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Short Term, Low Dose Thyroxin Treatment of Euthyroid Patients with Type 2 Diabetes improves Peripheral Blood Flow and Overall Insulin Sensitivity

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Abstract

Purpose: Variation of plasma thyroid hormone levels influences insulin sensitivity and peripheral glucose disposal. High thyroxin dose administration to healthy humans induces insulin resistance, whereas moderate doses increase peripheral glucose disposal. An open-labeled, randomized and placebo-controlled intervention was performed in euthyroid type 2-diabetic patients, to examine the effect of a small thyroxin dose within the euthyroid range on postprandial forearm muscle glucose uptake, insulin sensitivity, in vitro glucose uptake and GLUT4 recruitment in the plasma membrane of monocytes.

Methods: A meal was given to eleven euthyroid, treatment-naive, type-2 diabetic patients (aged 43 ± 3.8 yrs, BMI 27.48 ± 1.39 kg/m^2, T3 119 ± 5.7 ng/dl, T4 8,13 ± 0.46 μg/dl, TSH 1.51 ± 0.14 μU/ml, FT4 1.272 ± 0.047 ng/dl) before and after administration of 50 μg of thyroxin once daily for 2 months. Similarly, a placebo was given to eleven age, sex and BMI-matched euthyroid, type-2 diabetic patients. Blood was drawn for 300 min from a forearm deep vein and the radial artery for measurements of glucose, insulin, and GLUT4 abundance in peripheral monocytes. Forearm blood flow (BF) was measured with strain-gauge-plethysmography. Forearm glucose-uptake, and insulin sensitivity were assessed. After the first meal-tolerance-test, daily treatment with 50 μg of thyroxin or placebo was initiated for a 2-month period. Then a second identical test was repeated.

Results: TSH, glucose, insulin levels and HbA1c reduced significantly in the treatment group. Peak-baseline BF and Glucose-uptake (AUC0-300 min) increased significantly (1.685 ± 0.3 vs. 3.07 ± 0.15 ml/min per 100 cc tissue, p=0.0018) and (587 ± 68 vs. 1015 ± 131 μmol per 100 cc tissue, p=0.0051), respectively. All insulin-sensitivity indices improved post-treatment. Glucose uptake and GLUT4 abundance in monocytes also improved. The placebo group exhibited no change in all variables.

Conclusion: Administration of small, subthyrotoxic doses of thyroxin to euthyroid diabetic patients improves peripheral glucose disposal, blood flow responses and overall insulin sensitivity. This could be of therapeutic importance by reducing the burden of hyperglycaemia and possibly the long term complications of diabetes.

Keywords: Insulin resistance; Muscle blood flow; Glucose uptake; GLUT4; Thyroxin

Abbreviations

TSH: Thyroid Stimulating Hormone; HbA1c: Glycated Haemoglobin; GLUT4: Glucose Transporter 4; BMI: Body Mass Index; FPG: Fasting Plasma Glucose; FPI: Fasting Plasma Insulin; SEM: Standard Error of Mean

Introduction

It is well established that high levels of thyroid hormones in plasma induce insulin resistance [1-3]. This is due to increased rates of endogenous glucose production [4], as well as to impaired muscle insulin-stimulated glucose uptake, a defect which is corrected, at least in part, by the increases in blood flow [5].

In terms of intervention, the results remain controversial. Administration of high doses of thyroid hormones to healthy humans induced insulin resistance [4]. In contrast, delivery of moderate doses of thyroxin to healthy individuals, leading to short-term mild experimental hyperthyroidism, have been shown to increase peripheral glucose disposal during euglycaemia, which counteracts the increase in splachnic glucose release, and results in the maintenance of normal glucose tolerance overall [6]. Interestingly, administration of small doses of triiodothyronine to lean and obese insulin-resistant rats increased the expression of GLUT4 in skeletal muscle and had a beneficial effect on hyperinsulinaemia [7]. In addition, long term administration of small doses of levothyroxine to euthyroid horses has been shown to improve glucose dynamics [8]. The impact of administration of small physiological doses of thyroxine to healthy humans in vivo, which suppress TSH within the euthyroid range, has never been investigated.

