (9.5)	–	–
Years between blood collection and cancer diagnosis[2]	8.2 (4.4)	–	–	8.6 (4.7)	–	–
Height (cm)[2]	161.3 (6.3)	160.2 (6.6)	0.002	176.5 (6.7)	175.3 (6.3)	0.14
Body mass index (kg/m^2)[2]	26.1 (4.5)	25.5 (4.6)	0.02	26.4 (3.0)	26.4 (3.4)	0.87
Waist circumference (cm)[2]	81.9 (11.3)	80.3 (11.1)	0.01	94.6 (10.0)	94.5 (9.9)	0.95
Alcohol intake (g/day)[2]	6.1 (9.0)	7.5 (10.9)	0.02	17.5 (18.3)	19.5 (23.6)	0.48
Smoking status (%)[3]						
Never smoker	60	60	0.96	32	35	0.77
Ex smoker	21	21		35	35	
Current smoker	19	18		33	30	
Education level (%)[3]						
None or primary	42	41	0.83	30	30	0.99
Secondary or more	58	59		70	70	

[1]From logistic regression conditional on matching factors.
[2]Mean and standard deviation.
[3]Percentage excluding participants with missing variables.

Table 2. Spearman correlation coefficients between prediagnostic levels of inflammation markers[1] and age and anthropometric factors among controls and corresponding *p*-values[2] by gender

	Age	Alcohol intake	BMI	Waist circ.	Height	Leptin	Adiponectin	CRP	IL-10	IL-6
					Women					
Leptin	0.11	−0.12	**0.64**	**0.59**	−0.10					
Adiponectin	**0.21**	0.13	**−0.18**	**−0.23**	0.01	**−0.22**				
CRP	**0.17**	−0.07	**0.41**	**0.39**	−0.08	**0.36**	**−0.17**			
IL-10	0.01	−0.02	0.08	0.07	−0.04	0.07	−0.01	0.13		
IL-6	**0.15**	−0.13	**0.37**	**0.33**	−0.12	**0.25**	−0.10	**0.36**	**0.26**	
TNF-α	**0.17**	**−0.15**	**0.26**	**0.25**	**−0.17**	**0.20**	−0.07	**0.18**	**0.33**	**0.42**
					Men					
Leptin	0.16	0.05	**0.49**	**0.51**	0.08					
Adiponectin	0.23	0.03	−0.21	−0.18	0.12	0.02				
CRP	0.09	0.06	0.24	0.18	−0.10	**0.26**	−0.17			
IL-10	0.05	0.01	−0.02	0.02	−0.06	−0.04	−0.06	0.12		
IL-6	0.24	0.03	0.12	0.15	0.08	0.23	−0.15	**0.48**	0.21	
TNF-α	0.01	−0.03	0.05	0.03	0.01	0.00	−0.19	0.17	**0.44**	**0.38**

CRP, C-reactive protein; IL, interleukin; TNF, tumor necrosis factor.
[1]Adjusted for laboratory batch and age at blood donation (when appropriate).
[2]*p*-Values <0.0001 in bold.

the inflammation markers was substantially correlated with IL-10 or height.

Table 3 presents the association between tertiles of inflammation markers and TC risk, separately for women and men. Among women, a statistically significant inverse association was observed in the basic model between TC risk and adiponectin ($OR_{T3vs.T1} = 0.67$, 95% CI: 0.48–0.93, $P_{trend} = 0.02$) while IL-10 was positively associated ($OR_{T3vs.T1} = 1.54$, 95% CI: 1.10–2.16, $P_{trend} = 0.02$). Adjustment for TC risk factors did not substantially modify these findings ($OR_{T3vs.T1} = 0.69$,

Table 3. Odds ratio (OR) of differentiated TC by inflammation marker tertile in women and men

