Clinical aspects of endogenous hypothyroidism and subclinical hyperthyroidism in patients with differentiated thyroid carcinoma

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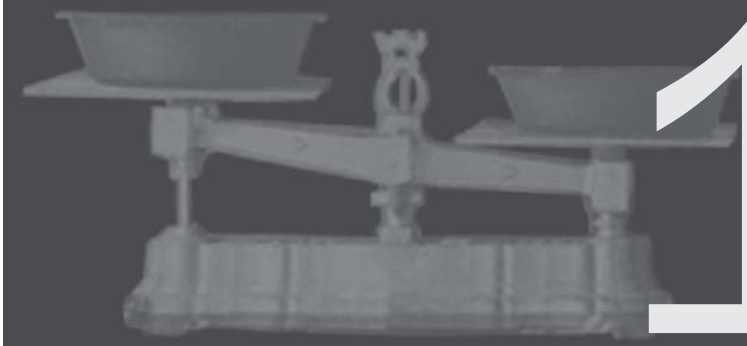

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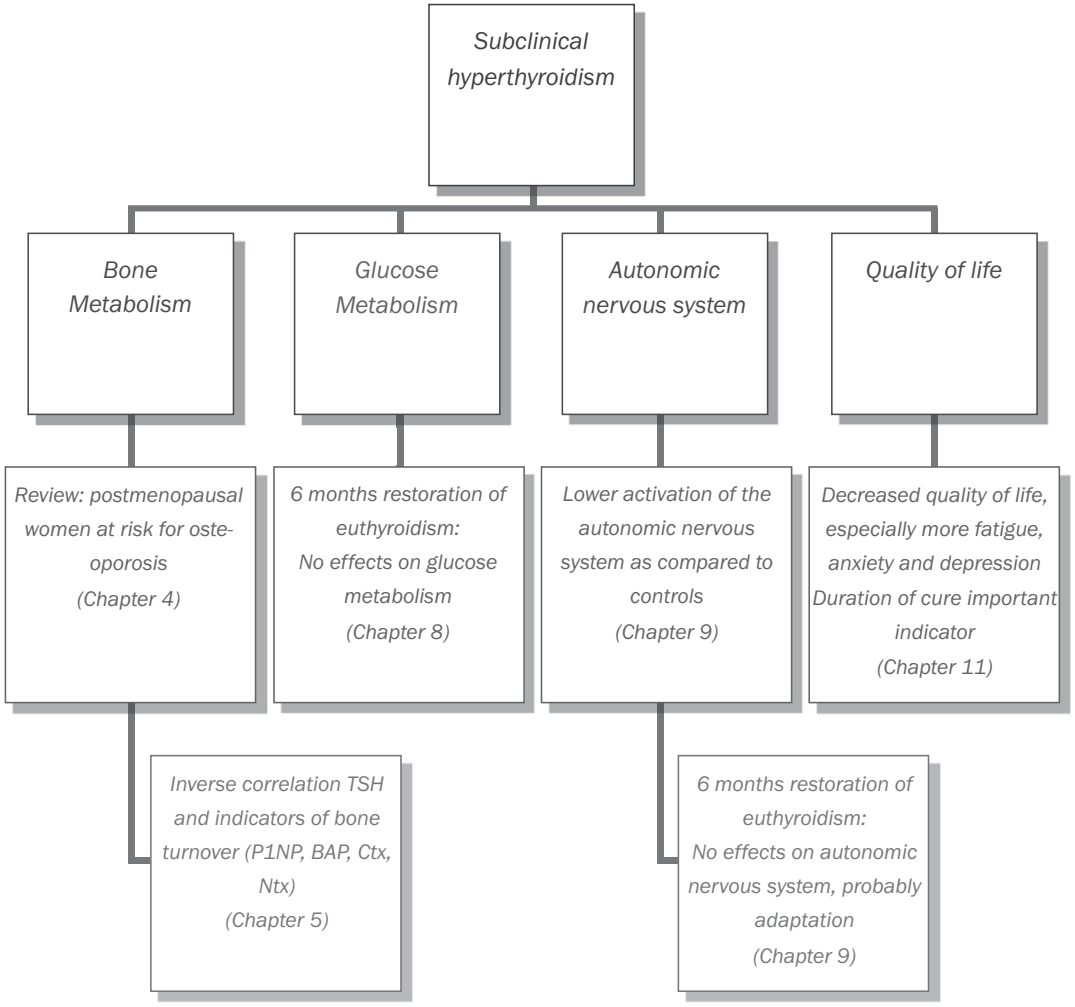
Summary and General Discussion