This study was undertaken in type 2 diabetic patients, to examine the hypothesis that treatment with small doses of thyroxin within the

euthyroid range can improve muscle glucose disposal and postprandial insulin sensitivity. This was investigated using the arteriovenous-difference technique after the consumption of a mixed meal [9,10] and the in vitro study of a glucose analogue (6-NBDG) uptake by the peripheral monocytes [11].

Subjects and Methods

Subjects

Eleven euthyroid, treatment naive, type-2 diabetic patients with a micronodular texture of the thyroid gland, (aged 42 ± 3.8 yrs, BMI 27.48 ± 1.39 kg/m^2, TSH 1.51 ± 0.14 μU/ml, FT4 1.272 ± 0.047 ng/dl, were studied before and after administration of 50 μg of thyroxin once daily for 2 months (Table 1).

In parallel, a placebo group was also studied. Eleven euthyroid treatment-naïve subjects with type 2 diabetes and a micronodular texture of the thyroid gland, matched for age, sex, BMI, and basal thyroid function, [aged 43 ± 3.74 yrs, BMI 27.8 ± 1.28 kg/m^2, FT4 1.3 ± 0.046 ng/dl, TSH 1.554 ± 0.181 μU/ml] were studied before and after administration of a placebo (placebo for 50 μg thyroxine, Unipharma, Greece) once daily for 2 months (Table 2). All participants were newly diagnosed, treatment-naïve type 2 diabetic patients. The diagnosis of diabetes was made according to the ADA/EASD criteria as confirmed by their glucose and HbA1c values (Tables 1 and 2).

In the present study we have used a small dose of thyroxin for a short period of time (2 months), under strict surveillance so as to maintain the patients' thyroid function within the euthyroid range. Special care was taken so that not even subclinical hyperthyroidism develops, as the latter could influence our results in terms of metabolic regulation. In order to choose the appropriate thyroxin dose we performed several pilot studies when the dose was calculated according to body weight. However, for doses more than 50 μg, some patients quickly suppressed TSH levels below the cut-off level of 0.27 uU/ml, which was unacceptable, and were excluded from the study. The treatment protocol was then adjusted so that the dose of thyroxin was small enough and could not cause subclinical hyperthyroidism. The dose was then fixed to a level of 50 μg for all subjects, since they had comparable body weight and basal TSH levels.

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

Experimental Protocol

Arteriovenous technique

All subjects were admitted to the hospital at 0700 h after an overnight fast and had the radial artery (A) and a forearm deep vein (V) catheterized. A meal (730 kcal, 50%carbohydrate, of which 38% was starch, 40% fat, and 10% protein) was given at least 1 h after catheter insertion and was consumed within 20 min. "The choice to use a mixed meal was based on reports that the relative importance of different tissues in carbohydrate metabolism may vary with the dose of oral glucose or the levels of glycaemia and insulinaemia during a clamp" [12].

Furthermore, the meal creates a metabolic environment that permits the interaction of insulin and substrates to be investigated under conditions as close to physiological as possible [10].

Blood samples were drawn from both sites before the meal (at -30 and 0 min) and at 30- to 60-min intervals for 300 min thereafter for measurements of thyroid hormones (Roche Diagnostics, GmbH Manheimm, Germany), insulin (Linco Research, St. Charles, MO), glucose (Yellow Springs Instruments, Yellow Springs, OH). All participants were relatively young, newly diagnosed, with no signs or symptoms of gastroparesis or any other known gastrointestinal dysfunction. Moreover, the meal tolerance test lasted for 300 min (5 hours), which is an adequate time period for full digestion.

Forearm blood flow was measured with strain-gauge plethysmography (Howkanson, Bellevue, WA), as previously described [4,9]. Immediately after taking an antecubital sample, a cuff was inflated to a pressure of 220 mmHg around the wrist for 2 min. In addition, a cool fan was used over the forearm for 10 min before measurements to minimize contamination with skin blood. With these manipulations, the contribution of skin and subcutaneous adipose tissue blood flow to muscle blood flow in the forearm is small and the variability of forearm blood flow measurements is reduced [4,9]. After the first meal tolerance test, treatment with 50 μg of thyroxin or placebo once daily was initiated for a 2-month period. Then a second identical test was repeated. Special care was taken in order to avoid the induction of subclinical hyperthyroidism, that is suppression of TSH below 0.26 μU/ml, as it has recently been shown that the latter may also be an insulin-resistant condition [13].

