

Thyroid autoimmunity has no negative impact on insulin dynamics in pre-diabetic patients with normal thyroid function

Krystallenia Alexandraki (✉ alexandrakik@gmail.com)

<https://orcid.org/0000-0003-2398-6768>

Georgios Boutzios

National and Kapodistrian University of Athens Faculty of Medicine

Ioanna Antonopoulou

National and Kapodistrian University of Athens Faculty of Medicine

Lamprini Iro Bartsioka

National and Kapodistrian University of Athens Faculty of Medicine

Panagiotis Moschouris

National and Kapodistrian University of Athens Faculty of Medicine

Angeliki Karapanagioti

National and Kapodistrian University of Athens Faculty of Medicine

Vasiliki Mavroeidi

National and Kapodistrian University of Athens Faculty of Medicine

Konstantinos Makrilakis

National and Kapodistrian University of Athens Faculty of Medicine

Leonidas Duntas

National and Kapodistrian University of Athens Faculty of Medicine

Gregory Kaltsas

National and Kapodistrian University of Athens Faculty of Medicine

Stavros Liatis

National and Kapodistrian University of Athens Faculty of Medicine

Research article

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Abstract

Background The association of autoimmune thyroid disease (AITD) with diabetes mellitus (DM) type 1 (DM1) has been previously documented. However, there is paucity of data regarding the association of AITD and DM type 2 (DM2). The aim of the study was to shed light on the role of AITD on DM2, we studied the impact of AITD in euthyroid patients with pre-diabetes.

Methods Euthyroid pre-diabetic patients were defined by impaired fasting glucose (IFG) (≥ 100 mg/dl) and/or glucose intolerance (IGT) [glucose 120min post-oral glucose tolerance test (OGTT) ≥ 140 mg/dl]. We assessed static and dynamic insulin resistance (IRI) and insulin secretion indices (ISI), and the disposition index (DI). Age, gender, waist circumference, waist-to-height ratio, body mass index, anti-hypertensive and hypolipidemic treatment were recorded.

Results Out of 166 patients studied (132 females) 53 (31.9%) had AITD; IFG prevalence was similar to non-AITD patients (92.2% versus 94.3%, $p=0.55$). In contrast, IGT was less prevalent in AITD compared to non-AITD patients (9.4% versus 28.3%, $p=0.008$). IRI did not differ between the two groups. The dynamic ISI: area under the curve (AUC) of insulin-to-glucose ratio (IncAUC_{ins/glu}), 1st-phase and 2nd-phase insulin release, and the DI showed a worse insulin secretion in non-AITD compared to AITD patients [0.54 (0.49, 0.2-2.42) versus 0.45 (0.41, 0.09-2.07), $p = 0.029$; 1029.3 (837.1, 323-2614.2) versus 864.3 (826.9, -282.5-2772.5), $p=0.033$; 354.8 (268.4, 132.8-851.1) versus 286.5 (254.7, 24.7-906.3), $p=0.035$; 0.09 (0.09, 0.006-0.55) versus 0.07 (0.06, -0.01-0.51), $p=0.02$, respectively]. On the other hand, hsCRP was higher in AITD versus non-AITD individuals [2.8 (5, 0-16) versus 1.2 (3, 0-13), $p=0.008$].

Conclusion Although individuals with prediabetes and AITD presented with higher levels of low-grade inflammation, their dynamic ISI and the DI, were less impaired, implying a better dynamic insulin secretion in patients with established abnormality of carbohydrate metabolism who carry an additional autoimmune metabolic component.