Contents

I. Introduction

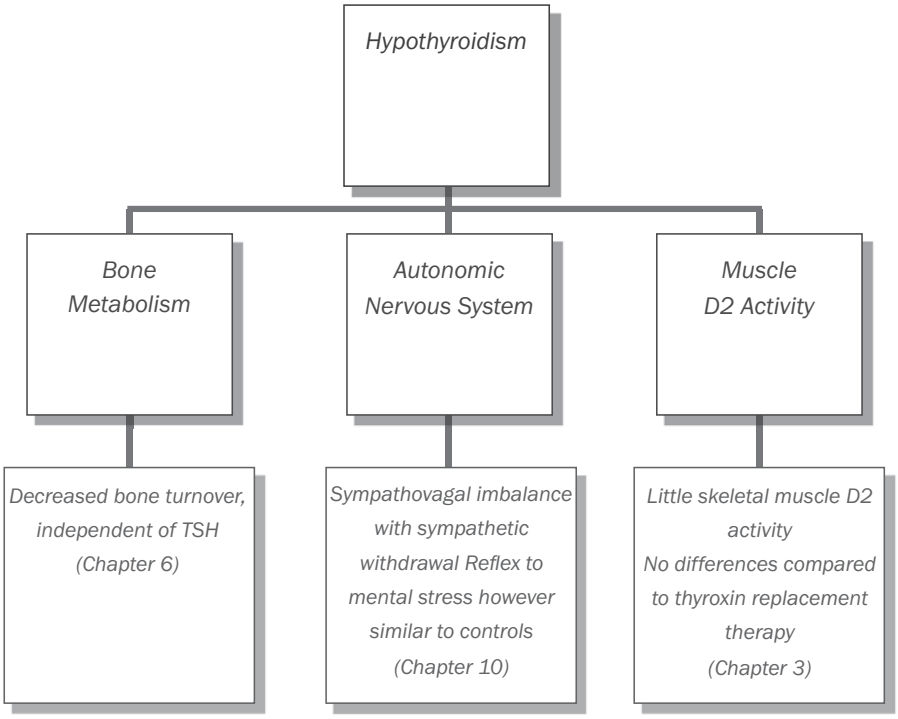
Patients with differentiated thyroid carcinoma (DTC) have an excellent prognosis due to the biological behaviour of the tumor as well as the efficacy of initial therapy. Although the recent publications of Dutch, European and American guidelines and consensus papers have improved the implementation of uniform protocols for diagnosis, treatment and follow-up, still many uncertainties are present. Some of these uncertainties are related to the fact that DTC is a low prevalent disease, which makes the conductance of randomized trials difficult. Other limitations are due to the fact that DTC is a unique malignant disease in which fascinating biological phenomena are present, including the pathophysiology of iodide transport and (controlled) alterations in thyroid hormone levels, which makes that general principles of clinical oncology cannot always be extrapolated to DTC. Another aspect is the decentralised approach of the treatment of the disease. Despite the low incidence, many centers treat patients with DTC.
Many uncertainties still exist with respect to the optimal follow-up strategy of DTC. In the context of DTC, most research is dealing with specific aspects of DTC detection and treatment. In this thesis, we have studied the diagnostic and prognostic value of serum thyroglobulin measurements. However, the main part of this thesis we focused on the consequences of thyroidectomy and subsequent thyroxin substitution therapy on physiological endpoints and quality of life in patients with DTC. DTC patients are treated with high doses of thyroxin to suppress thyrotropin (TSH) levels, because TSH is a growth factor for thyrocytes and increased TSH levels are associated with growth of residual thyroid cancer cells. However, long-term TSH suppression, which in fact represents a state of exogenous (subclinical) hyperthyroidism, may impact on bone metabolism, glucose metabolism, the autonomic nervous system and quality of life (Figure 1).

Figure 1 Overview of effects of subclinical hyperthyroidism on bone metabolism, glucose metabolism, the autonomic nervous system and quality of life.