Calculations

The values obtained from the two pre-prandial samples were averaged to give a 0' time value. The plasma levels of glucose were converted to whole blood by using fractional hematocrit [9].

Glucose uptake in the forearm tissues was calculated as: Glucose uptake = (A-V) glucose X (FMBF) and the Fractional Glucose Extraction as: (A-V) glucose/A glucose (this calculation is independent of BF) [5,9], where A-V stands for arteriovenous, FMBF for forearm muscle blood flow.

Results are presented as mean ± SEM of plasma levels or integrated postprandial responses [areas under curve (AUCs).

Insulin sensitivity in the fasting state was measured by homeostasis model assessment (HOMA-IR) [14] and hepatic-insulin-sensitivity-index (ISI HOMA) [15] and post-prandially by Gutt-index [ISI0-120] [16], as follows:

HOMA-IR= FPG×FPI/22.5, where FPG stands for fasting plasma glucose, FPI for fasting plasma insulin

Hepatic insulin sensitivity=k/FPG × FPI, where k=22.5 × 18,

Gutt=(m/MPG)/logMSI, Where m=[75000 mg+(0 min Glucose – 120 min Glucose)×0.19×body weight(kg)]/120 min

MPG=mean of 0 min+120 min Glucose (mmol/l)

MSI=mean of 0 min+120 min Insulin (mU/l)

Where MPG stands for mean plasma glucose, MSI for mean systemic insulin.

Gutt-Index is an accurate, generalizable, relatively easy to obtain, estimate of insulin sensitivity. It is more sophisticated than other indices of insulin sensitivity, and it correlates better with the insulin sensitivity index obtained from the euglycemic hyperinsulinemic clamp (M) [16]. This correlation is maintained across the spectrum of

glucose intolerance, and is unaffected by decreasing β-cell secretory capacity.

Forearm muscle blood flow peak-baseline was calculated as the difference between the maximum blood flow achieved following the mixed meal and the baseline blood flow. The maximum blood flow value was reached between 90-180 min after meal consumption.

Effect of insulin on GLUT expression and NBDG uptake – Flow cytometry analysis

Insulin exerts its action, at a cellular level, by a numerous steps intracellular mechanism, the insulin signaling pathway. Regarding glucose transport, the final step of insulin signaling is the enrichment of plasma membrane with GLUT4 isoform. Surface glucose transporter isoforms were determined after incubating cells with insulin and staining them with anti-GLUT4 antisera. The mononuclear cells were collected from all participants (fresh blood) on each study day when drawing the basal sample at the beginning of the meal tolerance procedure. In summary, mononuclear cells were aliquoted at the desired concentration (1×106 cells/ml) and incubated for 60 min, at 220°C, in a buffer (NaCl 140 mM, HEPES 20 mM, KCl 5 mM, $MgSO_4$ 2.5 mM, glucose 5.5 mM, pH 7.4), containing different concentrations (0,25,50,100 and 200 uU/ml) of insulin (Sigma Diagnostics, St. Louis, MO, USA). Termination of incubation was achieved with the addition of cytochalasin-B (10 μM) (Sigma Diagnostics, Missouri, USA). Cells were then stained with specific antiserum for GLUT4 and were analyzed by flow cytometry, as described previously, in detail [11].

For the glucose transport experiments, the tracer used to monitor glucose flux in monocytes was 6-[N-(7-nitrobenz-2-oxa-1, 3-diazol-4-yl) amino]-6-deoxyglucose (NBDG, Invitrogen, Carlsbad, California, USA). Cells were suspended to the above mentioned buffer, at the same concentration. Flow cytometric analysis was initiated immediately after the addition of NBDG (final concentration 30 μM) and insulin. The uptake of the fluorescent probe was recorded as MFI during a 600 sec interval, when the reaction reached a plateau [11].

