Endocrine (2011) 40:95–101
DOI 10.1007/s12020-011-9446-5

ORIGINAL ARTICLE

Insulin sensitivity, plasma adiponectin and sICAM-1 concentrations in patients with subclinical hypothyroidism: response to levothyroxine therapy

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Received: 17 January 2011 / Accepted: 15 February 2011 / Published online: 18 March 2011

Abstract Subclinical hypothyroidism is associated with an increased risk of atherosclerosis. The aim of this study was to investigate the concentration of plasma soluble intercellular adhesion molecule-1 and adiponectin in relation to insulin sensitivity in patients with subclinical hypothyroidism and to estimate if L-thyroxine treatment had an influence on these parameters. 13 women with subclinical hypothyroidism and 14 euthyroid controls were included in the study. A physical examination was conducted, hyperinsulinemic euglycemic clamp and plasma soluble intercellular adhesion molecule-1, adiponectin and lipids profiles were measured at baseline in both groups and in the group with subclinical hypothyroidism the above procedures were performed after L-thyroxine therapy (mean time of treatment 5.0 months) in stable euthyroid state. Insulin sensitivity and adiponectin were not different at baseline in the two studied groups. Plasma soluble intercellular adhesion molecule-1 concentration was significantly higher in the patients with subclinical hypothyroidism ($P = 0.011$). The comparison of lipids profiles revealed that only LDL-cholesterol concentration was higher ($P = 0.011$) in the group with subclinical hypothyroidism. After therapy, we observed an improvement of insulin sensitivity ($P = 0.012$) and a decrease of plasma glucose ($P = 0.019$) and soluble intercellular adhesion molecule-1 ($P = 0.01$), whereas adiponectin concentration remained unchanged. We concluded that L-thyroxine treatment in patients with subclinical hypothyroidism might exert a beneficial effect by reducing cardiovascular risk factors.

Keywords Subclinical hypothyroidism · Insulin sensitivity · sICAM-1

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Introduction

Subclinical hypothyroidism (SH) is characterized by elevated TSH level whereas fT3 and fT4 are within the normal range. Prevalence of this condition is high, from 1 to 10% of the adult population, with a strong link with gender (significantly higher in women), iodine consumption and age [1–6]. It is unclear whether SH is a risk factor for atherogenesis and cardiovascular diseases [7, 8]. A recently published study demonstrated that the prevalence of hypertension in SH patients is significantly higher in comparison to euthyroid subjects [9]. The data from the Rotterdam study showed that SH is an independent risk factor for atherosclerosis, which is not associated with proatherogenic lipid profile [10]. It is suggested that other factors could contribute to an increased risk of atherosclerosis in SH. Some authors proposed lipoprotein (a) or homocysteine as a link between SH and an increased risk for atherogenesis [11] but the results of other researchers did not confirm these hypotheses [6, 12, 13].

In recent years a great number of researchers have focused on hyperinsulinemia and insulin resistance as independent risk factors of atherosclerosis. There is no consistent data about insulin sensitivity in patients with SH. In experimental studies on rats, thyroid hormone deficiency resulted in a decrease in glucose uptake and glycogen synthesis in the muscles in response to insulin stimulation [14]. Studies in a cohort of euthyroid subjects revealed that there was a significant link between low to normal fT4 levels and an increase in insulin resistance assessed by HOMA-IR and the components of metabolic syndrome [15]. Tuzcu et al. [16] found higher fasting insulin level in patients with SH in comparison to controls, followed by an elevated serum level of high sensitive C reactive protein (hs-CRP), however HOMA-IR was not significantly different. The authors suggested that the elevated hsCRP, which might reflect low grade chronic inflammation, could be associated with fasting hyperinsulinemia before insulin resistance become evident in patients with SH [16]. This is in agreement with the hypothesis that low-grade chronic inflammation leads to the development of insulin resistance which is a key factor in the pathogenesis of type 2 diabetes mellitus and atherosclerosis [17]. Consistently with this hypothesis, an increased level of inflammatory factors (CRP, interleukin-6) predicted the development of insulin resistance and type 2 diabetes mellitus [18]. Impaired insulin sensitivity was also observed in patients with SH and rheumatoid arthritis [19].

Several lines of evidence support a crucial role played by adhesion molecules in the development of atherosclerosis [20]. Soluble forms of adhesion molecules, encompassing soluble intercellular adhesion molecule-1 (sICAM-1) were postulated to be biohumoral markers of the extent and severity of atherosclerosis [20]. Mayer et al. [21] found no difference in sICAM-1 plasma concentration in patients with coronary heart disease and SH in comparison to the euthyroid group. However, as was suggested by the authors, lack of difference might be influenced by concomitant therapies of coronary heart disease with aspirin and statins [21].