Background

Autoimmune thyroid disease (AITD) and disorders of carbohydrate metabolism are common endocrine disorders in the general population (1,2). In a large Nordic unselected population-based study the prevalence of positive thyroid peroxidase antibodies (TPOAb) was 13.9% in females and 2.8% in males (2). Similarly, in another large series of National Health and Nutrition Examination Survey (NHANES III) in the United States population, anti-thyroglobulin antibodies (TgAb) were positive in $10.4 \pm 0.5\%$ and TPOAb, in $11.3 \pm 0.4\%$; positive antibodies increased with age with a clear female preponderance (3). On the other hand, in a national study from a European country, Spain, revealed that 30% of the population had abnormal carbohydrate metabolism. In particular, the adjusted for age and sex prevalence of established diabetes mellitus (DM) type 2 (DM2) was 13.8% (95%CI: 12.8, 14.7%), whereas the prevalence of pre-diabetic states was 3.4% (95%CI: 2.9, 4.0%) for isolated impaired fasting glucose (IFG), 9.2% (95%CI: 8.2, 10.2%) for isolated impaired glucose tolerance (IGT) and 2.2% (95%CI: 1.7, 2.7%) for

combined IFG–IGT (4). Both DM2 and IGT increased significantly with age being also more prevalent in men than in women (4). Moreover, oral glucose tolerance test (OGTT)-based studies in Europe documented a prevalence of pre-diabetes (including IFG, IGT) ranging from 14% in Spain to 30% in Turkey (1).

The association between AITD and alterations in glucose homeostasis has been reported since 1979 (5,6). In 1310 adult persons with DM, 13.4% had thyroid dysfunction (clinical and subclinical hypothyroidism or hyperthyroidism) with the highest prevalence in DM type 1 (DM1) (31.4%) and the lowest in DM2 (6.9%) (7). The prevalence of thyroid dysfunction among Greek diabetic patients as defined by the need for thyroxin administration, use of antithyroid drugs, history of thyroidectomy, radioactive iodine treatment, was 12.3%, with women being more frequently affected than men (8). In another study, in diabetic patients, the prevalence of thyroid dysfunction defined by the presence of either hypothyroidism or hyperthyroidism (clinical and/or subclinical) was found to be 14.7% whereas TPOAb were positive in 10.8% (9). Inversely, DM2 was present in 27.8% of patients with AITD, whereas IFG or IGT occurred in 16.6% (10). Despite the apparent association of DM with thyroid dysfunction, specific studies addressing potential associations of AITD have been performed mainly in DM1 (11–13) whereas there is limited and occasionally contradictory information in DM2 patients (14).

Recently, in a large series of 9082 euthyroid subjects, AITD was positively related to HbA1c, IRI, obesity, central obesity, hyperlipidemia, and metabolic syndrome, especially in women (15). This specific study highlighted that AITD per se may be a potential risk factor for the development of cardiometabolic disorders since the investigators abrogated the negative effect of abnormal TSH (hypothyroidism or hyperthyroidism) on glucose metabolism (15–16).

Since these are relatively common diseases and some patients with apparent DM2 may have an underlying autoimmune component we aimed at investigating the possible impact of AITD on insulin secretion and insulin resistance indices (ISI, IRI, respectively), in a population of individuals with prediabetes.

Methods

Subjects

The study included subjects recruited from the Diabetic and Endocrine Outpatient Clinic of the Laiko University Hospital in Athens. The study was approved by the Scientific Committee of “Laiko” University Hospital (47/14.01.2013).

We recruited patients with pre-diabetes defined by the presence of one of the following criteria: i) IGT defined by serum glucose level at 120minutes ≥ 140 mg/dl but < 200 mg/dl, following a formal 75 g OGTT, ii) IFG defined by fasting serum glucose levels ≥ 100 but < 126 mg/dl) according to ADA criteria (17). Autoimmune thyroid disease was defined by the presence of TgAb and/or TPOAb antibodies. The population was divided into two groups, patients with AITD and patients without AITD.

All subjects under any medication known to affect glucose metabolism, those with abnormal thyroid function (thyroid stimulating hormone (TSH) ≥ 5 $\mu\text{IU/ml}$ defined as subclinical/ clinical hypothyroidism or TSH < 0.5 $\mu\text{IU/ml}$ as subclinical/ clinical hyperthyroidism) and individuals with thyroidectomy were excluded. Pregnant women and individuals with a history of hospitalization during the last 6 months, and hemoglobin levels less than 12 g/dl were also excluded from the study.