Statistical analysis

Grouped data are expressed as mean ± SEM. Differences between pre- and postreatment values within group were tested with two sided paired t-test. The comparison of GLUT4 levels and 6-NBDG uptake between pre- and post-treatment, within group, was also done by two sided paired t-test. 6-NBDG uptake is calculated as a % increase over baseline (baseline defined as the MFI of cells prior to the addition of the fluorescent analogue). The %increase of 6-NBDG uptake was plotted vs. the various concentrations of insulin and the area under the curve (AUC0-200) of NBDG uptake was calculated by the formula of the area of trapezium. The normal distribution of the data was verified by the Kolmogorov and Smirnov method. The statistical analysis was performed by the statistic software GraphPad InStat (San Diego, CA, USA).

Results

Plasma levels of glucose, insulin and thyroid hormones

TSH levels reduced significantly after 2-month thyroxin treatment only in the treatment group but still remained within the normal range. FT4 and T4 values increased significantly post-treatment as well. Plasma glucose levels for the whole postprandial period (AUC0-300 min) significantly dropped in the treatment arm, followed by a significant decrease in the Glycosylated Haemoglobin (HbA1c) value. Plasma insulin levels (AUC0-300 min) also reduced after treatment with thyroxin for the whole postprandial period, suggesting an improvement in insulin sensitivity (results shown in Table 1). No significant change was observed in the placebo group for either variable (Table 2).

Variables	Pre-treatment	Post-treatment	P value
TSH μU/ml	1.51 ± 0.11	0.79 ± 0.11	<0.0001
T4 μg/dl	8.13 ± 0.46	9.04 ± 0.502	0.0018
T3 mmol/l	1.3 ± 0.035	1.318 ± 0.055	0.5527
FT4 ng/dl	1.272 ± 0.046	1.454 ± 0.05	0.0016
BMI kg/m^2	27.48 ± 1.39	27.45 ± 1.4	0.6497
FPG mmol/l	7.7 ± 0.22	6.61 ± 0.27	0.0001
FPI μU/ml	16.26 ± 2	8.35 ± 0.9	0.004
HOMA-IR	5.55 ± 0.68	2.38 ± 0.2	0.0013
Hepatic ISI-HOMA	0.207 ± 0.024	0.44 ± 0.027	p<0.0001
Gutt Index (ISI 0-120)	34.36 ± 2.43	55 ± 5.8	0.0004
HbA1c %	8.26 ± 0.16	7.78 ± 0.22	0.0003
Glucose AUC0-300 min (mMmin)	2867 ± 117	2315 ± 109	<0.0001
Insulin AUC0-300 min (mUmin)	26390 ± 4513	15748 ± 3112	0.0001
"Peak-baseline BF (ml/min /100 cc tissue)"	1.685 ± 0.3	3.07 ± 0.15	0.0018
Glucose Flux AUC0-300 min (mmol/100cc tissue)	587 ± 68	1015 ± 131	0.0051
Fractional Glucose Uptake AUC0-300 min % × min	15.86 ± 2.05	27.06 ± 2.76	0.0048

Table 1: Metabolic variables before and after administration of 50 μg of thyroxin (treatment group). (FPG: Fasting Plasma Glucose, FPI: Fasting Plasma Insulin, FT4: Free T4)

Forearm blood flow and glucose uptake

Peak-baseline blood flow increased significantly after the administration of thyroxin (Table 1). Baseline values of the two groups did not differ (3.045 ± 0.13 vs 2.91 ± 0.18 ml/min per 100 cc tissue, p=0.5487).

The net uptake of glucose into the forearm (Glucose Flux AUC0-300 min) increased significantly in the treatment group whereas there was no change in the placebo group. The Fractional Glucose Uptake AUC0-300 min was significantly increased in the treatment group (data shown in Figure 1, Tables 1 and 2).