	Women				Men				P interaction by gender
	Tertiles[1]			P_{trend}[2]	Tertiles[1]			P_{trend}[2]	
Leptin (ng/ml)	<15.6	15.6–29.1	>=29.1		<7.6	7.6–11.6	>=11.6		0.79
Cases/ controls	113/250	147/250	128/249		26/73	25/73	24/72		
OR [95% CI]: basic model[3]	1.00	1.30 [0.95; 1.77]	1.14 [0.81; 1.59]	0.47	1.00	0.94 [0.50; 1.79]	0.92 [0.47; 1.79]	0.80	
OR [95% CI]: adj. model[4]	1.00	1.14 [0.82; 1.60]	0.87 [0.57; 1.31]	0.47	1.00	0.93 [0.48; 1.81]	0.93 [0.44; 1.95]	0.84	
Adiponectin (ng/ml)	<7,654.9	7,654.9–12,441.9	>=12,441.9		<4,259.7	4,259.7–6257.8	>=6,257.8		0.07
Cases/ controls	155/262	130/262	111/261		23/75	23/75	30/75		
OR [95% CI]: basic model[3]	1.00	0.79 [0.58; 1.07]	0.67 [0.48; 0.93]	0.02	1.00	1.01 [0.51; 2.01]	1.35 [0.67; 2.72]	0.38	
OR [95% CI]: adj. model[4]	1.00	0.84 [0.61; 1.14]	0.69 [0.49; 0.98]	0.04	1.00	1.02 [0.51; 2.04]	1.36 [0.67; 2.76]	0.37	
C-reactive protein (ng/ml)	<722.1	722.1–1,887.8	>=1,887.8		<754.7	754.7–2,055.5	>=2,055.5		0.34
Cases/ controls	135/262	121/261	140/261		28/75	29/75	19/74		
OR [95% CI]: basic model[3]	1.00	0.88 [0.65; 1.20]	1.05 [0.77; 1.41]	0.77	1.00	1.01 [0.54; 1.92]	0.68 [0.35; 1.34]	0.26	
OR [95% CI]: adj. model[4]	1.00	0.85 [0.62; 1.17]	0.94 [0.68; 1.30]	0.70	1.00	1.02 [0.53; 1.95]	0.68 [0.34; 1.36]	0.27	
IL-10 (pg/ml)	<0.22	0.22–0.31	>=0.31		<0.22	0.22–0.32	>=0.32		0.82
Cases/ controls	93/251	155/251	137/251		20/74	27/74	29/74		
OR [95% CI]: basic model[3]	1.00	1.69 [1.23; 2.32]	1.54 [1.10; 2.16]	0.02	1.00	1.61 [0.75; 3.46]	1.81 [0.81; 4.02]	0.17	

Table 3. Odds ratio (OR) of differentiated TC by inflammation marker tertile in women and men (Continued)

	Women				Men				P interaction by gender
	Tertiles[1]			P_{trend}[2]	Tertiles[1]			P_{trend}[2]	
OR [95% CI]: adj. model[4]	1.00	1.67 [1.21; 2.30]	1.59 [1.13; 2.25]	0.01	1.00	1.59 [0.74; 3.43]	1.78 [0.80; 3.98]	0.19	
IL-6 (pg/ml)	<0.34	0.34–0.53	>=0.53		<0.32	0.32–0.58	>=0.58		0.31
Cases/ controls	110/251	131/251	144/251		19/74	41/74	16/74		
OR [95% CI]: basic model[3]	1.00	1.23 [0.89; 1.68]	1.38 [0.99; 1.94]	0.06	1.00	2.11 [1.10; 4.06]	0.85 [0.39; 1.85]	0.67	
OR [95% CI]: adj. model[4]	1.00	1.20 [0.87; 1.66]	1.26 [0.88; 1.80]	0.21	1.00	2.09 [1.09; 4.03]	0.84 [0.39; 1.85]	0.68	
TNF-α (pg/ml)	<1.52	1.52–1.95	>=1.95		<1.63	1.63–2.04	>=2.04		0.53
Cases/ controls	125/252	121/251	138/251		26/74	27/74	23/74		
OR [95% CI]: basic model[3]	1.00	1.00 [0.72; 1.38]	1.17 [0.83; 1.64]	0.39	1.00	1.02 [0.53; 1.97]	0.87 [0.41; 1.85]	0.73	
OR [95% CI]: adj. model[4]	1.00	0.95 [0.68; 1.32]	1.08 [0.76; 1.53]	0.66	1.00	1.01 [0.52; 1.95]	0.86 [0.40; 1.84]	0.71	

[1]Tertiles based on the gender-specific distribution of controls.
[2]P_{trend} values were computed by assigning consecutive scores (scores of 1–3) to the categories.
[3]Logistic regression conditional on matching factors.
[4]Logistic regression conditional on matching factors and adjusted for BMI, and alcohol consumption.