During follow-up, patients are sometimes withdrawn of thyroxin replacement therapy or alternatively are treated by recombinant human TSH to assess residual or recurrence disease by TSH stimulated thyroglobulin levels and I131 scans. During thyroxin withdrawal, patients become overt hypothyroid, which may affect the systems mentioned above as well (Figure 2).

Figure 2. Effects of hypothyroidism on bone metabolism, glucose metabolism, the autonomic nervous system and Muscle D2 activity.



In addition to the clinical consequences of hyper- and hypothyroidism, patients with DTC are a unique substitution-model to investigate the effects of exogenous thyroid hormones, both deficiency and excess, on bone metabolism, glucose metabolism, the autonomic nervous system and quality of life. In this thesis the effects of exogenous subclinical hyperthyroidism and thyroxin withdrawal on bone metabolism, glucose metabolism, the autonomic nervous system and quality of life are discussed. In addition, we will discuss deiodinase 2 activity in skeletal muscle samples during hypothyroidism. Furthermore, we studied the contribution of the deiodinase 2 Thr92Ala-polymorphism on bone metabolism and thyroxin dosage.

II. Diagnostic & prognostic value of Thyroglobuline

Serum thyroglobulin (Tg) level is the most important diagnostic marker in the follow-up of DTC. Because a European consensus paper recommended to define institutional Tg cut-off levels and the prognostic value of Tg has been hardly studied, we investigated the prognostic and diagnostic value of thyroglobulin (Tg) measurements on recurrence and death in a large cohort of DTC patients by using ROC curves (chapter 2). Our findings indicate an excellent diagnostic accuracy of serum Tg values during TSH stimulation 6 months after initial therapy (sensitivity 100%), with a higher Tg cut-off level (10•0 µg/l) than commonly reported. The specificity and positive predictive value decreased considerably when the more commonly

Used cut-off level of 2 µg/l was used. Advantages of our study are the homogeneity of the patient group for initial therapy, the fact that multiple Tg measurements were analyzed at fixed time-points during follow-up, and the use of ROC analyses. The higher cut-off level may be explained by the lower initial ablation rate in our institute as compared to others. A Tg level pre-ablation of < 27•5 µg/l may predict cure irrespective of prognostic indicators such as stage T4, follicular histology, metastases and higher age. TSH-stimulated Tg measurements 6 months after initial therapy and at 2 and 5 years after initial therapy were independent predictors of thyroid carcinoma-related death. We found a less than 100 % specificity of Tg, which may be explained by the limitations of current imaging techniques to detect thyroid carcinoma. We therefore agree with the policy to administer high dose of RaI to patients with elevated Tg levels. We believe that Tg should be considered as a risk indicator and should therefore be included in the conventional panel of risk factors. A limitation of our study is that the analysis is based on retrospective data. The prognostic Tg cut-off values should therefore be interpreted with caution and prospective research should be performed.