Variables	Pre-treatment	Post-treatment	P value	P basal values treatment group vs. placebo
TSH μU/ml	1.55 ± 0.18	1.52 ± 0.15	0.5175	0.8458
T4 μg/dl	7.95 ± 0.33	7.96 ± 0.36	0.8884	0.7402
T3 mmol/l	1.3 ± 0.046	1.354 ± 0.056	0.1921	>0.999
FT4 ng/dl	1.25 ± 0.04	1.3 ± 0.04	0.211	0.774
BMI kg/m^2	27.8 ± 1.28	27.74 ± 1.22	0.4561	0.8683
FPG mmol/l	7.65 ± 0.2	7.55 ± 0.17	0.361	0.8931
FPI μU/ml	15.63 ± 1.7	14.9 ± 1.6	0.099	0.8166
HOMA-IR	5.274 ± 0.54	4.96 ± 0.49	0.061	0.7623
Hepatic ISI-HOMA	0.22 ± 0.024	0.224 ± 0.0023	0.3478	0.8968
Gutt Index (ISI 0-120)	34.48 ± 2.67	35.4 ± 3.2	0.3203	0.9757
HbA1c %	8.218 ± 0.14	8.14 ± 0.144	0.221	0.8359
Glucose AUC0-300 min mMmin	2865 ± 115	2829 ± 108	0.2317	0.988
Insulin AUC0-300 min mUmin	25351 ± 4205	27431 ± 3903	0.0725	0.868
Peak-baseline BF ml/min /100 cc tissue	1.863 ± 0.21	1.472 ± 0.21	0.0917	0.6394
Glucose Flux AUC0-300 min (mmol/ 100cc tissue)	540.5 ± 172	680 ± 211	0.203	0.8053
Insulin AUC0-300 min (mUmin).	15 ± 5.5	17.7 ± 6	0.308	0.8767

Table 2: Metabolic variables before and after administration of placebo. (FPG: Fasting Plasma Glucose, FPI: Fasting Plasma Insulin, FT4: Free T4)

Insulin indices

Fasting insulin sensitivity improved following thyroxin treatment, as expressed by the significant reduction in HOMA-IR index in the treatment arm and the increase in the hepatic ISI-HOMA. Postprandial insulin sensitivity improved as well, as reflected in the significant increase in the Gutt-Index (data shown in Figure 1 and Table 1). No change in values was detected in the placebo (Table 2).

For this specific time period, our patients did not demonstrate any weight change, sinus rhythm alteration, blood pressure changes or any other symptom that could imply a clinically significant result of the intervention.

GLUT expression and NBDG uptake

The glucose uptake by peripheral monocytes was studied by the usage of the fluorescent analogue 6-NBDG. The uptake of the analogue is presented as %increment of the fluorescence of the monocytes from baseline (time 0 sec, just before the addition of the analogue) to maximal fluorescence (time 600 sec, when uptake reaches plateau). The %increment of analogue's uptake in the treatment group was plotted versus the insulin's concentrations (Figure 2A). The trend lines in Figure 2A were fitted to the data by linear regression. The tilt is commensurate to the cells responsiveness to insulin. The area under the curve of the 6-NBDG uptake was significantly higher in the treatment arm 5597.068 ± 245.24 vs 8916 ± 1009, P<0.05), (Figure 2B) after the administration of thyroxin.

The insulin's effect of GLUT4 translocation from cytoplasmic depots to plasma membrane was studied by stimulating monocytes with various concentrations of the hormone (0, 25, 50, 100 and 200 uU/ml). The increment of enrichment of plasma membrane to GLUT4 is represented as %increment from baseline (0 mU/l) to maximal concentration (200 mU/l). The abundance of plasma membrane GLUT4 increased after the treatment (Figure 2C). The enrichment of plasma membrane to GLUT4 mediates the increment of glucose uptake by monocytes in the post-treatment state.

Discussion

There has recently been growing evidence that there might be a range in thyroid hormone values at which insulin sensitivity is optimal, and out of which metabolic disturbances may occur [1,2,5,13]. In euthyroid subjects it has been observed that thyroid hormone concentrations have an impact on cardiovascular health and plasma lipids, and that low free-T4 concentration may be an independent risk factor for atherosclerosis [17,18].