Oral glucose tolerance test was performed after a 10-h overnight fast. Serum glucose (mg/dl) and insulin levels ($\mu\text{IU/ml}$), were measured at baseline and at 30-min intervals (30', 60', 90', 120'). Glycosylated haemoglobin (HbA1c,%) and high-sensitive C-reactive protein (hs-CRP) (mg/L) were also measured. Plasma glucose, total cholesterol, High Density Lipoprotein (HDL)-Cholesterol, Low Density Lipoprotein (LDL)-cholesterol and triglycerides were measured as previously described (18). Percent concentration of HbA1c was performed in vitro in whole blood with an immunological method (tholosimetric suppression immunoanalysis, TINla) in an automatic analyzer of clinical chemistry (Hitachi 912, Roche, France). Serum insulin was measured with the immunoradiometric assay IRMA (DIAsource ImmunoAssays, Louvain-la-Neuve, Belgium). High-sensitivity CRP (hsCRP, mg/L, high sensitivity CRP enzyme immunoassay test kit, LI7500, Linear Chemicals, S.L., 08390 Montgat, Barcelona, Spain) serum levels were determined by enzyme-linked immunosorbent assay. The intra- and interassay coefficients of variance for hsCRP were 7.5 and 4.1% for low levels and 2.3 and 2.5% for high levels, respectively.

Arterial hypertension was diagnosed according to each individual's medical and drug history or in the presence of systolic blood pressure (SBP) ≥ 135 mmHg and/or diastolic blood pressure (DBP) ≥ 85 mmHg [mean value of latest European guidelines (SBP/DBP $> 140/90$ mm Hg) and the American guidelines (SBP/DBP $< 130/80$ mm Hg) (18–21). Dyslipidaemia was diagnosed on the basis of LDL levels ≥ 130 mg/dl and the administration of hypolidaemic drugs (22).

Body weight was measured using analogue scales in light clothing; height was measured bare-foot using a stadiometer. Body mass index (BMI, kg/m^2) was calculated to assess obesity and waist and waist-to-height ratio (WHR) to assess body fat distribution.

Antibodies to glutamic acid decarboxylase (anti-GAD) were randomly measured in the first 25 persons that were enrolled into the study to test for the case of persons with Latent Autoimmune Diabetes of Adults.

Indices Of Carbohydrate Metabolism

Insulin resistance indices (IRI)

Static IRI

1/fasting insulin, fasting glucose-to-insulin ratio (GIR), the quantitative insulin sensitivity check index (QUICKI), and insulin resistance Homeostasis model assessment (HOMA) (HOMA-IR) were used to assess insulin action (23) in the fasting state, using the following formulas:

QUICKI = $1 / (\log(\text{fasting insulin } \mu\text{U/mL}) + \log(\text{fasting glucose mg/dL}))$ (24)

HOMA-IR = $\text{fasting insulin } (\mu\text{IU/ml}) \times \text{fasting glucose (mmol/ml)} / 22.5$ (25).

Dynamic IRI

The Matsuda index was used to assess dynamic *insulin action* using the following formula:

Matsuda index = $(10,000 / \text{square root of } [\text{fasting glucose} \times \text{fasting insulin}] \times [\text{mean glucose} \times \text{mean insulin during OGTT}])$ (26)

β-cell secretion indices (ISI)

Static ISI (SISI)

HOMA index of β-cell function (HOMA-B) was calculated using the following formula:

HOMA-B = $20 \times \text{fasting insulin } (\mu\text{IU/ml}) / [\text{fasting glucose (mmol/ml)} - 3.5]$ (27).

Dynamic ISI

First phase and second phase insulin secretion and the incremental area under the insulin to glucose curve (incAUCins/glu) were used using the following formulas (28):

Predicted index of first phase of insulin secretion (1st PHIS) = $1283 + [1.289 \times \text{insulin at 30 minutes (pmol/L)}] - [138.7 \times \text{glucose at 30 minutes (mmol/L)}] + [3.772 \times \text{insulin at baseline (pmol/L)}]$ (29)

Predicted index of second phase of insulin secretion (2nd PHIS) = $287 + [0.4164 \times \text{insulin at 30 minutes (pmol/L)}] - [26.07 \times \text{glucose at 30 minutes (mmol/L)}] + [0.9226 \times \text{insulin at baseline (pmol/L)}]$ (29)

incAUCins/glu by the trapezoidal method from 0' to 120' min (23)

Combined index

The combined index of insulin action and β-cell secretion is expressed by the disposition index (DI) and the following formula is used:

Oral disposition index (DI) = $\Delta I_{0-30} / \Delta G_{0-30} \times 1 / \text{fasting insulin}$ (27).