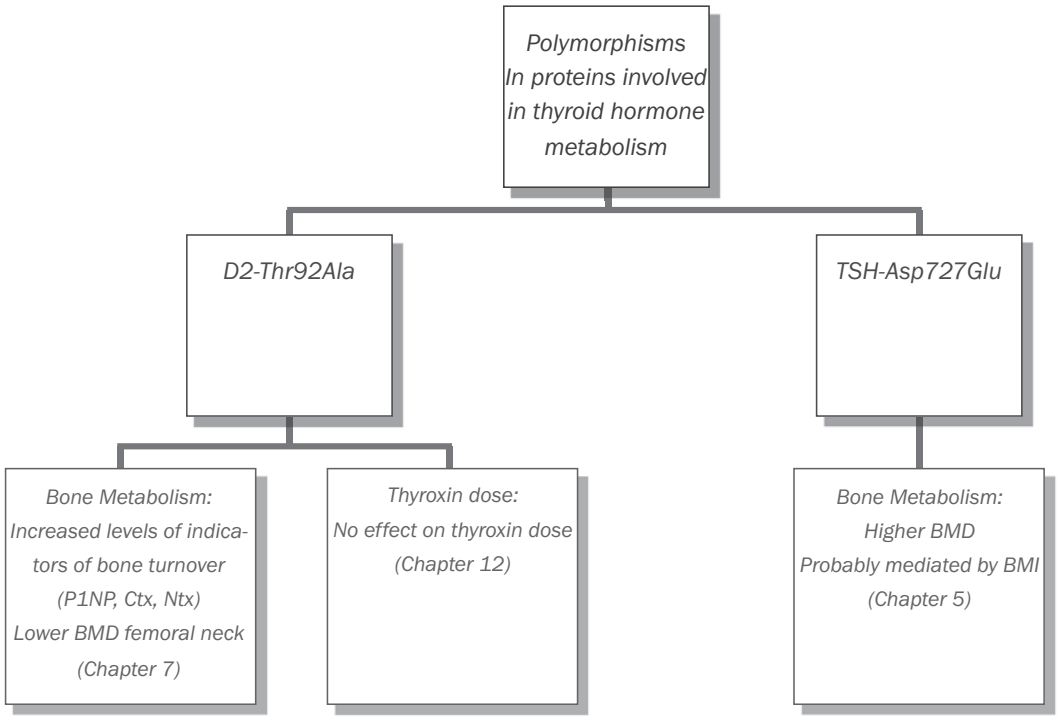
Clinical implications

Our study shows that it is important in the follow-up of DTC to define institutional Tg cut-off levels. A Tg level during TSH stimulation 6 months after initial therapy has an excellent diagnostic value. Tg levels are an independent prognostic indicator of disease free remission and death and using this strategy, allows the identification of high-risk patients in addition to conventionally used prognostic indicators.

Perspective

The methodological imperfections and the inter-institutional differences of the Tg measurements create the need for newer methods to detect tumor recurrence. In our opinion, harmonization of Tg measurements between institutes, at national and international level should be stimulated. RT-PCR to detect cells that produce other thyroid specific proteins, such as TPO could be novel methods to detect recurrent disease. However, more research into this field is needed to evaluate the role of these markers in the follow-up of DTC.

Figure 3. Polymorphism in proteins involved in thyroid hormone metabolism and their effects on bone metabolism and thyroxin dose.



III. Deiodinase 2 activity in skeletal muscle during hypothyroidism

Recently it was reported that D2 is a major source of T3 production during euthyroidism and could therefore play an important role during hypothyroidism (1). The peripheral conversion of T4 to T3 is increased during hypothyroidism, which might be the result of increased D2 activity. In rats, increased conversion of T4 to T3 by D2 was observed during hypothyroidism (2). Animal studies have shown significantly increased D2 activity in the cerebral cortex and pituitary during hypothyroidism. Because D2 activity has been reported in human skeletal muscles, we investigated D2 activity and expression of D2 and D3 mRNA in skeletal muscle samples during hypothyroidism and thyroxin replacement therapy in DTC patients (chapter 3). We found little D2 activity in skeletal muscle samples and no differences were observed in D2 activity during hypothyroidism or after thyroxin replacement therapy. To rule out interfering effects of lidocaine on D2 activity in the skeletal muscle samples, we analyzed the effects of increasing lidocaine concentrations on D2 activity expressed in D2-COS1 cells. This experiment showed no differences in D2 activity with increasing lidocaine concentrations. Nevertheless, we cannot exclude a local effect of lidocaine resulting in downregulation of D2 activity. No differences were observed in the expression of D2 and D3 mRNA during hypothyroidism compared to thyroxin replacement therapy. For that reason, elevated D2 activity in other tissues or elevated D1 activity must be responsible for the increased conversion of T4 to T3 in hypothyroid subjects.
In summary, D2 activity and expression in human skeletal muscle samples is not regulated by thyroid status. The low D2 activities in human skeletal muscle question the physiological relevance of D2 activity for extrathyroidal T3 production.

