

# Time-dependent efficacy of myo-inositol plus selenium in subclinical hypothyroidism

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**ABSTRACT — OBJECTIVE:** The beneficial effect of myo-inositol plus selenium (myo-ins + Se) in patients with subclinical hypothyroidism (SCH) has been recently investigated. In everyday practice, six-month supplementation of myo-ins + Se seems to be adequate for obtaining a therapeutic effect. This study aimed to evaluate the effectiveness of oral myo-ins + Se treatment at different time-points in patients with SCH.

**PATIENTS AND METHODS:** 52 patients with SCH meeting the inclusion criteria were selected to enter the study. Patients were given six-month follow-up with or without myo-ins + Se treatment, consisting in two visits at 3 and 6 months, after an initial visit. Endpoints were serum TSH, fT3, fT4, TPOAb and TgAb levels.

**RESULTS:** A significant decrease of TSH levels in the treated group was observed from baseline to T1 ( $4.38 \pm 0.31$  to  $3.42 \pm 0.3$  mIU/L,  $p \leq 0.05$ ) and T2 ( $4.38 \pm 0.31$  to  $3.11 \pm 0.2$  mIU/L,  $p \leq 0.0001$ ). In the treated group the levels of thyroid hormones had a progressive increment from the baseline until the end of the study. Reduction of TgAb levels was observed in treated group since the first timepoint, whereas in controls was observed a continuous increment. TPOAb decreased significantly in treated patients from  $229.27 \pm 52.89$  IU/ml at baseline to  $165.80 \pm 40.7$  IU/ml at T1 ( $p \leq 0.05$ ) and  $156.77 \pm 44.02$  IU/ml at T2 ( $p \leq 0.05$ ), but not in controls.

**CONCLUSIONS:** In patients with SCH treatment with myo-ins + Se reduced TSH and autoantibodies levels and increased thy-

roid hormones. An improvement of the hormonal and antibody titer was observed since after 3 months of therapy. However, at least 6 months of myo-ins + Se administration seems to be the most adequate length of treatment of SCH.

## KEYWORDS

Subclinical hypothyroidism, Myo-inositol, Selenium, Serum thyroid-stimulating hormone, Autoimmune thyroiditis.

## INTRODUCTION

Subclinical hypothyroidism (SCH) is a pathological condition in which serum thyroid-stimulating hormone (TSH) levels are increased and circulating thyroxine ( $T_4$ ) and tri-iodothyronine ( $T_3$ ) levels within their respective reference ranges<sup>1</sup>. In the general adult population, the reference range for serum TSH is between 0.4 and 4.0 mIU/L, however it has a circadian fluctuation in healthy and SCH individuals. SCH incidence may go up to 15% depending upon the gender, age and population, generally more frequent in females, elderly and in iodine-sufficient regions<sup>2-4</sup>. Chronic autoimmune thyroiditis (AIT), characterized by the presence of circulating antithyroglobulin antibodies (TgAb) and/or antithyroid peroxidase antibodies (TPOAb), is the most common cause of SCH. A specific treatment for SCH has not been defined yet. Repeated measurement of serum TSH, free  $T_4$ , and TPOAb, preferably after a 3-month interval, is recommended when values at first investigation are outside of range<sup>1</sup>. In patients with SCH a reduced quality of life has been reported along with fatigue, muscle weakness,

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weight gain, cold intolerance, depressive symptoms or cognitive and memory deficit<sup>2,5</sup>. The progression to overt hypothyroidism ranges from 2 to 6% per year<sup>4,6</sup>. SCH has been marked as a potentially risk factor of cardiovascular disease and mortality<sup>7-9</sup>. Similarly, higher levels of TSH have been associated to an increased risk of malignancy in patients with thyroid nodules<sup>10-12</sup>. Even though many patients with SCH do not need treatment, the reduction of TSH levels, when out of range, through a specific treatment seems quite important to avoid long-term adverse effects and complications. Combined treatment with myo-inositol and selenium (myo-ins + Se) has shown to ensure euthyroidism in SCH patients with autoimmune thyroiditis (AIT), lowering levels of TSH and thyroid autoantibodies<sup>13-15</sup>. Another recent study has observed the immune-modulatory effect of myo-ins + Se in patients with euthyroid AIT<sup>16</sup>. Treatment with Se alone has also shown to be useful in patients with AIT; however, these studies have shown only a reduction of TPOAb, but no effect was observed in TSH levels<sup>17-19</sup>. Therefore, the aim of this study was to evaluate the effectiveness of oral myo-ins + Se treatment at different timepoints in patients with SCH. Indeed, for the everyday practice is crucial to have the awareness about the effectiveness time-related of a specific therapy.