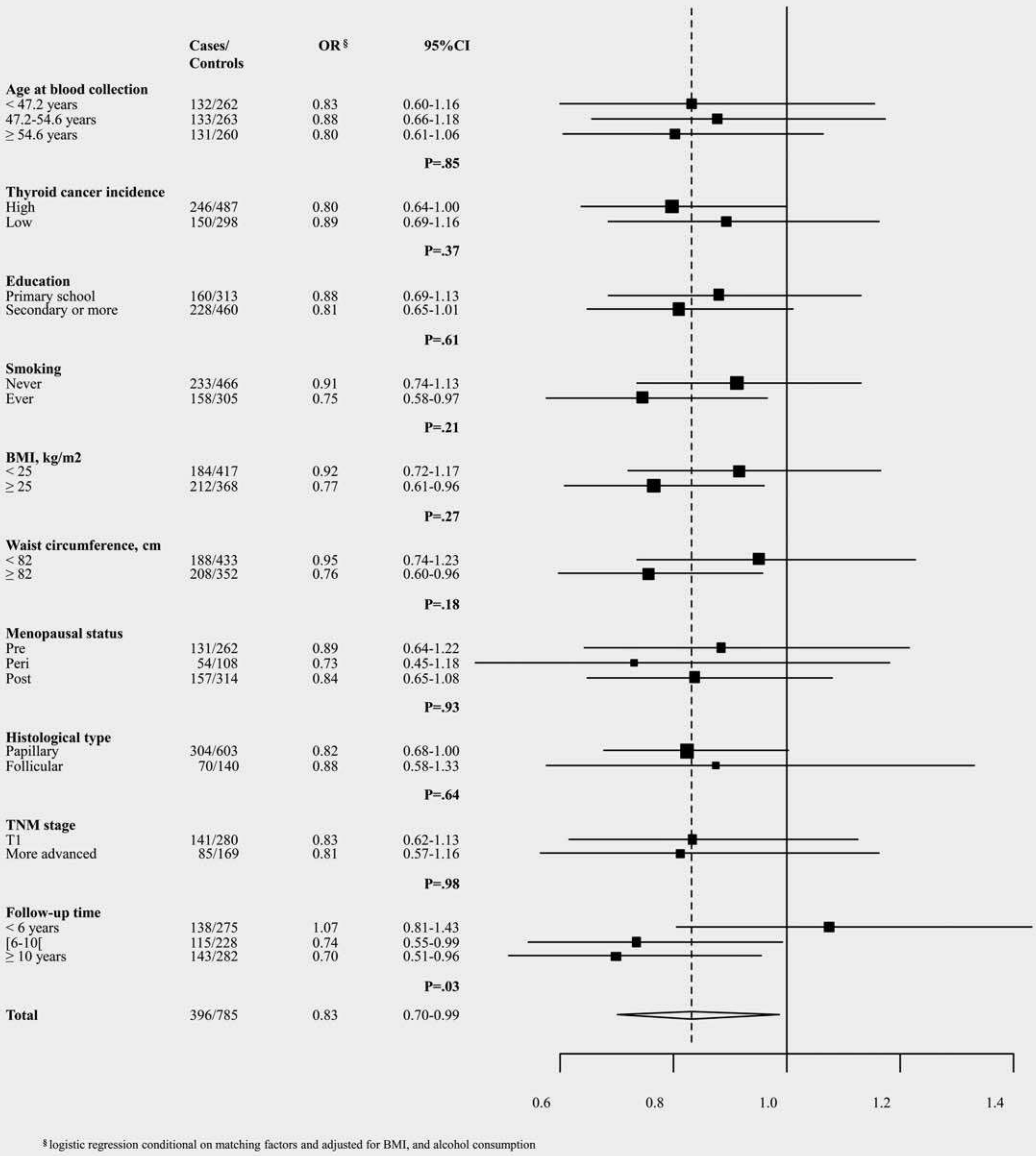

Figure 1. Odds ratios of differentiated TC for an increase in one tertile of adiponectin among women, stratified by selected variables.

