CLINICAL STUDY

Insulin resistance in hyperthyroidism: the role of IL6 and TNF α

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Abstract

Objective: Although insulin resistance is a common finding in hyperthyroidism, the implicated mechanisms are obscure. The aim of this study was to investigate whether interleukin 6 (IL6) and tumour necrosis factor α (TNF α) are related to the development of insulin resistance in hyperthyroidism of nonautoimmune origin.

Design and methods: A meal was given to ten hyperthyroid (HR) and ten euthyroid (EU) women. Plasma samples were taken for 360 min from the radial artery for measurements of glucose, insulin, and nonesterified fatty acids (NEFA). IL6 and TNF α were measured preprandially from the superficial epigastric vein and from the radial artery.

Results: i) In HR versus EU: (a) arterial glucose was similar (AUC_{0-360} 2087 ± 57 vs 2010 ± 43 mM $\times$ min), but insulin was increased (AUC_{0-360} $17\,267 \pm 2447$ vs $10\,331 \pm 666$ μ U/ml $\times$ min, $P=0.01$), (b) homeostasis model assessment (HOMA) was increased (2.3 ± 0.4 vs 1 ± 0.1 kg/m², $P=0.007$), (c) arterial NEFA were increased (AUC_{0-360} 136 ± 18 vs 89 ± 7 mmol/l $\times$ min, $P=0.03$), (d) arterial IL6 (2 ± 0.3 vs 0.9 ± 0.1 pg/ml, $P=0.0009$) and TNF α (4.2 ± 0.8 vs 1.5 ± 0.2 pg/ml, $P=0.003$) were increased, and (e) IL6 production from the subcutaneous adipose tissue (AT) was increased (18 ± 6 vs 5 ± 1 pg/min per 100 ml tissue, $P=0.04$). ii) (a) Subcutaneous venous IL6 was positively associated with HOMA (β -coefficient = 1.7 ± 0.7 , $P=0.049$) and (b) although TNF α was not produced by the subcutaneous AT, arterial TNF α was positively associated with NEFA (AUC_{0-360} ; β -coefficient = 0.045 ± 0.01 , $P=0.005$). **Conclusions:** In hyperthyroidism: i) glucose and lipid metabolism are resistant to insulin, ii) subcutaneous AT releases IL6, which could then act as an endocrine mediator of insulin resistance, iii) although there is no net secretion of TNF α by the subcutaneous AT, increased systemic TNF α levels may be related to the development of insulin resistance in lipolysis.

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Introduction

Adipose tissue (AT) is an active endocrine organ that, in addition to regulating fat mass and nutrient homeostasis, releases a number of bioactive mediators (adipokines) such as interleukin 6 (IL6) and tumour necrosis factor α (TNF α) (1).

Besides their potent proinflammatory effects in host defense, both IL6 and TNF α have been implicated in regulating insulin signaling and lipid metabolism in peripheral tissues (1, 2). IL6 has been reported to reduce insulin-dependent hepatic glycogen synthesis (3, 4) and glucose uptake in adipocytes (5), whereas it enhances insulin-dependent glycogen synthesis and glucose uptake in myotubes (6, 7). Evidence supporting a key role for TNF α in obesity-related insulin resistance came from studies showing that deletion of TNF α or TNF α receptors resulted in significantly improved insulin sensitivity in both diet-induced obese mice and leptin-deficient ob/ob mice (8). Neutralization of TNF α

increased insulin resistance in obese rats (9). However, infusion of TNF α -neutralizing antibodies to obese, insulin-resistant subjects, or patients with type 2 diabetes did not improve insulin sensitivity (10, 11). TNF α has been shown to inhibit lipoprotein lipase activity and decrease its production in adipocyte cell lines (12) as well as increase lipolysis (13, 14).

Although various conditions influence AT expression of these proteins, the hormonal regulation of their production is still obscure (2).

An interaction between thyroid hormones and AT-produced cytokines would be important for two reasons. First, thyroid hormones have marked effects on AT metabolism (15, 16). And secondly, since thyroid hormones induce insulin resistance (15, 17), an effect on production rates and plasma levels of these cytokines could provide an insight into the responsible mechanism(s).

Measurements of TNF α and IL6 in hyperthyroidism have shown conflicting results: these levels have been found normal (18–20) or increased (18–23).

However, the measurements have been done mostly in patients with autoimmune hyperthyroidism, which could have altered the effect of thyroid hormones on the adipocyte secretory pattern; these cytokines, in addition to their metabolic effects, are powerful modulators of the immune response involved in the host defense, and their secretion is therefore affected when the immune system is stimulated (24, 25).

The present study was undertaken: i) to investigate the effects of thyroid hormones *per se* on plasma levels and production rates from the subcutaneous AT of TNF α and IL6 in patients with hyperthyroidism of nonautoimmune origin, and ii) to correlate these changes with the level of tissue sensitivity to insulin.