## PATIENTS AND METHODS

### Patients

Between January 2016 and April 2017, 52 patients with SCH attended our outpatient clinic. During the study inclusion period, in keeping with the European Thyroid Association (ETA) guidelines<sup>1</sup>, patients were screened for thyroid hormones and autoantibodies. Patients were selected to enter the study if meeting the following criteria: TSH levels  $\geq 4.0$  mIU/L (TSH levels lower than 4.0 mIU/L were also considered for inclusion criteria when patients had high autoantibodies levels), TPOAb  $> 34$  UI/ml or TgAb  $> 115$  UI/ml, normal fT<sub>4</sub> and fT<sub>3</sub> levels. Patients under treatment with other drugs or with malabsorption and debilitating disorders were excluded. Enrolled subjects were divided in two groups: treated or control. At recruitment, patients were orally informed, about the composition (600 mg myo-ins plus 83  $\mu$ g Se - Tiroxil®, LO.LI. Pharma S.r.l., Rome, Italy) and the dosage (once daily by oral route for 6 months) of the tablet. Patients not treated received conservative advices maintaining their normal lifestyle. The main endpoint was serum TSH levels. Secondary endpoints were TPOAb, TgAb, fT<sub>3</sub> and fT<sub>4</sub> hormone concentrations. Visits for all patients were assessed by the same physician at baseline (T0) and during follow-up, consisting in two following visits at 3 and 6 months (T1 and T2) after starting the study. At each timepoint, patients were asked about the presence of any side-effect correlated to the treatment. Clinical data were recorded at each outpatient visit with

the same standardized criteria. In the phase of recruiting, both groups had similar values for age, hormones and antibodies levels. The present study complies with the current laws of the country in which it was performed and in accordance with the ethical principles of the Declaration of Helsinki. All data were anonymized and held securely in the outpatient care center.

### Laboratory and technical investigations

Blood samples were drawn from all patients at each timepoint (T0, T1 and T2). Serum concentrations of TSH, fT<sub>3</sub>, fT<sub>4</sub>, TgAb and TPOAb were measured by electro-chemiluminescence immunoassay (ECLIA) (Roche Diagnostics Ltd., Basel, Switzerland). Laboratory references ranges values: TSH 0.4-4.0 mIU/l, fT<sub>3</sub> 2.0-3.5 pg/ml, fT<sub>4</sub> 0.6-1.8 ng/dL, TPOAb 5-34 IU/ml and TgAb 5-115 IU/ml).

### Statistical Analysis

Data were expressed as mean  $\pm$  standard error of the mean (SEM). Both intragroup and intergroup statistical analyses were carried out. Repeated measures ANOVA were used for intragroup analysis and unpaired 't' test was used for intergroup analysis. *p*-value  $\leq 0.05$  was considered as statistically significant.