95% CI: 0.49–0.98, $P_{trend} = 0.04$ for adiponectin, and $OR_{T3vs.T1} = 1.59$, 95% CI: 1.13–2.25, $P_{trend} = 0.01$ for IL-10). None of the markers were significantly associated with TC risk among men ($P_{trend} \geq 0.17$) but the positive association with IL-10 was similar to the one seen among women ($OR_{T3vs.T1} = 1.81$, 95% CI: 0.81–4.02 in the basic model; $OR_{T3vs.T1} = 1.78$, 95% CI: 0.80–3.98 in the adjusted model). Similar results were observed when analyses were restricted to papillary TC (Supporting Information Table 2). When genders were combined, the negative association with adiponectin was no longer significant ($OR_{T3vs.T1} = 0.79$, 95% CI: 0.58–1.07, $P_{trend} = 0.13$), whereas a positive significant association with IL-10 was confirmed ($OR_{T3vs.T1} = 1.61$, 95% CI: 1.18–2.21, $P_{trend} = 0.005$) (Supporting Information Table 3).

No statistically significant heterogeneity was observed in the inverse association between adiponectin and TC risk in women by age at blood collection, national TC incidence, education, smoking, BMI, waist circumference, menopausal status, histologic type, TNM stage (Fig. 1) and country (data not shown). The negative association with adiponectin was, however, absent among women in whom the interval between blood collection and TC diagnosis was <6 years (OR: 1.07, 95% CI: 0.81–1.43).

Finally, we explored the effect of adiponectin (middle and highest tertile *vs.* lowest tertile) according to BMI (<25 or ≥25; data not shown). The OR was 0.96 (95% CI: 0.62–1.46) among normal-weight women and 0.66 (95% CI: 0.46–0.95) among overweight and obese women. Although, therefore, the negative association of high adiponectin level with TC