Subjects and methods

Subjects

Ten newly diagnosed female hyperthyroid (HR) subjects (due to multinodular goiter) before initiation of any treatment were studied (age 33 ± 2 years, body mass index (BMI) 22 ± 1 kg/m², tri-iodothyronine (T₃) 345 ± 47 ng/dl, thyroxine (T₄) 17 ± 2 μ g/dl, TSH undetectable). All HR subjects had negative antithyroglobulin (16.9 ± 3.5 U/ml) and anti-thyroperoxidase (10.4 ± 2.4 U/ml) antibodies. Ten female euthyroid (EU) subjects (age 32 ± 3 years, BMI 22 ± 0.7 kg/m², T₃ 107 ± 7 ng/dl, T₄ 8 ± 0.7 μ U/dl, TSH 1 ± 0.06 μ U/ml) were also studied as controls.

The thyroid hormones' reference range was 80–200 ng/dl for T₃, 5.1–14.1 μ g/dl for T₄, and 0.27–4.2 μ U/ml for TSH. The antibodies reference range was 0–34 U/ml for anti-thyroperoxidase and 0–115 U/ml for antithyroglobulin antibodies.

Total fat mass, assessed by bioelectric impedance, was not different between HR (15.9 ± 2.4 kg) and EU subjects (15.7 ± 2 kg).

All HR patients had multinodular goiter on clinical examination. Ultrasound showed an enlarged gland with several hypodense nodules of variable size. Thyroid scan with ⁹⁹Tc showed areas of increased uptake together with areas of decreased uptake interspersed by suppressed normal thyroid tissue.

The study was approved by the hospital ethics committee, and subjects gave informed consent.

Experimental protocol

The subjects were admitted to the hospital at 0700 h after an overnight fast, and had the radial artery and the superficial epigastric vein (which is draining the abdominal subcutaneous AT) catheterized as previously described (26, 27).

A mixed meal was administered to the subjects (730 kcal, 50% carbohydrate of which 38% was starch, 40% fat, and 10% protein), at least 1 h after catheter insertion; the meal was consumed within 20 min.

Blood samples were withdrawn before the meal (at -30 and 0 min) and at 30 – 60 min intervals for 360 min from the radial artery for measurements of insulin (RIA; Linco Research, St Charles, MO, USA), glucose (YSI Inc., Yellow Springs, OH, USA), and nonesterified fatty acids (NEFA; Roche Diagnostics). IL6 and TNF α (ELISA; R&D Systems, Oxon, UK) were measured in the fasting state (at -30 and 0 min) from the superficial epigastric vein and from the radial artery.

AT blood flow (BF) was measured immediately before each of the two preprandial blood samplings (-30 and 0 min). ¹³³Xe dissolved in sterile saline (4 MBq, DuPont, MDS Nordion, Fleurus, Belgium) was injected into the subcutaneous AT of the anterior abdominal wall within the drainage area of the cannulated abdominal vein (but on the opposite side), about 8 cm from the midline and 5 cm below the level of the umbilicus. The duration of the injection and the withdrawal of the needle were 2 min and 30 s respectively. The injection was given at least 45 min before the first measurement and the washout analyzed with a scintillation detector (Oakfield Instruments, Oxford, UK). During measurements, the subjects remained still (26, 27).

Calculations

The values obtained from the two preprandial samples were averaged to give a '0 time' value.

Insulin sensitivity was calculated by HOMA analysis (plasma glucose (mM) $\times$ plasma insulin (mU/l)/22.5).

AT production rates of cytokines from the AT were calculated as the differences between the plasma levels measured in the subcutaneous vein and the radial artery, and multiplied by BF in the AT.

Statistical analysis

Results are presented as mean $\pm$ S.E.M. All variables studied were normally distributed.

Differences between HR and EU subjects were tested with Student's nonpaired *t*-test. Differences between experiments within the same group were tested with Student's paired *t*-test.

Analysis of covariance was used to evaluate the association between IL6 and HOMA, TNF α and NEFA, and IL6 and TNF α . BMI and total fat mass did not differ between the groups. However, BMI and total fat mass values were used as covariates. The inclusion of covariates increases statistical power because it accounts for some of the variability. All statistical calculations were performed in SPSS (version 16; SPSS Inc., Chicago, IL, USA).

Results

Glucose, insulin, and NEFA

Fasting plasma insulin was increased in HR versus EU subjects (10.9 ± 2.3 vs 4.8 ± 0.8 μ U/ml, $P=0.02$), whereas fasting plasma glucose was similar

(5 ± 0.1 vs 4.7 ± 0.1 mM). Fasting plasma NEFA levels were increased in HR (634 ± 55 μ mol/l) versus EU (454 ± 56 μ mol/l, $P=0.03$).