Adiponectin, a protein secreted by adipose tissue plays an important role in the modulation of glucose and lipid metabolism in insulin-sensitive tissues and has anti-inflammatory and antiatherogenic properties [22]. In humans plasma levels of adiponectin are significantly lower in insulin-resistant states including different forms of obesity and type 2 diabetes [23] and also in patients with coronary artery disease [23]. Moreover, adiponectin and thyroid hormones share the same physiological actions—both induce an increase in thermogenesis and lipid oxidation.

Therefore, we aimed to investigate plasma sICAM-1 and adiponectin concentration in relation to insulin sensitivity in patients with SH. Then, we tested the hypothesis if L-thyroxine (L-T4) treatment had an influence on these parameters.

Patients and methods

Patients

Thirteen women aged 51.84 ± 14.08 with diagnosed SH (TSH concentration higher than 5 μIU/l observed at least twice in independent measurements: 3.5 ± 1.6 weeks before the commencement of the study and on entry to the study) and who were not receiving L-T4 before the baseline visit, were recruited from the Outpatient Clinic of the Department of Endocrinology, Diabetology and Internal Medicine of the Medical University in Białystok. Four of them had Hashimoto's thyroiditis, nine developed SH after radioiodine treatment (I^{131}). In the group previously treated with radioactive iodine, five were diagnosed as having Graves' disease, whereas four as toxic nodular goitre. The mean time span between I^{131} the therapy and the entry into the study was 12.57 ± 5.16 months. All the patients suffering from Hashimoto and Graves diseases were positive for antithyroid peroxidase autoantibodies (TPO-Abs). The control group, recruited mainly from the Outpatient Clinic and medical staff, consisted of 14 healthy women—without abnormalities of the thyroid gland on clinical examination and with normal thyroid hormones and TPO-Abs levels. All the study participants were in good health, without a known history of any serious disease (apart from a history of thyroid disease in the SH group) and not taking medication which could affect glucose or lipid metabolism. All the subjects were on an unrestricted diet and the SH patients were advised not to change their dietary habits during participation in the study. The local ethics committee approved the protocol of the study and all the participants signed informed written consent forms before enrolment in the project. Characteristics of the SH and control groups are presented in Table 1.

Protocol

All the patients were examined in the morning after an overnight fast. The clinical examination including anthropometric measurements, waist and hip circumferences were taken; body mass index (BMI) and waist to hip ratio (WHR) were calculated. After that, blood samples were drawn for the determination of serum TSH, fT_4, fT_3, TPO-Abs, total cholesterol (TC), HDL-cholesterol (HDL-C), triglycerides (TG), glucose, sICAM-1 and adiponectin concentrations. LDL-cholesterol (LDL-C) was calculated using the Friedewald formula [24]. Next, in all the study

Table 1 Clinical and biochemical characteristics of all study participants

Parameter	Control group $n = 14$	Subclinical hypothyroidism $n = 13$
Age (years)	41.14 ± 10.75	51.84 ± 14.08
Weight (kg)	71.27 ± 15.00	76.26 ± 12.33
BMI (kg/m^2)	27.11 ± 5.10	28.52 ± 4.55
WHR	0.79 ± 0.05	0.81 ± 0.08
SBP (mmHg)	127.85 ± 20.25	141.53 ± 19.61
DBP (mmHg)	82.14 ± 10.86	85.76 ± 9.09
Glucose (mg/dl)	91.85 ± 9.11	89.17 ± 5.74
M (mg x kg^{-1} x min^{-1})	5.90 ± 2.52	4.83 ± 1.66
Adiponectin (μg/ml)	19.76 ± 2.89	25.29 ± 8.63
sICAM-1 (ng/ml)	264.00 ± 84.74	350.46 ± 78.78*
TC (mg/dl)	187.67 ± 34.12	205.41 ± 67.74
HDL-C (mg/dl)	53.51 ± 17.98	48.54 ± 8.52
LDL-C (mg/dl)	112.83 ± 31.33	158.23 ± 53.43*
TG (mg/dl)	107.28 ± 64.97	92.61 ± 37.62
TPO-Abs (IU/ml)	66.64 ± 40.62	802.41 ± 902.31*
TSH (μIU/ml)	1.07 ± 0.85	9.03 ± 2.33*
fT_4 (ng/dl)	1.00 ± 0.15	0.82 ± 0.15*
fT_3 (ng/ml)	2.58 ± 0.61	1.98 ± 0.59*