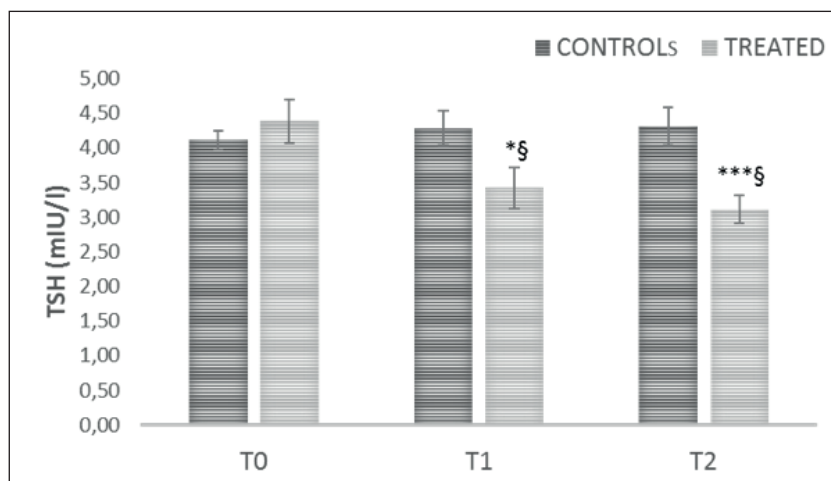
## RESULTS

Fifty-two age-matched patients with SCH, 49 females and 3 men were enrolled in this prospective controlled study. During the study, 4 patients dropped out of the study: one got pregnant, one had suspected overt hypothyroidism and two had no follow-up information available. Therefore, in total 48 patients completed the study (treated group: No. 26; control group: No. 22). As shown in Table 1, at baseline both groups were comparable for age, thyroid hormones and autoantibodies

**Table 1.** Trimester-specific screening of serum TSH, fT<sub>4</sub> and fT<sub>3</sub> levels.

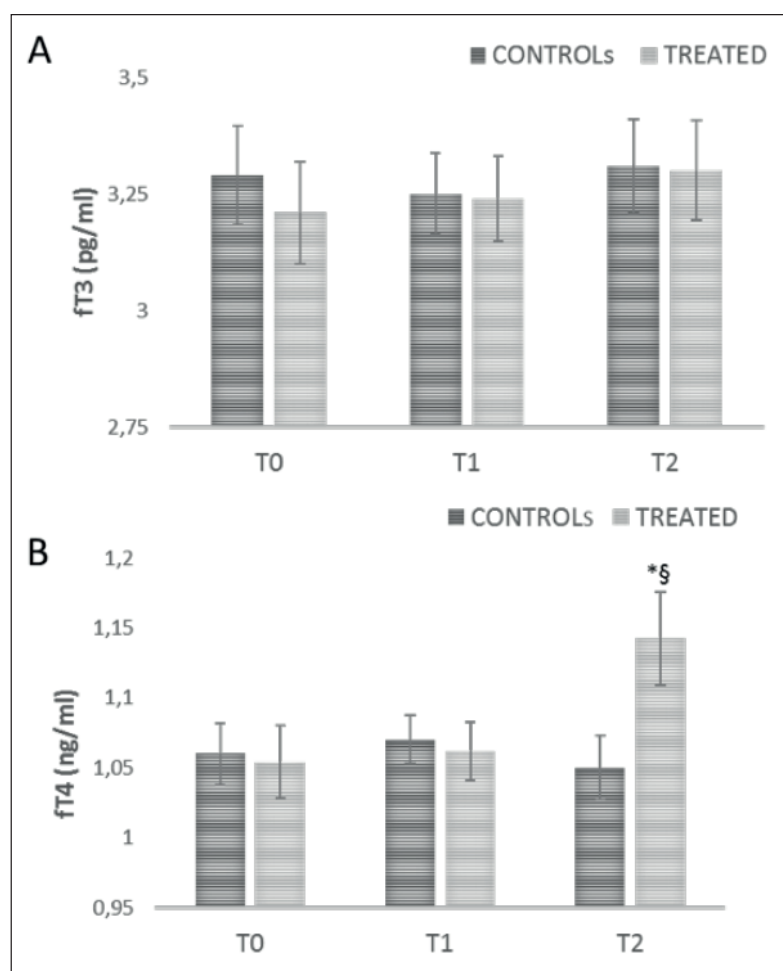
Characteristics	Controls	TREATED	p-value
Age (years)	48.95 $\pm$ 2.36	50.65 $\pm$ 2.24	0.8231
Females, No. (%)	20 (90) 4 dropouts	25 (96)	
Men, No. (%)	2 (10)	1 (4)	
TSH (mIU/L)	4.11 $\pm$ 0.14	4.38 $\pm$ 0.31	0.4708
fT <sub>3</sub> (pg/ml)	3.29 $\pm$ 0.11	3.21 $\pm$ 0.11	0.7451
fT <sub>4</sub> (ng/ml)	1.06 $\pm$ 0.02	1.05 $\pm$ 0.02	0.8771
TPOAb (IU/ml)	331.64 $\pm$ 66.76	229.27 $\pm$ 52.89	0.2529
TgAb (IU/ml)	416.23 $\pm$ 45.61	371.58 $\pm$ 105.78	0.5539