The presence of insulin resistance was defined as previously described (28): HOMA-IR > 2.16 and/or QUICKI < 0.34 values.

Assays

The serum TSH levels were measured by a sensitive two-site chemiluminescent immunometric assay with analytical sensitivity: 0.004 μ IU/mL, and the coefficient of variation (CV) is less than 5.5% for TSH values comprising between 0.3 and 10 μ IU/mL. Thyroid antibodies: TgAb < 40 U/mL with analytical sensitivity 20 U/mL with an intra- and inter-assay CV of 3.2% and 4.6%, respectively and TPOAb < 30 U/mL with analytical sensitivity 10 U/mL with an intra- and inter-assay CV of 5.2% and 3.2%, respectively (IMMULITE 2000 SIEMENS Healthcare Diagnostics Products Ltd. Llanberis, Gwynedd LL55 4EL United Kingdom). High-sensitive CRP (reference range <5 mg/L) was determined with a highly sensitive latex-based immunoassay. Anti-GAD antibodies were measured by ELISA (Anachem Ltd, Luton, UK).-

Statistical analysis

A between-group comparison of non-continuous variables was carried out by Chi-squared test corrected by Fisher's exact test when appropriate, while for continuous variables a t-test student for parametric and Mann-Whitney U test for non-parametric variables was used. Parametric variables are presented as mean value \pm standard deviation (SD), and non-parametric variables as median values, interquartile range (IQR), minimum-to-maximum values range). A p value < 0.05 was considered as statistically significant. SPSS software (SPSS 16. Inc. Chicago, IL) was used for the statistical analysis.

Results

Total population

One hundred sixty-six patients with pre-diabetes were recruited; 132 (79.5%) were females. The median age and BMI were 50 (20, 18–79) years and 32.1 (10, 18–59) kg/m^2 , respectively. Demographic and biochemical characteristics stratified by the presence or absence of AITD are shown in Table 1. The two groups did not differ in terms of age, gender, BMI, waist and waist-to-height ratio (Table 1).

Table 1
Demographic and biochemical characteristics of the population studied.

	Prediabetics (n = 166)	AITD (n = 53)	Non-AITD (n = 113)	p-value
Age (years)	50 (20, 18–79)	49.5 (20, 18–77)	53 (18, 31–79)	0.43
Females (%)	132 (79.5%)	44 (83%)	88 (77.9%)	0.44
BMI (kg/m ²)	32.1 (10, 18–59)	31.4 (12, 18–59)	32.4 (7, 22–58)	0.25
Waist (cm)	97.5 ± 16.4	98.6 ± 14.8	96.9 ± 17.4	0.58
Waist-to-height ratio	0.58 (0.13, 0-0.88)	0.58 (0.15, 0-0.88)	0.59 (0.11, 0-0.81)	0.51
TSH (μIU/ml)	2.3 ± 1.1	2.3 ± 1.0	2.3 ± 1.1	0.065
Thyroxin replacement (%)	70 (42.2)	31 (58.5)	39 (34.5)	0.004*
Dyslipidemia	68 (56.2)	22 (51.2)	46 (59)	0.41
Total Cholesterol (mg/dl)	204.4 ± 38	208 ± 43.5	202.4 ± 34.7	0.48
HDL (mg/dl)	55.2 ± 15.3	56.1 ± 16.4	54.7 ± 14.8	0.65
LDL (mg/dl)	127.3 ± 33.1	131.9 ± 38	124.7 ± 30	0.31
Triglycerides (mg/dl)	109.5 (60, 41–409)	110 (51, 41–320)	109 (75, 45–409)	
Hypertension	53 (44.2)	3 (7.1)	13 (16.7)	0.17
SBP (mmHg)	134 (21, 108–166)	135 (21, 108–166)	131 (25, 112–166)	
DBP (mmHg)	88.2 ± 11.6	86.1 ± 11.2	89.4 ± 11.8	0.18
HR (bpm)	76 (21, 43–128)	78 (20, 43–128)	76 (19, 59–112)	
Family History of Diabetes	56 (46.7)	19 (44.2)	37 (48.1)	0.68
TgAb and /or TPOAb (%)	53 (31.9)	53 (100%)	0	N/A