After the meal, plasma insulin was increased in HR versus EU subjects (AUC_{0-360} $17\,267 \pm 2447$ vs $10\,331 \pm 666$ μ U/ml $\times$ min, $P=0.01$; Fig. 1A). Plasma glucose was similar (AUC_{0-360} 2087 ± 57 vs 2010 ± 43 mM $\times$ min; Fig. 1B). Plasma NEFA levels were increased in HR (AUC_{0-360} 136 ± 18 mmol/l $\times$ min) versus EU subjects (89 ± 7 mmol/l $\times$ min, $P=0.03$), suggesting increased lipolysis (Fig. 1C).

HOMA was increased (2.3 ± 0.4 vs 1 ± 0.1 kg/m 2 , $P=0.007$), indicating insulin resistance in hyperthyroidism.

Figure 1 Arterial concentrations of insulin (A), glucose (B), and NEFA (C) in hyperthyroid and euthyroid subjects. Results are presented as mean $\pm$ S.E.M. Differences between hyperthyroid and euthyroid subjects were tested with Student's nonpaired *t*-test (SPSS version 16; SPSS Inc., Chicago, IL, USA; * $P<0.05$).

Adipose tissue blood flow

AT BF was increased in HR versus EU subjects (3.9 ± 0.7 vs 2.1 ± 0.4 ml/min per 100 ml tissue, $P=0.004$).

IL6

In both EU and HR groups, the IL6 plasma levels were higher in the abdominal vein than in the radial artery suggesting that IL6 is produced by the subcutaneous AT (Fig. 2).

Both arterial and subcutaneous venous IL6 levels of HR subjects were significantly higher compared to those in the EU subjects (Fig. 2).

IL6 production from the subcutaneous AT was increased in HR versus EU subjects (18 ± 6 vs 5 ± 1 pg/min per 100 ml tissue, $P=0.04$).

Subcutaneous venous IL6 was positively associated with HOMA index (β -coefficient = 1.7 ± 0.7 , $P=0.049$; Fig. 3), although there was no association between arterial IL6 and HOMA index in the HR subjects.

No association was found between IL6 (arterial or venous) and HOMA index in the EU subjects.

TNF α

In the HR patients TNF α concentrations in plasma were significantly increased in relation to those found in EU subjects both in arterial and in subcutaneous venous samples (Fig. 4).

In both groups, there were no significant differences in the concentration of TNF α between the arterial and the subcutaneous venous samples (Fig. 4). As a result, there was no significant TNF α release from the subcutaneous AT studied either in HR (0.35 ± 0.37 pg/min per 100 ml tissue) or in EU subjects (0.23 ± 0.4 pg/min per 100 ml tissue).

Figure 2 Arterial and subcutaneous venous concentrations of IL6 in hyperthyroid and euthyroid subjects. Results are presented as mean $\pm$ S.E.M. Differences between hyperthyroid and euthyroid subjects were tested with Student's nonpaired *t*-test. Differences between experiments within the same group were tested with Student's paired *t*-test (SPSS version 16; SPSS Inc., Chicago, IL, USA).

Figure 3 Analysis of covariance (ANCOVA) evaluating the association of subcutaneous venous IL6 concentrations to HOMA in the hyperthyroid subjects (β -coefficient = 1.7 ± 0.7 , $P=0.049$; SPSS version 16; SPSS Inc., Chicago, IL, USA).

However, $\text{TNF}\alpha$ arterial levels were positively associated with arterial plasma NEFA levels (AUC_{0-360}) (β -coefficient = 0.045 ± 0.01 , $P=0.005$; Fig. 5). Subcutaneous venous $\text{TNF}\alpha$ levels were also positively associated with plasma NEFA levels (AUC_{0-360} ; β -coefficient = 0.037 ± 0.01 , $P=0.029$).

No association was found between $\text{TNF}\alpha$ (arterial or venous) and IL6 (arterial or venous) levels in both HR and EU subjects.

Discussion

Although insulin resistance is a common finding in hyperthyroidism, the implicated mechanisms are obscure. Our study indicates a possible link between cytokine levels and insulin resistance in hyperthyroidism.

Figure 4 Arterial and subcutaneous venous concentrations of $\text{TNF}\alpha$ in hyperthyroid and euthyroid subjects. Results are presented as mean $\pm$ S.E.M. Differences between hyperthyroid and euthyroid subjects were tested with Student's nonpaired *t*-test. Differences between experiments within the same group were tested with Student's paired *t*-test (SPSS version 16; SPSS Inc., Chicago, IL, USA). NS, not statistically different.