Data are presented as a mean ± SD

* $P < 0.05$ statistically significant difference

SBP systolic blood pressure, *DBP* diastolic blood pressure

participant's insulin sensitivity was evaluated by means of the hyperinsulinemic normoglycemic clamp technique according to DeFronzo et al. [25] as previously described [26]. On the morning of the study, two venous catheters were inserted into antecubital veins, one for the infusion of insulin and glucose and the other in the contralateral hand, which was heated to ~60°C, for blood sampling. Insulin (Actrapid HM, NovoNordisk, Copenhagen, Denmark) was given as a primed-continuous intravenous infusion for 2 h at 40 mU/m^2/min resulting in constant hyperinsulinemia of approximately 550 pmol/l. Arterialized blood glucose was obtained every 5 min and 20% dextrose (1.11 mmol/l) infusion was adjusted to maintain plasma glucose levels at 5.0 mmol/l. The glucose infusion rate approached stable values during the final 40 min of the study and the rate of the whole-body glucose uptake (M value) was calculated as the mean glucose infusion rate from 80 to 120 min, corrected for glucose space.

From the following day, L-T_4 treatment (Euthyrox, Merck Darmstadt, Germany) was introduced in the SH group. The therapy always started with 25 μg of L-T_4 per day, than the dose being gradually increased on the basis of clinical examination and TSH measurements, repeated every 6 weeks until reaching a stable euthyroid state. The mean L-T4 dose leading to euthyroidism was 66.18 ± 24.9 μg/day, whereas the mean duration of therapy before re-evaluation was 5.0 ± 1.53 months. One patient was lost in the follow up (after I^{131} therapy), so all the procedures described above were then repeated in the remaining 12 SH patients, except for insulin sensitivity which was re-evaluated in nine cases.

Biochemical measurements

Serum TSH, fT3 and fT4 were measured by Microparticle Enzyme Immunoassay (MEIA) (IMx system, Abbott Laboratories, Wiesbaden, Germany). The normal range for estimated hormones was as follows: TSH, 0.47–5.01 μIU/ml; fT4, 0.71–1.83 ng/dl; fT3, 1.68–3.54 ng/ml. TPO-Abs (TPO-ELISA, Warsaw, Poland), sICAM1 (Human Parameter Colorimetric Sandwich, R&D Systems, Minneapolis, USA) were determined by the use of Enzyme Linked Immunosorbent Assays (ELISA). Serum adiponectin was measured with radioimmunoassay kit (Linco Research, St. Charles, MO, USA). Glucose, TC, HDL-C and TG were assayed using enzymatic methods (ANALCO, Warsaw, Poland).

Statistical analysis

The statistical analyses were performed with the STATISTICA 8.0 program (StatSoft, Krakow, Poland). The variables, which did not have normal distribution (TG, TPO-Abs, TSH) were log-transformed prior to statistical analysis. For the purpose of the data presentation, these variables were transformed to absolute values in the "Results" section. The differences between the groups were estimated with unpaired Student *t* test. Differences between variables before and after L-thyroxine therapy were assessed with paired Student's *t*-test. The relationships between variables were studied with the Pearson product-moment correlation analysis and with multiple regression analysis. The level of significance was accepted at $P < 0.05$.

Results

Clinical and biochemical characteristics of all study participants are summarized in Table 1. SH group had significantly higher levels of TSH ($P < 0.0001$), TPO-Abs ($P = 0.005$), and lower fT_4 ($P = 0.005$) and fT_3 ($P = 0.023$) serum concentrations. The comparison of lipid profiles revealed that only LDL-C serum concentration ($P = 0.011$) was significantly higher in the SH group. Insulin sensitivity was not different between the groups, whereas sICAM-1 serum concentrations were significantly higher in SH patients ($P = 0.011$). There were no significant differences in either serum adiponectin or plasma

glucose concentrations at the baseline. After adjustment for age the difference in plasma sICAM-1 concentration was still statistically significant ($P = 0.029$); only the difference in the plasma LDL-C concentration lost statistical significance after adjustment for age. When we compared the studied parameters based on the etiology of SH, we did not observe statistically significant differences between the groups in plasma adiponectin, sICAM-1 and the insulin sensitivity index (*M* value).