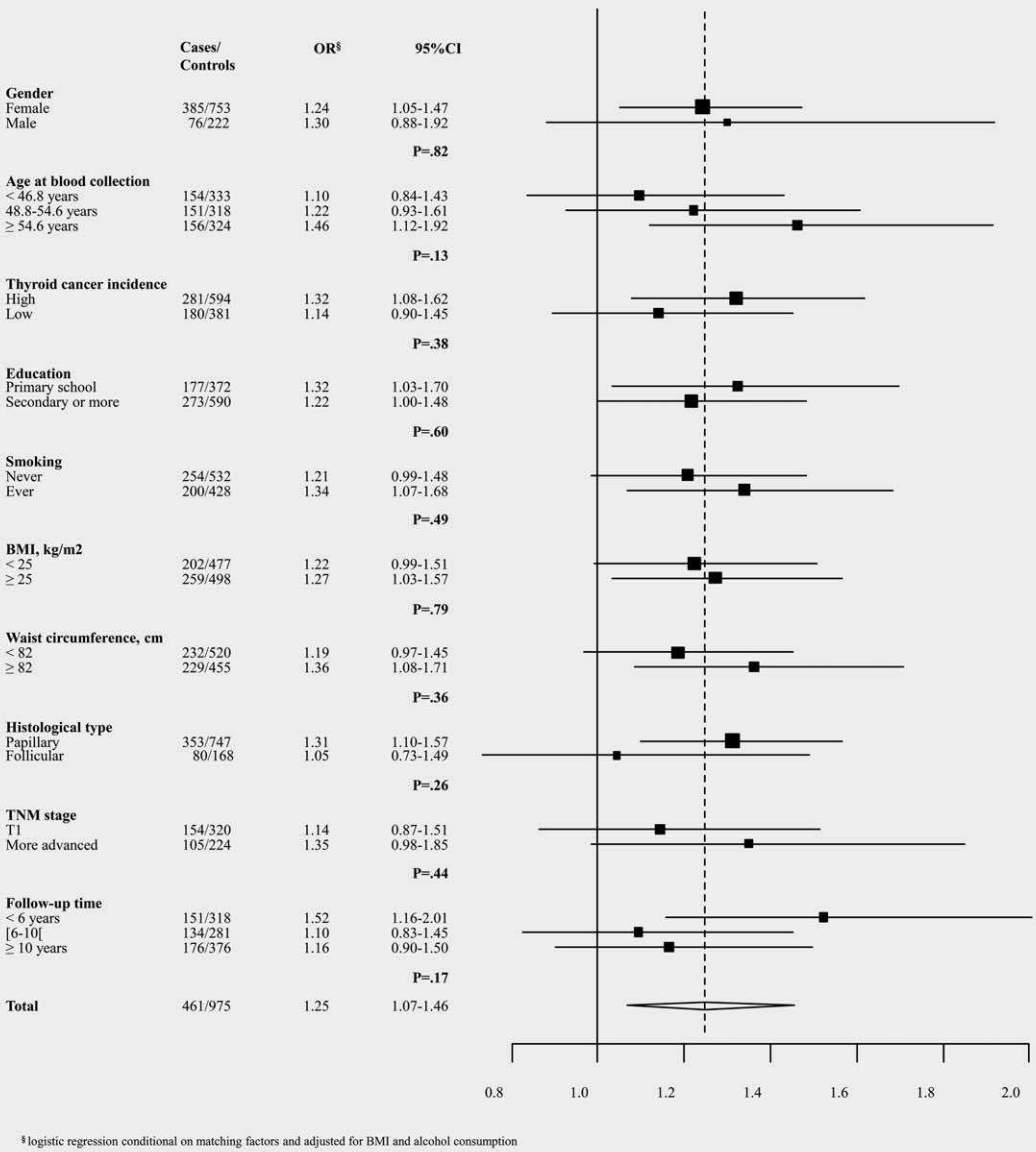

Figure 2. Odds ratios of differentiated TC for an increase in one tertile of IL-10, stratified by selected variables.

risk was only significant the heaviest women the 95% CI of the two ORs broadly overlap. Figure 2 (which includes both women and men) shows lack of significant heterogeneity in the positive association of TC risk with IL-10 by the same variables as in Figure 1 and country (data not shown).

Discussion

We showed for the first time in a prospective study that high adiponectin levels were associated with a lower risk of TC among women while elevated IL-10 levels were associated with an increased risk of TC probably in both genders. Leptin, CRP, IL-6 and TNF-α were not associated with TC risk in either gender.

Our results on adiponectin are consistent with the inverse association observed with papillary TC in a previous case-control study.[28] Although Mitsiades *et al.*[28] did not present data for women only, 90% of the 175 TC cases included were women. The lack of significant association with adiponectin in men, who were a minority also in our study, may be due to chance or may suggest that the association may be more relevant in women, as possibly suggested by the substantially higher adiponectin levels compared to men. Prospective studies have shown a negative association of adiponectin levels with the risk of women's cancers related to weight excess such as postmenopausal breast and endometrial cancers.[18,31–33] A recent metaanalysis of 107 studies[34] showed however a negative association of adiponectin with a broad range of cancers, including gastrointestinal and prostate cancers, in both case-control and prospective studies.

Several experimental *in vivo* and *in vitro* studies also reported the expression of adiponectin receptors on thyroid cancer cells and tissue.[28,35] Adiponectin is an adipokine with

antiinflammatory, and also antidiabetic, antiatherogenic and antiangiogenic properties.[36–38] The mechanisms by which adiponectin may act on thyroid cancer still remain to be identified but may include a protection against the development of insulin resistance,[39] in particular through the activation of the adenosine monophosphate kinase (AMPK) pathway.[35,40,41] Adiponectin has also been shown to directly inhibit angiogenesis and promote apoptosis *in vivo*, through the activation of the caspase cascade.[42] Adiponectin has been proposed as a possible mediator in the association between BMI and cancer risk.[31] Alternative pathways that may mediate the BMI-TC association include hyperinsulinemia, metabolic syndrome, insulin-like growth factors or sex steroids.[9]

In our study, adiponectin was inversely correlated with BMI and waist circumference in both genders, but the negative association with TC risk was only significant in women with BMI $\geq$25. However, the 95% CIs of the ORs in women whose BMI was $<$25 or $\geq$25 broadly overlapped. We cannot therefore draw conclusions on whether adiponectin plays a role in TC in addition to or independently of excess weight. A number of relatively small studies have compared levels of leptin, circulating cytokines, including IL-1β, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-13, TNF-α, IFN-γ and CRP, among TC cases and controls but results were not consistent across studies.[23–25,27,43–45] In prediagnostic samples, we observed no significant associations with leptin, CRP, IL-6 and TNF-α. Only IL-10 was associated with an increased risk of TC consistently in women and men, although the association was statistically significant only in women.