**Figure 1.** Thyroid-stimulating hormone (TSH) levels of patients with subclinical hypothyroidism. TSH levels at 3 timepoints. T0: baseline, T1 after 3 months and T2 after 6 months of treatment with or without myo-inositol + selenium. Treated group (No. 26); control group (No. 22). Values are expressed as mean ( $\pm$  SEM). Concentration comparison of TSH was performed intra-group (\*) and inter-group (§);  $p \leq 0.05$ ,  $p \leq 0.001$ .



levels; no significant differences were observed. There was a significant decrease of mean TSH concentrations in the treated group from baseline to T1 ( $4.38 \pm 0.31$  mIU/L to  $3.42 \pm 0.3$  mIU/L,  $p \leq 0.05$ ) and T2 ( $4.38 \pm 0.31$  mIU/L to  $3.11 \pm 0.2$  mIU/L,  $p \leq 0.0001$ ) (Fig. 1). Inter-group analysis has shown a statistical difference ( $p \leq 0.05$ ) between groups in either T1 and T2 timepoints (Fig. 1). During follow-up a minor increase of TSH levels in the control group was observed (from 4.11

$\pm 0.14$  mIU/L up to  $4.31 \pm 0.27$  mIU/L) (Fig. 1). However, the change was statistically insignificant. Patients after treatment with myo-ins + Se reduced their TSH levels by 21% at T1 and -29% at T2 in respect to baseline, whereas in the control group TSH levels increased by +1% and +5% after 3- and 6-month of treatment, respectively. In the treated group the levels of thyroid hormones had a progressive increment from the baseline until the end of the study (Fig. 2). However, only



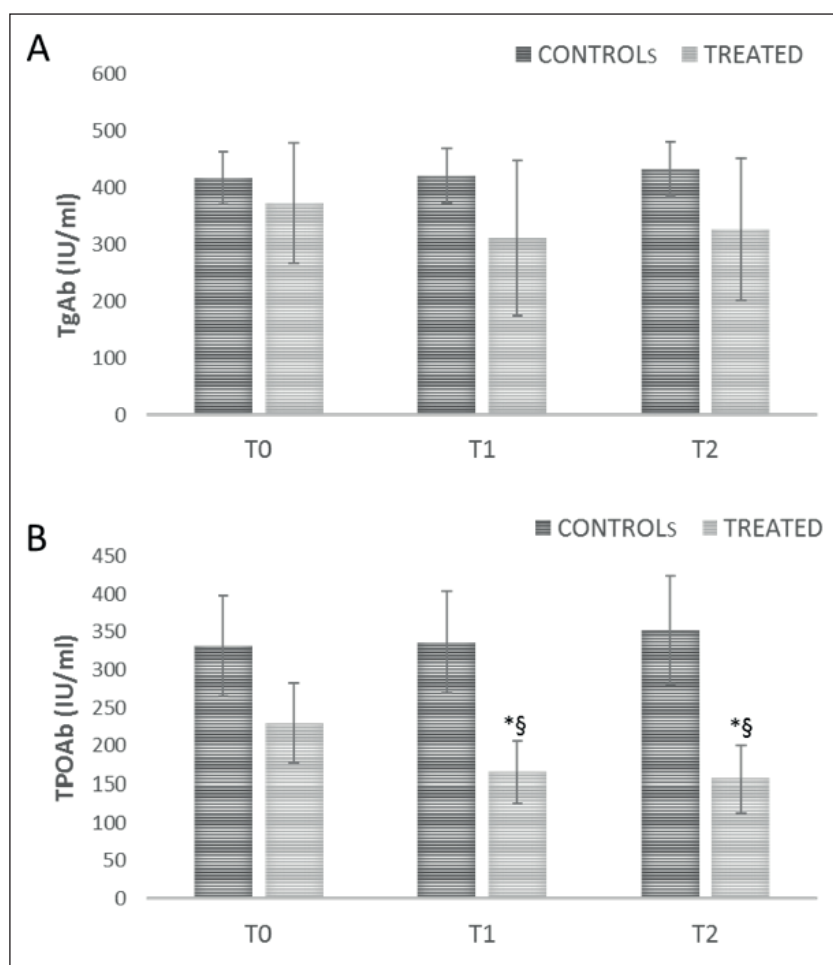
**Figure 2.** Thyroid hormones levels of patients with subclinical hypothyroidism. A. Serum levels of free-triiodothyronine (fT<sub>3</sub>) and B: free-thyroxine (fT<sub>4</sub>) at 3 timepoints. T0: baseline, T1 after 3 months and T2 after 6 months of treatment with or without myo-inositol + selenium. Treated group (No. 26); control group (No. 22). Values are expressed as mean ( $\pm$  SEM). Concentration comparison of fT<sub>3</sub> (A) and fT<sub>4</sub> (B) was performed intra-group (\*) and inter-group (§);  $p \leq 0.05$ .