*statistical significant value

Footnotes: AUC: area under the curve; BMI body mass index, DBP: diastolic blood pressure; HbA1c: glycosylated hemoglobin; HDL: high-density lipoprotein; HOMA: Homeostasis Model Assessment; hsCRP: high sensitivity C reactive protein; HR: heart rate; IFG: impaired fasting glucose; IGT impaired glucose tolerance; IR; insulin resistance; LDL: low-density lipoprotein; QUICKI: quantitative insulin sensitivity check index; TSH: thyroid stimulating hormone; SBP: systolic blood pressure

	Prediabetics (n = 166)	AITD (n = 53)	Non-AITD (n = 113)	p-value
Glucose 0 min(mg/dl)	104 (9, 74–125)	104 (10, 74–125)	104 (9, 86–124)	0.36
Glucose 60 min(mg/dl)	163 (60, 65–275)	157 (54, 65–237)	169 (63, 78–275)	0.06
Glucose 120 min(mg/dl)	108.5 (46, 40–199)	112 (59, 40–199)	100 (40, 56–199)	0.07
Insulin 0 min(μIU/ml)	12.5 (9, 3–34)	12.1 (9, 3–34)	13 (8, 3–28)	0.42
Insulin 60 min(μIU/ml)	92.2 (82, 15–688)	81.5 (82, 15–688)	103.8 (124, 23–598)	0.059
Insulin 120 min(μIU/ml)	52.5 (67, 0-405)	51.8 (67, 4-284)	55.8 (82, 0-405)	0.068
hsCRP (mg/L)	1.7 (4, 0–16)	2.8 (5, 0–16)	1.2 (3, 0–13)	0.008*
HbA1c (%)	5.7 (0.5, 4.2–6.5)	5.7 (0.7, 4.2–6.5)	5.7 (0, 5–6)	0.58
IFG (%)	153 (92.2)	50 (94.3)	103 (91.2)	0.55
IGT (%)	37 (22.3)	5 (9.4)	32 (28.3)	0.004*
Insulin resistance (HOMA-IR/QUICKI)	121 (72.9%)	41 (77.4%)	80 (70.8%)	0.37
Statical Insulin Resistance Indices (statical IRI)				
Glucose/insulin (GIR)	8.5 (6, 3–41)	9.2 (7, 3–41)	7.9 (5, 4–32)	0.37
QUICKI	0.32 (0.04, 0.28–0.42)	0.32 (0.04, 0.28–0.42)	0.32 (0.03, 0.29–0.40)	0.49
HOMA-IR	3.3 (2.5, 0.6–9.5)	3.1 (2.6, 0.6–9.5)	3.5 (2.2, 0.8–7.7)	0.49
1/fasting insulin	0.08 (0.07, 0.03–0.4)	0.08 (0.07, 0.03–0.4)	0.08 (0.05, 0.04–0.3)	0.42
Dynamic Insulin Resistance Index (dynamic IRI)				
MATSUDA index	3.03 (2.68, 0.72-13)	3.03 (3.03, 0.83-13)	2.8 (2.3, 0.72–10.83)	0.31
*statistical significant value				

Footnotes: AUC: area under the curve; BMI body mass index, DBP: diastolic blood pressure; HbA1c: glycosylated hemoglobin; HDL: high-density lipoprotein; HOMA: Homeostasis Model Assessment; hsCRP: high sensitivity C reactive protein; HR: heart rate; IFG: impaired fasting glucose; IGT impaired glucose tolerance; IR; insulin resistance; LDL: low-density lipoprotein; QUICKI: quantitative insulin sensitivity check index; TSH: thyroid stimulating hormone; SBP: systolic blood pressure