The changes in clinical and biochemical parameters of the SH group after L-T_4 treatment are shown in Table 2. After therapy, serum TSH levels decreased significantly ($P = 0.0017$), which meant a return to the normal range. Serum fT_4 and fT_3 concentrations increased after L-T_4 treatment but only for fT_4 the change was statistically significant ($P = 0.012$). Plasma sICAM-1 concentration ($P = 0.01$) and fasting plasma glucose ($P = 0.019$) decreased significantly after L-T4 treatment. An improvement in insulin sensitivity after therapy was also observed ($P = 0.012$) (Fig. 1). Adiponectin level remained unchanged after L-T4 treatment.

In the subgroup of patients with SH, insulin sensitivity at baseline correlated significantly with sICAM-1 before ($r = -0.71$, $P = 0.013$) and after L-T4 treatment ($r = -0.73$, $P = 0.025$).

Table 2 Comparison of clinical and biochemical parameters in SH patients before and after L-T_4 treatment

Parameter	Before treatment $n = 12$	After treatment $n = 12$
Weight (kg)	76.04 ± 12.85	77.09 ± 15.00
BMI (kg/m^2)	28.13 ± 4.84	28.25 ± 5.86
Waist (cm)	89.79 ± 16.36	89.33 ± 16.84
SBP (mmHg)	140.00 ± 19.66	136.25 ± 25.15
DBP (mmHg)	85.42 ± 9.40	83.08 ± 11.18
Glucose (mg/dl)	88.43 ± 6.47	81.95 ± 4.31*
M (mg x kg^{-1} x min^{-1})$^{\#}$	4.82 ± 1.58	6.49 ± 2.79*
TC (mg/dl)	209.20 ± 69.31	218.17 ± 62.10
HDL-C (mg/dl)	47.91 ± 8.58	45.25 ± 14.02
LDL-C (mg/dl)	163.66 ± 51.92	155.83 ± 61.66
TG (mg/dl)	95.61 ± 37.28	80.00 ± 21.91
TPO-Abs (IU/ml)	730.96 ± 903.19	581.87 ± 885.23
Adiponectin (μg/ml)	24.67 ± 8.79	27.10 ± 9.87
sICAM-1 (ng/ml)	353.67 ± 81.39	302.17 ± 44.74*
TSH (μIU/ml)	8.83 ± 2.33	3.76 ± 3.58*
fT_4 (ng/dl)	0.79 ± 0.13	0.96 ± 0.18*
fT_3 (ng/ml)	2.01 ± 0.60	2.22 ± 0.46

Data are presented as a mean ± SD, *$P < 0.05$ statistically significant difference

SBP systolic blood pressure, *DBP* diastolic blood pressure

$^{\#}$ Comparison of insulin sensitivity parameters was performed for 9 patients

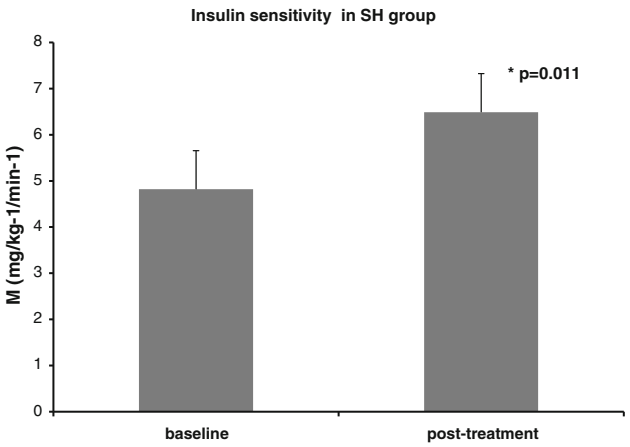

Fig. 1 Insulin sensitivity before and after L-T4 treatment in patients with subclinical hypothyroidism M (mg x kg^{-1} x min^{-1})—insulin sensitivity index calculated from the clamp

Discussion

The main finding of our study is an increase of plasma sICAM-1 levels in patients with SH in comparison to euthyroid controls. Replacement therapy with L-T4 caused a significant improvement in insulin sensitivity with a concurrent reduction in plasma sICAM-1 concentrations, whereas adiponectin levels remained unchanged.