serum levels of  $fT_4$  significantly increased at T2 respect to baseline (from  $1.05 \pm 0.02$  ng/dl to  $1.142 \pm 0.03$  ng/dl,  $p \leq 0.05$ ) (Fig. 2B). Inter-group concentration comparison of mean  $fT_4$  showed a significant difference at T2 ( $p \leq 0.05$ ) (Fig. 2B). In the control group serum levels of  $fT_4$  remained stable at each timepoint ( $1.06 \pm 0.02$  ng/dl at T0,  $1.07 \pm 0.02$  ng/dl at T1,  $1.05 \pm 0.02$  ng/dl at T2) (Fig. 2B). No significant changes were recorded on  $fT_3$  at each timepoint (Fig. 2A). At baseline, after 3 months and 6 months of treatment with myo-ins + Se,  $fT_3$  values scored were  $3.29 \pm 0.11$  pg/ml,  $3.24 \pm 0.09$  pg/ml and  $3.30 \pm 0.11$  pg/ml, respectively. In control group were  $3.21 \pm 0.11$  pg/ml,  $3.25 \pm 0.09$  pg/ml and  $3.31 \pm 0.10$  pg/ml, at each timepoint (Fig. 2A). The mean values of serum TgAb concentration in treated group decreased from  $371.58 \pm 105.78$  IU/ml to  $310.50 \pm 136.12$  IU/ml at T1, with a minor rise to  $326.12 \pm 124.83$  IU/ml after 6 months (Fig. 3A). In controls was observed a continuous increment of TgAb levels from  $416.23 \pm 45.61$  IU/ml at baseline to  $419.91 \pm 48.51$  IU/ml after 3 months, and  $432.09 \pm 46.84$  IU/ml after 6 months (Fig. 3A). Comparison intra- and inter-group did not show significant difference for this outcome. There was a significant decrease in mean TPOAb concentrations in treated patients from  $229.27 \pm 52.89$  IU/ml at baseline to  $165.80 \pm 40.7$  IU/ml at T1 ( $p \leq 0.05$ )

and  $156.77 \pm 44.02$  IU/ml at T2 ( $p \leq 0.05$ ) (Fig. 3B). A non-significant increase was observed in controls from  $331.64 \pm 66.76$  IU/ml at T0 to  $339.14 \pm 66.98$  IU/ml at T1 and  $364.45 \pm 70.97$  IU/ml at T2. Comparison inter-group showed a significant difference between groups either at T1 and T2 ( $p \leq 0.05$ ). No side-effects related to the treatment were recorded during follow-up (Data not shown).

## DISCUSSION

The results of this study confirm that oral administration of myo-ins + Se significantly decreases serum TSH and TPOAb levels, as well as increases  $fT_4$  concentration. In the treated group, TSH levels were reduced by 21% at T1 and 29% at T2 in respect to baseline, whereas in the control group TSH increased by 1% at first timepoint and 5% after 6-month of follow-up. Data show that after 3 months of treatment patients already had beneficial effect, but a greater efficacy was observed after six months of therapy. These results perfectly agree with other previous studies<sup>13-16</sup>, in which was reported a significant reduction of TSH levels and TPOAb in respect to basal values in AIT patients after 6 months treatment with myo-ins in association with Se.