	Prediabetics (n = 166)	AITD (n = 53)	Non-AITD (n = 113)	p-value
Statical β -cell secretion index (statical ISI)				
HOMA-B	38.9 (27.3, 5.3-124.5)	35.8 (28.6, 5.3-124.5)	42.1 (23.3, 7.8-87.7)	0.37
Dynamic β -cell secretion indices (dynamic ISI)				
1st phase insulin release	917.6 (909.6, -282.5-2772.5)	864.3 (826.9, -282.5-2772.5)	1029.3 (837.1, 323-2614.2)	0.033
2nd phase insulin release	316.5 (275.6, 24.7-906.3)	286.5 (254.7, 24.7-906.3)	354.8 (268.4, 132.8-851.1)	0.035
AUC insulin/glucose	0.49 (0.45, 0.09-2.42)	0.45 (0.41, 0.09-2.06)	0.54 (0.49, 0.20-2.42)	0.029
Combined Insulin Resistance and β -cell secretion index				
Disposition index	0.08 (0.07, -0.01-0.55)	0.07 (0.06, -0.01-0.51)	0.09 (0.09, 0.006-0.55)	0.02
*statistical significant value				
Footnotes: AUC: area under the curve; BMI body mass index, DBP: diastolic blood pressure; HbA1c: glycosylated hemoglobin; HDL: high-density lipoprotein; HOMA: Homeostasis Model Assessment; hsCRP: high sensitivity C reactive protein; HR: heart rate; IFG: impaired fasting glucose; IGT impaired glucose tolerance; IR; insulin resistance; LDL: low-density lipoprotein; QUICKI: quantitative insulin sensitivity check index; TSH: thyroid stimulating hormone; SBP: systolic blood pressure				

Fifty-three patients (31.9%) had AITD, and 34 (64.2%) of those with positive antibodies had positive TgAbs, and 50 (94.3%) had positive TPOAbs and 55.8% had both antibodies involved. Seventy (42.2%) patients were on thyroxine replacement treatment. All the participants obtained normal thyroid function tests and the serum TSH levels did not differ between the two groups. As expected, more patients with AITD were on thyroxine replacement therapy, compared to non-AITD ($p < 0.001$). One patient (4%) out of 25 had positive anti-GAD antibodies.

High sensitivity CRP levels were higher in AITD compared to non-AITD patients [2.8 (5, 0-16) versus 1.2 (3, 0-13), $p = 0.008$].

One hundred twenty-nine (92.2%) patients had IFG and 37 (22.3%) had IGT (Table 1). Seventy-seven (46.4%) patients had isolated IFG, 13 (7.8%) had isolated IGT with the remaining 54.2% presenting both abnormalities. IFG prevalence was similar in both groups (AITD: 94.3% versus 91.2%, in non-AITD $p = 0.55$), as opposed to IGT which was less prevalent in AITD (9.4% versus 28.3% in non-AITD, $p = 0.008$). Fasting serum glucose was similar between AITD and non-AITD individuals [AITD 104 (10, 74-125) mg/dL; non-AITD 104 (9, 86-124) mg/dL, $p = 0.36$]. However, 1-h and 2-h post load serum glucose was higher in the non-AITD group, both reaching statistical significance [glucose 60 min: AITD 157 (54, 65-

227) mg/dL; non-AITD 169 (63, 78–275) mg/dL, $p = 0.06$; glucose 120 min: AITD 112 (59, 40–199) mg/dL; non-AITD 100 (40, 56–199) mg/dL, $p = 0.07$]. One hundred twenty-one (72.9%) patients had IR, either by HOMA-IR and/or QUICKI criteria (Table 1)

Static IRI, dynamic IRI and static ISI did not differ between patients with and without AITD. In contrast, dynamic ISI indices, namely IncAUC_{ins/glu}, 1st -phase and 2nd -phase insulin release as well as DI were significantly higher in non-AITD compared to AITD patients (refer to table). DISI indices in AITD individuals were higher irrespectively of gender, BMI and HbA1c (data not shown).