There is a limited number of studies examining insulin sensitivity assessed by the euglycemic insulin clamp technique in SH patients [27, 28]. Studies of Amati et al. [28] showed no difference in insulin sensitivity between eight obese SH and eight obese euthyroid patients. This is in agreement with our results as we did not observe a difference in insulin sensitivity between SH and matched euthyroid patients. In the second study, 11 patients with SH and 12 patients with overt hypothyroidism (OH) were studied. Insulin sensitivity was not different between the groups in basal state [27]. In other studies, insulin resistance was calculated by indirect methods, in the vast majority using HOMA-IR index [29–31]. In two studies, the difference in insulin sensitivity between SH and euthyroid patients was not observed [29, 30]. However, Maraton et al. [31] observed significantly higher HOMA-IR in 12 OH and 13 SH patients in comparison to the euthyroid group. Furthermore, they found a decreased GLUT4 level in plasma membrane of monocytes in SH and OH patients and impaired glucose transport in comparison to the euthyroid patients. The authors concluded that the decreased translocation of glucose transporters into plasma membrane caused an impairment of glucose transportation into the cell and thus reduced insulin action. This observation is in line with the results from previous experimental studies in which a beneficial effect of thyroid hormones on glucose transporters was observed [32, 33]. We demonstrated that after L-T4

treatment, insulin sensitivity improved significantly in SH patients. Handisuraya et al. [27] observed that L-T4 replacement therapy resulted in an improvement of insulin sensitivity only in the group with OH, in the SH group insulin sensitivity remained unchanged. As we have written in the "Introduction" section insulin resistance is considered to be an independent risk factor of atherosclerosis. Also several more recent meta-analyses found an association between SH and coronary artery disease [34–36]. Thus, an improvement of insulin resistance should be considered important in population with high risk for atherosclerosis. In line with this result (improvement of insulin sensitivity) is the observation about the decrease of fasting glucose concentration. However, the precise mechanism underlying the effect of thyroid hormone therapy on insulin sensitivity remains to be elucidated. Interestingly, L-T4 treatment had no influence on many other factors which are related to insulin sensitivity like BMI and waist circumference, so it does not seem likely that a reduction in obesity is related to the improvement of insulin sensitivity.

The data from prospective studies indicated a predictive role of an elevated circulating level of sICAM-1 in initially healthy people. The atherosclerosis risk in communities (ARIC) study first identified sICAM-1 as a significant predictor for future cardiovascular events [37]. In addition, sICAM-1 significantly correlated with carotid intima-media thickness (IMT), the index of early atherosclerosis [38]. It is in agreement with the study published by Monzani et al. [39] who reported significantly higher mean IMT of carotid artery in SH patients in comparison to euthyroid controls. They also found that after 6 months of restored euthyroidism carotid artery intima-media complex was reduced by almost 10% [39].

The results obtained in the current study may indicate that insulin resistance and an elevated level of sICAM-1 could contribute to the relationship between SH and the risk of cardiovascular disease. The only parameter (except from thyroid hormones and TSH) which decreased together with an improvement of insulin sensitivity after L-T4 treatment was sICAM-1 level. However, taking into account the limited number of study participants, this hypothesis needs further investigation. Also it is not known why adhesion molecules are elevated in SH patients—is it caused by relative hormone deficiency or is it a reflection of inflammatory activity? Data from studies estimating sICAM-1 levels in thyroid diseases support the inflammatory hypothesis. In one study, the mean sICAM-1 concentration in patients with hypothyroidism due to chronic thyroiditis was significantly elevated versus control subjects and not different from untreated hyperthyroid patients [40]. Other authors reported an increase in sICAM-1 levels in both hyperthyroidism and hypothyroidism, while patients with euthyroid nodular goiter were within the normal range [41].

We did not observe any statistically significant difference in the adiponectin level between the SH and control groups in our study. Replacement therapy did not affect adiponectin concentration in the SH patients. These results are in agreement with other studies [42–44]. In all other studies, the comparison of adiponectin serum concentration in euthyroid, hypo- and hyperthyroid patients did not reveal any significant differences [42–44]. In additional, the treatment of hypothyroidism did not influence adiponectin concentration [43]. All the quoted data suggest that adiponectin is not related to metabolic changes associated with thyroid dysfunction.

We confirmed data obtained by other researchers regarding the higher concentration of LDL-C in patients with SH [45]. They also observed a reduction of LDL-C after L-T4 therapy. These are excellently reviewed by Biondi and Cooper [45].

One of the limitations of our study is the difference in age between the studied groups and although not statistically significant, this factor could influence the menopausal status of the studied groups. However, after adjustment for age the difference in sICAM-1 was still statistically significant.

We concluded that in SH, non-classical risk factors like insulin resistance and elevated sICAM-1 concentration could contribute to the development of atherosclerosis. L-T4 treatment in SH patients might exert a beneficial effect by reducing cardiovascular risk factors.

Acknowledgements This work was supported by a grant form the Medical University of Bialystok, Poland No 3-50951.

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