Insulin resistance in hyperthyroidism: the role of IL-6 and TNFα

Running title: IL-6 and TNFα in hyperthyroidism

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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 IL-6 and TNFα are related to the development of insulin resistance in hyperthyroidism of non-autoimmune origin. Design and methods: A meal was given to ten hyperthyroid (HR) and ten euthyroid women (EU). Plasma samples were taken for 360min from the radial artery for measurements of glucose, insulin and non-esterified-fatty-acids (NEFA). IL-6 and TNFα were measured preprandially from the superficial epigastric vein and from the radial artery. Results: (1) In HR vs EU: (a) arterial glucose was similar (AUC$_{0-360}$2087±57 vs 2010±43mM*min), but insulin was increased (AUC$_{0-360}$17267±2447 vs 10331±666µU/ml*min, p=0.01) (b) HOMA was increased (2.3±0.4 vs 1±0.1 kg/m$^2$, p=0.007) (c) arterial NEFA were increased (AUC$_{0-360}$136±18 vs 89±7mmol/l*min, p=0.03) (d) arterial IL-6 (2±0.3 vs 0.9±0.1pg/ml, p=0.0009) and TNFα (4.2±0.8 vs 1.5±0.2pg/ml, p=0.003) were increased (e) IL-6 production from the subcutaneous adipose tissue (AT) was increased (18±6 vs 5±1pg/min/100mltissue, p=0.04). (2) (a) subcutaneous venous IL-6 was positively associated with HOMA ($β$-coefficient=1.7±0.7, p=0.049) (b) although TNFα was not produced by the subcutaneous AT, arterial TNFα was positively associated with NEFA (AUC$_{0-360}$) ($β$-coefficient=0.045±0.01, p=0.005). Conclusions: In hyperthyroidism: (1) glucose and lipid metabolism are resistant to insulin (2) subcutaneous AT releases IL-6 which could then act as an endocrine mediator of insulin resistance (3) although there is no net secretion of TNFα by the subcutaneous AT, increased systemic TNFα levels may be related to the development of insulin resistance in lipolysis.
Introduction

Adipose tissue is an active endocrine organ that, in addition to regulating fat mass and nutrient homeostasis, releases a number of bioactive mediators (adipokines) such as IL-6 and TNFα (1).

Besides their potent proinflammatory effects in host defence, both IL-6 and TNFα have been implicated in regulating insulin signaling and lipid metabolism in peripheral tissues (1,2). IL-6 has been reported to reduce insulin-dependent hepatic glycogen synthesis (3,4) and glucose uptake in adipocytes (5), whereas enhances insulin dependent glycogen synthesis and glucose uptake in myotubes (6,7). Evidence supporting a key role for TNFα in obesity-related insulin resistance came from studies showing that deletion of TNFα or TNFα receptors resulted in significantly improved insulin sensitivity in both diet-induced obese mice and leptin-deficient ob/ob mice (8). Neutralization of TNFα increased insulin resistance in obese rats (9). However, infusion of TNFα-neutralizing antibodies to obese, insulin-resistant subjects, or patients with type 2 diabetes, did not improve insulin sensitivity (10,11). TNFα has been shown to inhibit lipoprotein lipase activity and decrease its production in adipocytes cell lines (12) as well as increase lipolysis (13,14).

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

An interaction between thyroid hormones and adipose tissue-produced cytokines would be important for two reasons. Firstly, thyroid hormones have marked effects on adipose tissue 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 TNFα and IL-6 in hyperthyroidism have shown conflicting results: these levels have been found normal (18,19,20) or increased (18,19,20,21,22,23). 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 defence and their secretion is therefore affected when the immune system is stimulated (24,25).

The present study was undertaken: (a) to investigate the effects of thyroid hormones per se on plasma levels and production rates from the subcutaneous adipose tissue of TNFα and IL-6 in patients with hyperthyroidism of non-autoimmune origin, and (b) to correlate these changes with the level of tissue sensitivity to insulin.

Subjects and methods

Subjects

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

The thyroid hormones’ reference range was 80-200ng/dl for T₃, 5.1-14.1μg/dl for T₄ and 0.27-4.2μU/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 hyperthyroid (15.9±2.4kg) and euthyroid subjects (15.7±2kg).

All hyperthyroid patients had multinodular goiter on clinical examination. Ultrasound showed 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 07:00 AM after an overnight fast, and had the radial artery and the superficial epigastric vein (which is draining the abdominal subcutaneous adipose tissue) catheterized as previously described (26,27).