Discussion

The present study provides data suggesting that individuals with pre-diabetes and AITD display a better β -cell insulin secretory profile compared to patients without AITD, for the same level of insulin resistance and despite having a higher level of low-grade chronic inflammation. This finding may simply suggest that thyroid autoimmunity does not confer any additional β -cell secretory dysfunction or, that prediabetics with AITD, in order to overcome a similar insulin resistance state as a population without AITD, have to increase their overall β -cell secretion. Since, however, as DI which is a more accurate marker of β -secretory capacity (as it takes into account the level of insulin resistance) was significantly higher in the AITD group, it seems reasonable to assume that β -cell “health” in the AITD group is better than in the non-AITD group. Indeed, this is reflected in the plasma glucose values at 1 h and 2 h post-OGTT, which were lower in individuals with AITD.

Nevertheless, since both clinical and subclinical thyroid disorders have been associated with insulin resistance, in the present study we included patients with normal thyroid function (30–32) either with or without L-thyroxine replacement treatment to eliminate this confounding factor.

In previous studies, the inter-correlation between DM and thyroid dysfunction has been extensively studied mostly in regards to DM1 where a causative pathogenic mechanism has been speculated; common susceptibility genes were identified in both autoimmune diseases (16). An apparent correlation between AITD and DM2 has been speculated in some but not all the studies, although no pathogenic mechanism has been identified (13,14,33–37). In a recent study one in five DM2 subjects had AITD as opposed to one in twenty seen in control subjects (14). Although there are studies in agreement with this finding (13,38,39), other studies did not document any association between AITD and DM2 as in the present study (13,36,37,40). In addition, the presence of metabolic syndrome, with or without obesity, has been shown to be related to AITD in some but not all studies (41–43) while similarly discordant results were published regarding the association of dyslipidemia with AITD (15,41,42,44,45). In favor of an association between AITD and cardiovascular risk factors is the fact that AITD has been related to indirect indices of atherosclerosis such as carotid intima-media thickness (46).

Several reasons have been put forward to account for the above-mentioned contradictory findings, such as differences in the assays used to determine thyroid auto-antibodies (more sensitive methodologies may result in a higher association), along with inherent variability of the diabetic populations studied,

such as duration of diabetes, diabetic control, and medications used (14). In order to eliminate the impact of the above differences on indices of insulin secretion and/or action (IRI or ISI) and to clarify the relationship of AITD with carbohydrate metabolism abnormalities we have recruited euthyroid patients with prediabetes not receiving any antidiabetic or insulin-sensitizing medication.

Our data showed that 30% of euthyroid patients with pre-diabetes have AITD, a figure that is similar to the 32.9% figure found in a cohort of non-pregnant patients with DM2 with unknown thyroid function (47). As a high proportion of patients with prediabetes are likely to develop frank DM, we have speculated that the presence of AITD may confer to prediabetics a higher risk of progression to DM. However, despite the fact that the AITD group exhibited a higher (low-grade) inflammatory state, no difference in insulin resistance between the two groups was observed, while, even further, thyroid autoimmunity was associated with a better β -cell secretory profile which was reflected in the higher DI, the lower glycemic excursions at 1 h and 2 h post-OGTT and, consequently, in the lower prevalence of IGT in the AITD group. In other words, dysglycemia in individuals with AITD was mainly associated with impaired fasting rather than impaired tolerance of glucose. Clinical studies suggest that the site of insulin resistance varies between IFG and IGT, the first being related mainly with severe hepatic insulin resistance with normal or near-normal muscle insulin resistance, while the latter with only mild hepatic insulin resistance but with severe insulin resistance at the periphery (48). AITD may have some impact and inflammatory factors (49) such as the levels of serum interleukin (IL)-6, tumor necrosis factor- α (TNF- α), IL-12, IL-10, and HOMA-IR were higher in patients with AITD and hypothyroidism compared to ones with AITD and normal levels of thyroid hormones (50). On the other hand, we have shown similar TSH levels between groups suggesting that TSH did not affect our results. Thyroid replacement treatment per se may also affect our findings (51) since more patients on AITD group were on treatment; however, the subgroups of AITD with and without replacement treatment did not differ in any of the parameters studied (data not shown). Another factor that may be involved is the changes in metabolomic patterns and fatty acid metabolism that may promote insulin resistance. However, the isolated TSH increase, as was often observed in obesity or euthyroid patients with AITD is not correlated with insulin metabolism. Slight changes in thyroid hormones may centrally interact with AMP-activated protein kinase (AMPK) decreasing peripheral glucose production and linking glucose regulation to fatty acids synthesis via the carboxylation of acetylCoA to form malonyl-CoA, which is catalysed by acetyl-CoA carboxylase (52).