Animal studies with euthyroid rodents and horses have demonstrated that short or long-term treatment with T3 or L-T4 can improve glucose transport in skeletal muscle, as well as overall insulin sensitivity and glucose disposal, respectively, even without an evident glucose-lowering effect [7,8].

In our study, thyroxin administration to patients with Type 2 diabetes and a euthyroid goiter resulted in a suppression of TSH within normal limits, and subsequently improved overall insulin sensitivity as

well as peripheral glucose uptake and disposal in vitro and in vivo. To our knowledge, this is the first in vivo study in humans addressing the question whether further suppression of normal TSH could result in a beneficial metabolic outcome. In the treatment group, there was a straightforward glucose lowering and insulin sensitizing effect of this small thyroxin dose. Forearm muscle blood flow increased significantly, leading to an increase in glucose uptake in the forearm tissues. To examine the possibility that this increase masked a defect in insulin-stimulated glucose uptake at the tissue level, we calculated fractional glucose extraction within the forearm tissues (which is independent of BF) [5]. The latter also improved significantly in the treatment group, suggesting an insulin sensitizing effect at a tissue level, which was even more pronounced by the subsequent increase in blood flow.

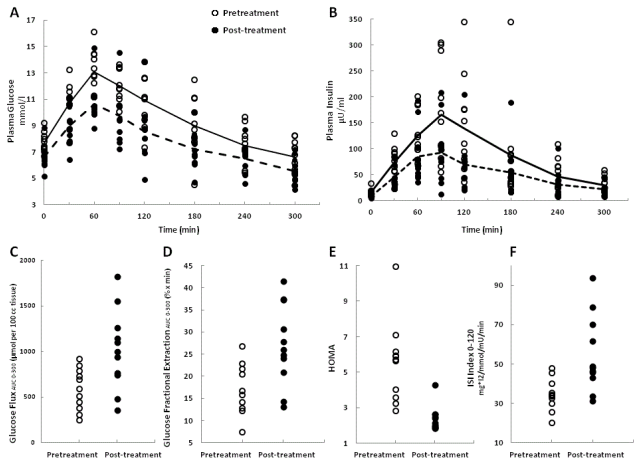

Figure 1: Pretreatment and post-treatment values of DM: (A) Plasma Glucose, (B) Plasma Insulin, (C) Forearm Muscle Glucose Flux (AUC 0-300) (D) Forearm Glucose Fractional Extraction (0-300). (E) HOMA_IR, (F) ISI-Index (0-120). Differences between pre- and post-treatment values were tested with Student's paired t-test (GraphPad InStat, San Diego, CA, USA). P<0.05 at all time points in (A), (B), and between pre- and post- treatment in (C), (D), (E), (F).

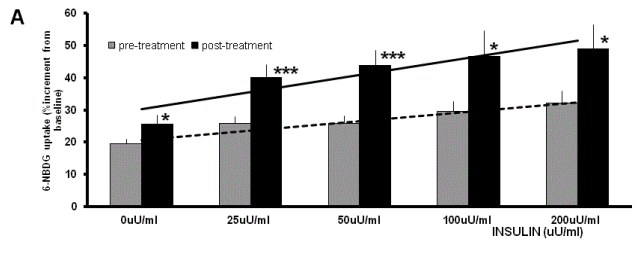

Figure 2A: Thyroxin administration improves glucose analogue's uptake by peripheral monocytes. 6-NBDG uptake by peripheral monocytes under insulin stimulation. The trend line is fitted by linear regression. *p<0.05, ***p<0.005

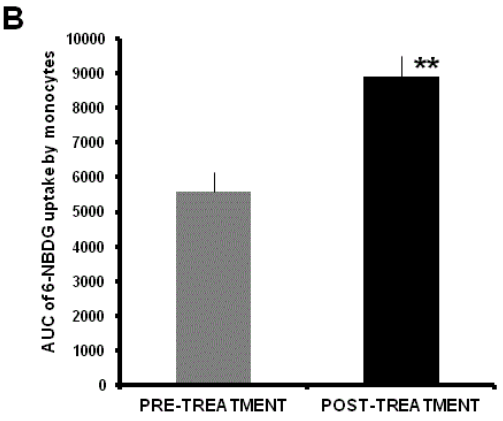

Figure 2B: The statistical analysis regards differences between pre – and post treatment in each insulin concentration and was done by paired t-test. The area under the curve of 6-NBDG uptake by monocytes under insulin stimulation (0,25,50,100 and 200 uU/ml). **p<0.01