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

Blood samples were withdrawn before the meal (at -30 and 0min) and at 30-60min intervals for 360min from the radial artery for measurements of insulin (RIA; Linco Research, St. Charles, USA), glucose (Yellow Springs Instruments, Ohio, USA) and nonesterified fatty acids (NEFA; Roche Diagnostics, Mannheim, Germany). IL-6 and TNFα (ELISA; R&D Systems, Oxon, UK) were measured in the fasting state (at -30 and 0min) from the superficial epigastric vein and from the radial artery.

Adipose tissue blood flow (BF) was measured immediately before each of the two preprandial blood samplings (-30 and 0min). 133Xe dissolved in sterile saline (4MBq, DuPont, MDS Nordion, Belgium) was injected into the subcutaneous adipose
tissue of the anterior abdominal wall within the drainage area of the cannulated abdominal vein (but on the opposite side), about 8cm from the midline and 5cm below the level of the umbilicus. The duration of the injection and the withdrawal of the needle were 2min and 30sec respectively. The injection was given at least 45min 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] x plasma insulin [mU/L] /22.5).

Adipose tissue production rates of cytokines from the adipose tissue was calculated as the differences between the plasma levels measured in the subcutaneous vein and the radial artery, and multiplied by blood flow in the adipose tissue.

Statistical analysis

Results are presented as mean±SEM. All variables studied were normally distributed.

Differences between hyperthyroid and euthyroid subjects were tested with Student’s non-paired t-test. Differences between experiments within the same group were tested with Student’s paired t-test.

Analysis of covariance (ANCOVA) was used to evaluate the association between IL-6 – HOMA, TNFα – NEFA and IL-6 – TNFα. 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).

Results

Glucose, insulin and NEFA

Fasting plasma insulin was increased in hyperthyroid vs euthyroid subjects (10.9±2.3 vs 4.8±0.8µU/ml, p=0.02), whereas fasting plasma glucose was similar (5±0.1 vs 4.7±0.1mM). Fasting plasma NEFA levels were increased in HR (634±55mmol/l) vs EU (454±56µmol/l, p=0.03).

After the meal, plasma insulin was increased in hyperthyroid vs euthyroid subjects (AUC₀⁻₃₆₀ 17267±2447 vs 10331±666µU/ml*min, p=0.01) (Figure 1A). Plasma glucose was similar (AUC₀⁻₃₆₀ 2087±57 vs 2010±43mM*min) (Figure 1B). Plasma NEFA levels were increased in hyperthyroid (AUC₀⁻₃₆₀ 136±18mmol/l*min) vs euthyroid subjects (89±7mmol/l*min, p=0.03), suggesting increased lipolysis (Figure 1C).

HOMA was increased (2.3±0.4 vs 1±0.1 kg/m², p=0.007), indicating insulin resistance in hyperthyroidism.

Adipose tissue blood flow

Adipose tissue blood flow was increased in hyperthyroid vs euthyroid subjects (3.9±0.7 vs 2.1±0.4ml/min/100ml tissue, p=0.004).

IL6

In both euthyroid and hyperthyroid groups, the IL-6 plasma levels were higher in the abdominal vein than in the radial artery suggesting that IL-6 is produced by the subcutaneous adipose tissue (Figure 2).
Both arterial and subcutaneous venous IL-6 levels of hyperthyroid subjects were significantly higher compared to those in the euthyroid subjects (Figure 2).

IL-6 production from the subcutaneous AT was increased in hyperthyroid vs euthyroid subjects (18±6 vs 5±1pg/min/100ml tissue, p=0.04).

Subcutaneous venous IL-6 was positively associated with HOMA index (β-coefficient=1.7±0.7, p=0.049) (Figure 3), although there was no association between arterial IL-6 and HOMA index in the hyperthyroid subjects.

No association was found between IL-6 (arterial or venous) and HOMA index in the euthyroid subjects.

**TNFα**

In the hyperthyroid patients TNFα concentrations in plasma were significantly increased in relation to those found in euthyroid subjects both in arterial and in subcutaneous venous samples (Figure 4).

In both groups there were no significant differences in the concentration of TNFα between the arterial and the subcutaneous venous samples (Figure 4). As a result, there was no significant TNFα release from the subcutaneous adipose tissue studied either in hyperthyroid (0.35±0.37pg/min/100ml tissue) or in euthyroid subjects (0.23±0.4pg/min/100ml tissue).

However, TNFα arterial levels were positively associated with arterial plasma NEFA levels (AUC_{0-360}) (β-coefficient=0.045±0.01, p=0.005) (Figure 5). Subcutaneous venous TNFα 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 TNFα (arterial or venous) and IL-6 (arterial or venous) levels in both hyperthyroid and euthyroid 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.

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 hyperthyroid state. These findings are in agreement with previous studies in hyperthyroid subjects showing that glucose production from the liver (28) and glucose uptake by peripheral tissues (skeletal muscle and adipose tissue) (16,17) is resistant to insulin.