Therefore, it may be speculated that the increased low-grade inflammation of the AITD group is preferentially inducing insulin resistance at the hepatic rather than the muscle or the adipose tissue level. It cannot be excluded of course that our findings may be the result of plain chance. The small sample size, the lack of more specific inflammatory markers (49,53) as well as the fact that insulin resistance was not assessed by more sophisticated techniques, does not allow for robust conclusions to be drawn (54).

Our study has some further number of limitations that need to be considered. Patients with AITD were studied cross-sectionally, being at various periods of disease evolution and AITD effect on thyroid function. We also recruited patients from the endocrine and diabetic outpatient clinics which presents a

source of bias. This explains the higher number of females participated in the study which could be a potential bias for the assessment of autoimmunity of the population participated in the study. However, despite the small numbers of patients studied in subpopulations analysis, the fact that similar results were seen in normal-weight and lean patients implies that this relationship is real and independent from obesity. Moreover, we found out one patient confirming the previously documented 4% presence of anti-GAD (38) that inclusion or exclusion did not affect the results (data not shown).

Conclusions

In conclusion, prediabetic patients with AITD exhibit a better β -cell dependent secretory profile, as assessed by mathematical models associated and in spite of an increased inflammatory state. Future studies need to be conducted using a prospective design in a larger cohort to confirm our findings and identify the exact mechanism that thyroid autoimmunity affecting more insulin-resistance than β -cell secretion targeting in the different pathophysiologic mechanisms.

Abbreviations

AITD

Autoimmune thyroid disease

anti-GAD

Antibodies to glutamic acid decarboxylase

AMPK

AMP-activated protein kinase)

BMI

Body mass index

DBP

diastolic blood pressure

DI

disposition index

DM

diabetes mellitus

DM1

diabetes mellitus type 1

DM2

diabetes mellitus type 2

GIR

glucose-to-insulin ratio

HbA1c

Glycosylated haemoglobin

HDL

High density lipoprotein
HOMA
Homeostasis model assessment
hs-CRP
high-sensitive C-reactive protein
IFG
impaired fasting glucose
IGT
impaired glucose tolerance
IL
interleukin
incAUCins/glu
incremental area under the insulin to glucose curve
IRI
Insulin resistance indices
ISI
 β -cell secretion indices
OGTT
oral glucose tolerance test
QUICKI
quantitative insulin sensitivity check index
SBP
systolic blood pressure
TgAb
anti-thyroglobulin antibodies
TNF- α
tumor necrosis factor- α
TPOAb
thyroid peroxidase antibodies
TSH
thyroid stimulating hormone
WHR
waist-to-height ratio
1st PHIS
Predicted index of first phase of insulin secretion
2nd PHIS
Predicted index of second phase of insulin secretion

Declarations

Ethics approval and consent to participate

The study was approved by the Scientific Committee of “Laiko” University Hospital (47/14.01.2013). Verbal consent was taken from all the participants as part of an audit study and since their data were retrospectively accessed.

Consent for publication

Yes, we have.

Availability of data and materials

The datasets generated and/or analysed during the current study are not publicly available due absence of institutional electronic record but are available from the corresponding author on reasonable request and they will be retrieved by the file of each individual patients.

Competing interests

Georgios Boutzios is an Associate Editor and Stavros Liatis a Section Editor of BMC Endocrine Disorders.

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Authors' contributions

KIA, GB equally contributed to the design of the study, to patients recruitment, analysis of the findings and writing of the manuscript; IA, LIB, PM, AK, VM all contributed to the collection of the data; KM, LD contributed to the design of the study and revision of the manuscript; GAK, SL equally contributed to the design of the study, to patients recruitment, and revision of the manuscript. All authors read and approved the final manuscript.

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Disclosure statement

The authors have no conflict of interest to declare.

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