Three case-control studies ($N < 23$ TC cases) examined the association between circulating levels of IL-10 and TC risk.[24,44,45] Only one of them reported significantly higher IL-10 levels among cases than among healthy controls.[24] In addition, two out of three other case-control studies observed a significant difference between TC cases and controls in IL-10–1082 G/A (rs1800896), a genetic polymorphism associated with an increase in the production of IL-10.[46–48] *In vitro* studies have shown that autocrine production of IL-10 might favour thyroid cancer cell survival and proliferation[49] and higher IL-10 levels have been observed in patients with persistent/recurrent disease.[50] IL-10 is an antiinflammatory cytokine characteristic of Th2 immunity and might therefore counteract Th1 immune response and favour the escape of tumor cells from immune destruction.[51] IL-10 has been shown to favour the progression and metastasis of several tumors,[51] while inconsistent effects have been observed on the proliferation of breast tumor cells.[52,53]

The main strength of our study includes its large sample size and its prospective design. As circulating levels of inflammation markers might be affected by the presence of the tumor, as well by diagnostic and therapeutic procedures, pre-diagnostic concentrations are essential when examining the etiologic role of inflammation in TC development to avoid reverse causation bias. Our study has also limitations, particularly the fact that we only measured inflammation markers at one point in time. However, most inflammation markers have shown good reproducibility in samples collected at different time points.[54] Repeated measurements of inflammation markers and/or anthropometry and more in-depth assessment of fat distribution would nevertheless improve the classification of individuals and therefore lead to stronger associations with TC risk. The limited sample size in men that reflects however the epidemiology of TC limit the interpretation of the results in this group. In addition, although information was available on histological subtypes of TC, EPIC data did not include history of benign thyroid diseases, thyroidectomy among control subjects and use of drugs that could interfere with thyroid function. Likewise, individual information on iodine deficiency and past exposure to ionizing radiations have not been collected but we know that severe iodine deficiency is rare in EPIC countries and that exposure to ionizing radiation due to previous cancer treatment is unlikely because we excluded prevalent cancer cases other than nonmelanoma skin cancers. Finally, as the increase in incidence observed over the last decades in differentiated thyroid carcinomas is largely driven by changes in diagnostic practices,[2] an additional limitation is that we do not have any indication on how the cancers have been diagnosed.

In conclusion, our results suggest a possible negative association of TC risk with prediagnostic circulating levels of adiponectin in women and an overall positive association with those of IL-10. Longer follow-up in EPIC or the combination of EPIC data with additional cohort studies are necessary to understand whether adiponectin and, possibly, other inflammation markers play a role in TC risk in combination with or independently of excess weight.

Acknowledgements

The authors thank all participants in the EPIC cohort for their invaluable contribution to the study. The authors also thank Bertrand Hémon (IARC) for his precious help with the EPIC database. For information on how to submit an application for gaining access to EPIC data and/or biospecimens, please follow the instructions at http://epic.iarc.fr/access/index.php.

REFERENCES

1. Ferlay J, Soerjomataram I, Dikshit R, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer* 2015;136:E359–86.
2. Vaccarella S, Franceschi S, Bray F, et al. Worldwide thyroid-cancer epidemic? The increasing impact of overdiagnosis. *N Engl J Med* 2016;375:614–7.
3. Sinnott B, Ron E, Schneider AB. Exposing the thyroid to radiation: a review of its current extent, risks, and implications. *Endocr Rev* 2010;31:756–73.
4. Franceschi S, Preston-Martin S, Dal Maso L, et al. A pooled analysis of case-control studies of thyroid cancer. IV. Benign thyroid diseases. *Cancer Causes Control* 1999;10:583–95.
5. Schmid D, Ricci C, Behrens G, et al. Adiposity and risk of thyroid cancer: a systematic review