IV. Bone metabolism

The impact of subclinical hyperthyroidism on bone metabolism in DTC patients is important because DTC is associated with a good prognosis and patients are therefore long-term treated with TSH suppressive therapy. Studies investigating this subject are inconsistent because of limitations in study design, variations in patient groups, methodology, follow-up time and choice of outcome parameters. Although there are numerous studies, no attempts have been made to categorize the studies according to the parameters mentioned above. We therefore performed a systematic review including all studies investigating the effect of subclinical hyperthyroidism on bone metabolism in DTC patients focusing on the changes in bone mineral density (BMD) (chapter 4).
Most studies in premenopausal women and men found no differences in BMD during TSH suppressive therapy, whereas several studies in postmenopausal women reported changes in BMD. This may suggest that there is a clinical effect of TSH suppressive therapy on BMD in postmenopausal women. Other studies in postmenopausal women have not reported differences in BMD. This may be the result of the instability of TSH levels over the years (3) or of differences in additional determinants of BMD such as dietary factors, physical exercise, endogenous factors or genetic susceptibility. These determinants become relevant after the contribution of estrogens has disappeared in postmenopausal women. Estrogen deprivation results in an increased release of cytokines and therefore an increased production of M-CSF and RANKL, which are essential cytokines for the formation and activation of osteoclasts. Subclinical hyperthyroidism could enhance this effect (4-6).

It was found that TSH reduced RANKL mRNA levels and increased OPG mRNA levels resulting In a decreased osteoclast formation and survival (4;7). TSH is suppressed during subclinical hyperthyroidism which could result in a decreased inhibition of bone resorption.

We also studied the relationship between TSH and indicators of bone turnover in 148 thyroidectomized DTC patients (chapter 5). The advantage of DTC patients is that they have more uniform thyroid hormone levels. We found a significant inverse relationship between serum TSH levels and indicators of bone formation (bone specific alkaline phosphatase (BAP) and procollagen type 1 aminoterminal propeptide (P1NP)) and indicators of bone resorption (C-cross-linking terminal telopeptide of type 1 collagen (CTx) and N-telopeptide of collagen cross-links (NTx)), independently of serum thyroid hormone levels. There was no relationship between TSH levels and BMD. This could be explained by the fact that BMD is acquired by a lifelong process, whereas indicators of bone turnover reflect short-term effects. To detect instability of TSH levels over the years, we calculated the slope of TSH levels for each patient. The slope of TSH levels was -0.0001 (range -0.004 to 0) per year, indicating stable TSH levels over the years.

The TSH-Asp727Glu polymorphism is associated with lower TSH levels, but not with differences in thyroid hormone levels (8;9). We, therefore, hypothesized that DTC patients with uniform TSH and thyroid hormone levels would be a good model to study the relationship between the TSH-Asp727Glu polymorphism and bone metabolism. We found significantly higher BMD in carriers of this polymorphism. However, after correction for BMI this relationship was no longer significant, which may suggest that the effect of the polymorphism is mediated by BMI. This is consistent with the study of van der Deure *et al*.(10).

Nonetheless, controversy exists about the net contribution of TSH on BMD and bone metabolism (11;12). Bassett *et al*. found that thyroid hormone receptor (TR)-α knockout mice have osteosclerosis with decreased osteoclastic bone resorption in the presence of normal thyroid hormone- and TSH levels. TR-β knockout mice were osteoporotic with increased bone resorption in the presence of elevated thyroid hormone- and TSH levels. T3 target gene expression showed skeletal hypothyroidism in TRα knockout mice, whereas TRβ mice showed skeletal hyperthyroidism. Furthermore, Bassett *et al*. reported that Pax -/- mice and hyt/hyt mice, two mouse models of congenital hypothyroidism in which the feedback between TSH and thyroid hormones was intact or disrupted, both displayed delayed ossification, reduced cortical bone, a trabecular bone remodeling defect and reduced bone mineralization, indicating that the effects of congenital hypothyroidism on bone are also independent of TSH (13). Moreover, they showed that osteoblasts and osteoclasts express TSH-receptors, but TSH did not affect a cAMP response or the differentiation or function (14).

To investigate the consequences of controlled hypothyroidism on bone metabolism and to discriminate between potential effects mediated by decreased thyroid hormone levels versus those mediated by increased TSH levels, we studied eleven DTC patients during short-term thyroxin withdrawal and 8 weeks after thyroxin replacement therapy versus eleven age-, gender- and BMI-matched DTC patients after injection with recombinant human TSH (rhTSH) resulting in increased TSH levels preserving normal thyroid hormone levels (chapter 6). To our knowledge, this is the first study comparing these two situations in age-, gender- and BMI-matched patients. During hypothyroidism, a significant decrease in C-cross-linking terminal telopeptide of type I collagen levels was found accompanied by increased levels of osteoprotegerin (OPG), indicating decreased bone resorption. This is in agreement with most studies. After rhTSH-injections, no differences in indicators of bone metabolism were observed. A recent study found only a weak expression of the TSH receptor in human osteoblasts (15). This might explain why we found no differences in parameters of bone turnover after rhTSH injection.