IL-6 and TNFα have been implicated in regulating insulin signaling and lipid metabolism in peripheral tissues (1).

Previous studies regarding the effect of hyperthyroidism on levels of IL-6 have primarily focused on plasma measurements; in these studies IL-6 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 approximately 25% of the systemic levels of IL-6 originate from subcutaneous adipose tissue. Adipose tissue secretion of IL-6 levels has been previously studied with subcutaneous fat biopsies, in vitro in hyperthyroid subjects with Graves’ disease (18). In that study serum concentrations as well as adipose tissue release of IL-6 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 adipose tissue secretion of IL-6, the question whether the observed increase in adipose tissue release of IL-6 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 adipose tissue production rates of IL-6 in patients with hyperthyroidism of non-autoimmune origin compared with the euthyroid subjects. The release of IL-6 from the subcutaneous adipose tissue, which is partly contributing to the increased circulating levels of IL-6 in the hyperthyroid subjects, suggests a direct effect of thyroid hormones in IL-6 production independent of autoimmunity.

In our study there was no association between arterial IL-6 and HOMA index in the hyperthyroid subjects. However, the fact that the increased subcutaneous venous IL-6 levels were positively associated with HOMA index, suggests a possible link between IL-6 production from adipose tissue and the development of insulin resistance in the hyperthyroid state.

In our hyperthyroid patients TNFα levels were significantly increased in relation to those found in euthyroid subjects.

In previous studies, focusing mainly in Graves’ disease, TNFα plasma levels have been found increased (20,21,22,23) or unchanged (18,19). Given that TNFα 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).

Adipose tissue 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 hyperthyroid subjects suggesting increased lipolysis. These findings correspond well with a previous study using the arteriovenous difference technique in
the abdominal subcutaneous adipose tissue depot, suggesting that adipose tissue lipolysis is resistant to insulin in the hyperthyroid state (16).

In the present study arterial and subcutaneous venous TNFα levels were positively associated with arterial plasma NEFA levels, suggesting a possible link between increased TNFα levels and the development of insulin resistance in lipolysis. This is supported by previous observations in euthyroid subjects showing that TNFα inhibits lipoprotein lipase activity and increases lipolysis (12,13,14).

Given that there was no secretion of TNFα by the subcutaneous adipose tissue depot studied, we suggest that TNFα, produced by other tissues or cells, influence 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 co-morbidities (diabetes, hypertension, dyslipidemia, obesity, 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 co-morbidities. 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 hyperthyroid patients with a control group and not with themselves after therapy.

Another limitation of our study is that although our hyperthyroid 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 hyperthyroid group.
In conclusion in hyperthyroidism: (1) glucose and lipid metabolism are resistant to insulin (2) subcutaneous adipose tissue releases IL-6 which could then act as an endocrine mediator of insulin resistance (3) although there is no net secretion of TNFα by the subcutaneous adipose tissue, in vivo, increased systemic TNFα 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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References


Figures

Figure 1

Arterial concentrations of insulin (A), glucose (B) and NEFA (C) in hyperthyroid and euthyroid subjects. Results are presented as mean±SEM. Differences between hyperthyroid and euthyroid subjects were tested with Student’s non-paired t-test (SPSS version 16; SPSS Inc., Chicago, IL) (*: p<0.05).

Figure 2

Arterial and subcutaneous venous concentrations of IL-6 in hyperthyroid and euthyroid subjects. Results are presented as mean±SEM. Differences between hyperthyroid and euthyroid subjects were tested with Student’s non-paired t-test. Differences between experiments within the same group were tested with Student’s paired t-test (SPSS version 16; SPSS Inc., Chicago, IL).

Figure 3

Analysis of covariance (ANCOVA) evaluating the association of subcutaneous venous IL-6 concentrations to HOMA in the hyperthyroid subjects (β-coefficient=1.7±0.7, p=0.049) (SPSS version 16; SPSS Inc., Chicago, IL).

Figure 4

Arterial and subcutaneous venous concentrations of TNFα in hyperthyroid and euthyroid subjects. Results are presented as mean±SEM. Differences between hyperthyroid and euthyroid subjects were tested with Student’s non-paired t-test. Differences between experiments within the same group were tested with Student’s paired t-test (SPSS version 16; SPSS Inc., Chicago, IL).

(NS: not statistically different)

Figure 5
Analysis of covariance (ANCOVA) evaluating the association of arterial TNFα concentrations to plasma NEFA levels (AUC_{0-360}) in the hyperthyroid subjects ($\beta$-coefficient=0.045±0.01, p=0.005) (SPSS version 16; SPSS Inc., Chicago, IL).
Figure 2.
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Figure 4.
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Figure 5.
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