**Figure 3.** Thyroid auto-antibodies levels of patients with subclinical hypothyroidism. *A*, Serum levels of anti- thyroglobulin (TgAb) and *B*: anti-thyroid peroxidase (TPOAb) at 3 timepoints. T0: baseline, T1 after 3 months and T2 after 6 months of treatment with or without myo-inositol + selenium. Treated group (No. 26); control group (No. 22). Values are expressed as mean ( $\pm$  SEM). Concentration comparison of TgAb (*A*) and TPOAb (*B*) was performed intra-group (\*) and inter-group (§);  $p \leq 0.05$ .

Myo-ins is an isomer of a C6 sugar alcohol, belonging to the inositol family. It is the most abundant form in nature, exerting significant physiological functions, as cellular and tissue development and metabolism regulation<sup>20</sup>. It plays a critical role on the TSH hormone signaling improving its sensitivity. TSH induces the uptake of myo-ins by the thyroid cell, tuning the iodine organification. An impairment of inositol-dependent TSH signaling pathway may lead to TSH resistance and hypothyroidism<sup>21</sup>. The beneficial role of myo-ins for panic disorder, obsessive-compulsive disorder<sup>22,23</sup>, polycystic ovary syndrome<sup>24</sup> and infertility<sup>25,26</sup> is well-known, along with its proven clinical safety<sup>27</sup>. The absence of side-effects is determinant for patients undergoing long and repeated therapies. For a better achievement of the desired healthcare outcomes it is important to improve the patients' compliance. Often adverse-effects or long treatment duration reduce the rate of adherence to a prescribed therapeutic regimen. Here comes the need to find a fast and effective treatment combined with a great therapeutic compliance. During this study patients did not have side-effect related to the treatment and adhered to it for up to six months.

Se is a trace element, embedded in several proteins, indispensable for the well-functioning of thyroid<sup>28</sup>. In the thyroid, it plays an antioxidant function and is involved in the metabolism of thyroid. Different studies have found an inverse relationship between the thyroid volume or its hypoechogenicity with the levels of Se in blood or urine<sup>29-31</sup>. Low concentration of Se may lead to an elevated risk of thyroid disease. Supplementation with Se has been shown to be clinically beneficial for mild to moderate Graves' orbitopathy<sup>28</sup> and AIT associated to SCH<sup>17-19</sup> as it reduces antibody titer, mainly TPOAb. TPOAb seems to be cytotoxic to the thyroid<sup>32</sup>. When autoantibodies bind to thyroid cell membranes cause cell lysis and inflammatory reactions altering thyroid gland function by humoral and cell-mediated mechanisms. The main thyroid autoantigens involved in AIT are TPO, Tg and the TSH receptor. Hashimoto's and atrophic thyroiditis are mainly caused by the detrimental action of TPOAb on the tissue<sup>32,33</sup>. Considering all these evidences, a physiological concentration of Se should be maintained to prevent AIT diseases and an adequate intake seems to be beneficial in immunological mechanisms.

Since myo-ins combined with Se decreases TSH and the auto-antibodies titer and raises thyroid hormones, together they may also provide indirect protection to cardiovascular complications, as  $fT_3$  and  $fT_4$  regulate heart rate and metabolism<sup>34,35</sup>. When the thyroid gland becomes inefficient such as in early hypothyroidism, the TSH levels are elevated and the thyroid hormones may remain within the normal range. The increase of TSH represents the pituitary gland's response to a decrease of circulating thyroid hormone; this is usually the first indication of thyroid gland failure. During supplementation of myo-ins

+ Se the TSH levels decreased and the thyroid hormones increased. This might be due to the recovery of the thyroid gland restoring also the circulating thyroid hormones. This study is in line with the previous results, with a further investigation on the efficacy of short-term treatment with oral myo-ins + Se for SCH. An improvement has been observed on each parameter analyzed after only 3 months of myo-ins + Se treatment, however at the end of the study the treated patients had a significant amelioration of the hormonal and antibody titer compared to controls. Therefore, for SCH patients it would be suitable to continue the treatment with myo-ins + Se at least for 6 months.

## CONCLUSIONS

The extension of myo-ins + Se supplementation for 3 more months resulted in an additional 8% decrease in the TSH concentrations. This time-dependent effectiveness of myo-ins + Se for SCH highlights the need to continue treatment at least up to 6 months. These encouraging results go along with a great adherence to this therapeutic regimen.

## CONFLICT OF INTERESTS:

The Author declares that has no conflict of interests.

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