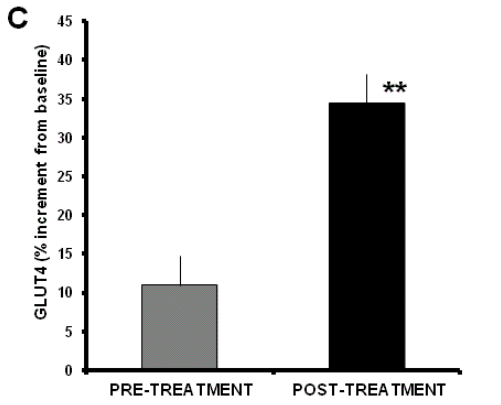

Figure 2C: GLUT4 increment of the abundance in plasma membrane of monocytes from baseline (0 uU/ml) to maximum (200 uU/ml) insulin.**P<0.01

Free T4 levels significantly increased, as expected, within the time interval of the intervention, and TSH was suppressed. However both alterations remained within the normal euthyroid range and represented only a boost in thyroid function to a "higher normal state". Although TSH has for long been considered as just a marker of thyroid function, recent findings imply that it may have an extra-thyroidal direct effect on adipocytes [19]. The TSH receptor is normally expressed in adipocytes; elevated TSH levels can promote a pro-inflammatory response in these cells, by stimulating the release of cytokines, such as IL-6 [19]. Thus, high TSH levels can induce adipose tissue inflammation, impair adipose tissue function such as lipolysis, and predispose to endothelial dysfunction and cardiovascular disease [19]. Furthermore, studies with TSH-receptor knockout mice have shown that inactivation of the receptor results in reduced lipolytic

effect of TSH and an increased adipocyte size, implying that TSH-receptor is important for the growth and metabolism of adipocytes [20].

Thyroid hormones influence glucose metabolism in muscle via the positive transcriptional regulation of GLUT4 [18]. Moreover, as recently suggested, thyroid hormone levels interfere with endothelial function per se [18]. At a cellular level, we found that glucose analogue uptake and GLUT4 abundance in plasma membrane of monocytes were improved post-treatment. This in-vitro effect supports the in vivo results. The rational of using monocytes to study insulin sensitivity is that they provide an easily accessible and reliable model for metabolic studies. These cells have insulin receptors that quickly respond to changes in insulin concentrations and, in the presence of insulin rapidly increase their rates of glucose disposal. Moreover, monocytes express all GLUT isoforms found in muscle and adipose tissue, and the increases in glucose transport in response to insulin in these cells correspond well with those observed in tissues quantitatively important for glucose disposal [10,13].

In our study, the insulin sensitizing effect of thyroid hormone "boosting" within normal levels, improved glycaemia, insulin sensitivity indices and peripheral glucose uptake and disposal in subjects with type 2 diabetes. A limitation of the study is the rather small sample size of participants. However, this is an interventional, labor-consuming study, with straight forward results which reached statistical significance. The sample size is similar to that of comparable studies in the literature, because of the difficulty of the actual procedure [5,9,11]. Moreover, the study of a placebo group further strengthens our results.

In conclusion, our study shows that administration of small subthyrotoxic doses of thyroxin to treatment-naive diabetic euthyroid subjects, can improve glucose disposal in forearm muscle and overall insulin sensitivity. The latter could be of therapeutic importance in subjects with insulin resistance by reducing the burden of hyperglycaemia and possibly the long term complications of diabetes.

Author Contribution

VL and EM wrote the manuscript and researched data, FS researched data, EV researched data. GM researched data, EH researched data, PM researched data, GD reviewed the manuscript.

Declaration of Interest

The authors fully declare any financial or other potential conflict of interest

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