and meta-analysis. *Obes Rev* 2015;16:1042–54.
6. Kitahara CM, McCullough ML, Franceschi S, et al. Anthropometric factors and thyroid cancer risk by histological subtype: pooled analysis of 22 prospective studies. *Thyroid* 2016;26:306–18.
7. Lauby-Secretan B, Scoccianti C, Loomis D, et al. Body fatness and cancer—viewpoint of the IARC Working Group. *N Engl J Med* 2016;375:794–8.
8. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011;144:646–74.
9. Renehan AG, Roberts DL, Dive C. Obesity and cancer: pathophysiological and biological mechanisms. *Arch Physiol Biochem* 2008;114:71–83.
10. Wellen KE, Hotamisligil GS. Obesity-induced inflammatory changes in adipose tissue. *J Clin Invest* 2003;112:1785–8.
11. Stern JH, Rutkowski JM, Scherer PE. Adiponectin, leptin, and fatty acids in the maintenance of metabolic homeostasis through adipose tissue crosstalk. *Cell Metab* 2016;23:770–84.
12. Guarino V, Castellone MD, Avilla E, et al. Thyroid cancer and inflammation. *Mol Cell Endocrinol* 2010;321:94–102.
13. Liotti F, Visciano C, Melillo RM. Inflammation in thyroid oncogenesis. *Am J Cancer Res* 2012;2:286–97.
14. Siddiqi A, Monson JP, Wood DF, et al. Serum cytokines in thyrotoxicosis. *J Clin Endocrinol Metab* 1999;84:435–9.
15. Lumachi F, Basso SM, Orlando R. Cytokines, thyroid diseases and thyroid cancer. *Cytokine* 2010;50:229–33.
16. Dossus L, Becker S, Rinaldi S, et al. Tumor necrosis factor (TNF)-alpha, soluble TNF receptors and endometrial cancer risk: the EPIC study. *Int J Cancer* 2011;129:2032–7.
17. Dossus L, Rinaldi S, Becker S, et al. Obesity, inflammatory markers, and endometrial cancer risk: a prospective case-control study. *Endocr Relat Cancer* 2010;17:1007–19.
18. Cust AE, Kaaks R, Friedenreich C, et al. Plasma adiponectin levels and endometrial cancer risk in pre- and postmenopausal women. *J Clin Endocrinol Metab* 2007;92:255–63.
19. Dossus L, Jimenez-Corona A, Romieu I, et al. C-reactive protein and postmenopausal breast cancer risk: results from the E3N cohort study. *Cancer Causes Control* 2014;25:533–9.
20. Ose J, Schock H, Tjonneland A, et al. Inflammatory markers and risk of epithelial ovarian cancer by tumor subtypes: the EPIC cohort. *Cancer Epidemiol Biomarkers Prev* 2015;24:951–61.
21. Aleksandrova K, Boeing H, Nothlings U, et al. Inflammatory and metabolic biomarkers and risk of liver and biliary tract cancer. *Hepatology* 2014;60:858–71.
22. Aleksandrova K, Jenab M, Boeing H, et al. Circulating C-reactive protein concentrations and risks of colon and rectal cancer: a nested case-control study within the European Prospective Investigation into Cancer and Nutrition. *Am J Epidemiol* 2010;172:407–18.
23. Beksac K, Sonmez C, Cetin B, et al. Evaluation of proinflammatory cytokine and neopterin levels in women with papillary thyroid carcinoma. *Int J Biol Markers* 2016;31:e446–50.
24. Provatopoulou X, Georgiadou D, Sergentanis TN, et al. Interleukins as markers of inflammation in malignant and benign thyroid disease. *Inflamm Res* 2014;63:667–74.
25. Kobawala TP, Trivedi TI, Gajjar KK, et al. Significance of interleukin-6 in papillary thyroid carcinoma. *J Thyroid Res* 2016;2016:6178921
26. Kobawala TP, Trivedi TI, Gajjar KK, et al. Significance of TNF-alpha and the adhesion molecules: l-selectin and VCAM-1 in papillary thyroid carcinoma. *J Thyroid Res* 2016;2016:8143695
27. Lee S, Choe JW, Kim HK, et al. High-sensitivity C-reactive protein and cancer. *J Epidemiol* 2011;21:161–8.
28. Mitsiades N, Pazaitou-Panayiotou K, Aronis KN, et al. Circulating adiponectin is inversely associated with risk of thyroid cancer: in vivo and in vitro studies. *J Clin Endocrinol Metab* 2011;96:E2023–8.
29. Riboli E, Hunt KJ, Slimani N, et al. European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. *Public Health Nutr* 2002;5:1113–24.
30. Rinaldi S, Plummer M, Biessy C, et al. Thyroid-stimulating hormone, thyroglobulin, and thyroid hormones and risk of differentiated thyroid carcinoma: the EPIC study. *J Natl Cancer Inst* 2014;106:dju097.