In our study, fasting arterial glucose levels were not altered by hyperthyroidism, despite hyperinsulinemia. Moreover, HOMA index was increased suggesting the development of insulin resistance in the HR state. These findings are in agreement with previous studies in HR subjects showing that glucose production from the liver (28) and glucose uptake by peripheral tissues (skeletal muscle and AT) (16, 17) are resistant to insulin.

IL6 and $\text{TNF}\alpha$ have been implicated in regulating insulin signaling and lipid metabolism in peripheral tissues (1).

Previous studies regarding the effect of hyperthyroidism on levels of IL6 have primarily focused on plasma measurements; in these studies, IL6 plasma levels have been found increased (18, 19, 22) or unchanged (20, 23).

Based on *in vivo* experiments in obese subjects (29), it has been estimated that $\sim 25\%$ of the systemic levels of IL6 originate from subcutaneous AT. AT secretion of IL6 levels has been previously studied with subcutaneous fat biopsies, *in vitro* in HR subjects with Graves' disease (18). In that study, serum concentrations as well as AT release of IL6 were increased, both before and during anti-thyroid treatment as compared with control subjects. Although the results of this study suggest a role of thyroid hormone excess in regulating AT secretion of IL6, the question whether the observed increase in AT release of IL6 is due to a direct effect of thyroid hormones or can be explained by factors related to the autoimmune nature of Graves' disease remains unanswered.

Our study showed increased plasma levels and AT production rates of IL6 in patients with hyperthyroidism of nonautoimmune origin compared with the EU subjects. The release of IL6 from the subcutaneous AT, which is partly contributing to the increased circulating levels of IL6 in the HR subjects, suggests a direct effect of thyroid hormones in IL6 production independent of autoimmunity.

Figure 5 Analysis of covariance (ANCOVA) evaluating the association of arterial $\text{TNF}\alpha$ concentrations to plasma NEFA levels (AUC_{0-360}) in the hyperthyroid subjects (β -coefficient = 0.045 ± 0.01 , $P=0.005$; SPSS version 16; SPSS Inc., Chicago, IL, USA).

In our study, there was no association between arterial IL6 and HOMA index in the HR subjects. However, the fact that the increased subcutaneous venous IL6 levels were positively associated with HOMA index suggests a possible link between IL6 production from AT and the development of insulin resistance in the HR state.

In our HR patients, TNF α levels were significantly increased in relation with those found in EU subjects.

In previous studies, focusing mainly in Graves' disease, TNF α plasma levels have been found increased (20–23) or unchanged (18, 19). Given that TNF α is a powerful modulator of the immune response, mediating the induction of adhesion molecules and other cytokines, the results of these studies could be attributed to the autoimmune nature of Graves' disease (30).

AT plays a crucial role buffering daily influx of dietary fat in the postprandial period, suppressing the release of NEFA into the circulation, and increasing triacylglycerol clearance (31). In our study, plasma NEFA levels were increased in the HR subjects suggesting increased lipolysis. These findings correspond well with a previous study using the arteriovenous difference technique in the abdominal subcutaneous AT depot, suggesting that AT lipolysis is resistant to insulin in the HR state (16).

In the present study, arterial and subcutaneous venous TNF α levels were positively associated with arterial plasma NEFA levels, suggesting a possible link between increased TNF α levels and the development of insulin resistance in lipolysis. This is supported by previous observations in EU subjects showing that TNF α inhibits lipoprotein lipase activity and increases lipolysis (12–14).

Given that there was no secretion of TNF α by the subcutaneous AT depot studied, we suggest that TNF α , produced by other tissues or cells, influences lipolysis through endocrine mechanisms (29).

The small size of the sample is a limitation of our study. It is well known that hyperfunctioning multinodular goiter mainly occurs in older age. However, older patients often have metabolic comorbidities (diabetes, hypertension, dyslipidemia, obesity, and cardiovascular diseases) and take medication therapy, which could affect glucose and lipid metabolism. This is the reason why we chose to study a relatively small group of young, lean subjects without comorbidities. In addition, in the literature, there are reports showing that hyperthyroidism is associated with abnormalities of carbohydrate metabolism, which are not restored to normal after antithyroid therapy (32, 33). As a result, we chose to compare our HR patients with a control group and not with themselves after therapy.

Another limitation of our study is that although our HR patients had negative autoantibodies, there is a percentage of Graves' disease patients where autoantibodies may not be detected; inclusion of some Graves' disease patients might have contributed to the increased cytokine levels in the HR group.

In conclusion in hyperthyroidism: i) glucose and lipid metabolism are resistant to insulin, ii) subcutaneous AT releases IL6, which could then act as an endocrine mediator of insulin resistance, iii) although there is no net secretion of TNF α by the subcutaneous AT, *in vivo*, increased systemic TNF α levels may be related to the development of insulin resistance in lipolysis.

Declaration of interest

There is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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