31. Dalamaga M, Diakopoulos KN, Mantzoros CS. The role of adiponectin in cancer: a review of current evidence. *Endocr Rev* 2012;33:547–94.
32. Tworoger SS, Eliassen AH, Kelesidis T, et al. Plasma adiponectin concentrations and risk of incident breast cancer. *J Clin Endocrinol Metab* 2007;92:1510–6.
33. Macis D, Guerrieri-Gonzaga A, Gandini S. Circulating adiponectin and breast cancer risk: a systematic review and meta-analysis. *Int J Epidemiol* 2014;43:1226–36.
34. Wei T, Ye P, Peng X, et al. Circulating adiponectin levels in various malignancies: an updated meta-analysis of 107 studies. *Oncotarget* 2016;7:48671–91.
35. Cheng SP, Liu CL, Hsu YC, et al. Expression and biologic significance of adiponectin receptors in papillary thyroid carcinoma. *Cell Biochem Biophys* 2013;65:203–10.
36. Tilg H, Moschen AR. Adipocytokines: mediators linking adipose tissue, inflammation and immunity. *Nat Rev Immunol* 2006;6:772–83.
37. Matsubara M, Namioka K, Katayose S. Decreased plasma adiponectin concentrations in women with low-grade C-reactive protein elevation. *Eur J Endocrinol* 2003;148:657–62.
38. Chandran M, Phillips SA, Ciaraldi T, et al. Adiponectin: more than just another fat cell hormone? *Diabetes Care* 2003;26:2442–50.
39. Yadav A, Kataria MA, Saini V, et al. Role of leptin and adiponectin in insulin resistance. *Clin Chim Acta* 2013;417:80–4.
40. Yamauchi T, Kamon J, Minokoshi Y, et al. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. *Nat Med* 2002;8:1288–95.
41. Zakikhani M, Dowling RJ, Sonenberg N, et al. The effects of adiponectin and metformin on prostate and colon neoplasia involve activation of AMP-activated protein kinase. *Cancer Prev Res (Phila)* 2008;1:369–75.
42. Brakenhielm E, Veitonmaki N, Cao R, et al. Adiponectin-induced antiangiogenesis and antitumor activity involve caspase-mediated endothelial cell apoptosis. *Proc Natl Acad Sci USA* 2004;101:2476–81.
43. Rehem RA, Elwafa WA, Elwafa RA, et al. Study of serum leptin in well-differentiated thyroid carcinoma: correlation with patient and tumor characteristics. *World J Surg* 2014;38:2621–7.
44. Zivancevic-Simonovic S, Mihaljevic O, Majstorovic I, et al. Cytokine production in patients with papillary thyroid cancer and associated autoimmune Hashimoto thyroiditis. *Cancer Immunol Immunother* 2015;64:1011–9.
45. Linkov F, Ferris RL, Yurkovetsky Z, et al. Multiplex analysis of cytokines as biomarkers that differentiate benign and malignant thyroid diseases. *Prot Clin Appl* 2008;2:1575–85.
46. Erdogan M, Karadeniz M, Ozbek M, et al. Interleukin-10 gene polymorphism in patients with papillary thyroid cancer in Turkish population. *J Endocrinol Invest* 2008;31:750–4.
47. Cunha LL, Tincani AJ, Assumpcao LV, et al. Interleukin-10 but not interleukin-18 may be associated with the immune response against well-differentiated thyroid cancer. *Clinics (Sao Paulo)* 2011;66:1203–8.
48. Cil E, Kumral A, Kanmaz-Ozer M, et al. Interleukin-10–1082 gene polymorphism is associated with papillary thyroid cancer. *Mol Biol Rep* 2014;41:3091–7.
49. Todaro M, Zerilli M, Ricci-Vitiani L, et al. Autocrine production of interleukin-4 and interleukin-10 is required for survival and growth of thyroid cancer cells. *Cancer Res* 2006;66:1491–9.
50. Stanciu AE, Serdarevic N, Hurduc AE, et al. IL-4, IL-10 and high sensitivity-CRP as potential serum biomarkers of persistent/recurrent disease in papillary thyroid carcinoma with/without Hashimoto's thyroiditis. *Scand J Clin Lab Invest* 2015;75:539–48.
51. Mingomataj EC, Bakiri AH. Regulator versus effector paradigm: interleukin-10 as indicator of the switching response. *Clin Rev Allergy Immunol* 2016;50:97–113.
52. Fernandes JV, Cobucci RN, Jatoba CA, et al. The role of the mediators of inflammation in cancer development. *Pathol Oncol Res* 2015;21:527–34.
53. Toomer KH, Chen Z. Autoimmunity as a double agent in tumor killing and cancer promotion. *Front Immunol* 2014;5:116.
54. Clendenen TV, Arslan AA, Lokshin AE, et al. Temporal reliability of cytokines and growth factors in EDTA plasma. *BMC Res Notes* 2010;3:302.