D2 activity is found in osteoblasts, which might imply a role for D2 in bone metabolism. Because the D2 Thr-92Ala polymorphism has been associated with a decreased enzyme velocity, we studied the relationship between the D2-Thr92Ala polymorphism and BMD and indicators of bone turnover in DTC patients (chapter 7). We found a 6% lower BMD of the femoral neck in the homozygote carriers of the D2-THr92Ala polymorphism. Furthermore, increased levels of indicators of bone formation (P1NP) and indicators of bone resorption (Ctx and Ntx) were observed in homozygote carriers independent of other determinants of bone metabolism, such as age, gender, BMI, estrogen status, calcium and vitamin D and also independent of TSH. This association was also independent of thyroid hormone levels indicating a true effect of the D2-Thr92Alal polymorphism. No relationship between the D2-Thr92Alal polymorphism and BMD of the lumbar spine was observed, which may be explained by the fact that BMD measurements of the lumbar spine are influenced by osteoarthritis and therefore cannot be correctly assessed. A recent study found an association between the D2-Thr92Alal polymorphism and osteoarthritis strengthening the role for D2 in bone metabolism. As the D2-Thr92Ala polymorphism is associated with a lower enzyme velocity in vitro in thyroid and skeletal muscle samples, and T3 stimulates osteoblastic proliferation and osteoblasts are the primary T3 target cells that regulate bone turnover (16-20), we expected to find decreased bone turnover in homozygote carriers of the D2-Thr92Ala polymorphism. We found, however, increased bone turnover in homozygote carriers of the D2-Thr92Ala polymorphism. D2 activity is found in mature osteoblasts, but not in osteoclasts (21). For this reason, the increased bone resorption could not be explained by direct effects on osteoclasts but more likely, indirectly, through alterations in the interaction between osteoblasts and osteoclasts. This study shows that the relationship between local T3 availability through the action of D2 and bone metabolism is complex, and can not be explained from the traditionally observed direct effects of T3 on bone cells. Multiple known and unknown components of the bone microenvironment may be involved. We believe the findings need to be reconfirmed in another study. However, as we used regression analysis to investigate this correlation and we corrected for all covariates, we feel that this study may add important data to the role of D2 and the D2-Thr92Ala polymorphism in bone metabolism.

In conclusion, we found no effect of TSH suppressive replacement therapy on bone mineral density in men and premenopausal women. However, postmenopausal women are at risk for osteoporosis. Although we found an inverse relationship between TSH and indicators of bone turnover in DTC patients on thyroxin replacement therapy, we found no effect of TSH on bone metabolism during TSH stimulation. This is in line with the studies of Bassett in mice. A possible explanation could be that in the cross-sectional study investigating the relationship between thyroid hormone levels and indicators of bone turnover postmenopausal women were included, whereas in the hypothyroidism study only men and premenopausal women were studied. Another explanation could be differences in study design, since we studied the effects hypothyroidism after short-term withdrawal (4 weeks), whereas the cross-sectional study included patients treated long-term (median (range) 9.3 (1.2-43) years) with TSH suppressive therapy. We believe that alterations in thyroid hormone levels are of more importance for bone turnover then TSH levels. We found that homozygote carriers of the D2-Thr92Ala polymorphism have lower BMD of the femora neck with increased indicators of bone turnover. These results point to a functional role of D2 in humans for the local availability of T3 in bone. However, this association is complex and is probably explained indirectly by the interaction between osteoclasts and osteoblasts.

Clinical implications

Our findings indicate a higher risk for bone loss in post-menopausal women on TSH suppressive therapy. In these patients screening at baseline and regular intervals during

TSH suppressive therapy is advised to allow timely intervention with bone protective agents. Since we have reported a relationship between TSH and indicators of bone turnover, we believe that restoration to euthyroidism should be propagated in patients with low-risk of recurrence or after long-term follow-up.

V. Glucose metabolism

There are only limited data available on the consequences of subclinical hyperthyroidism on glucose metabolism. For that reason, we performed a prospective randomized placebo-controlled study to investigate the effects of restoration of euthyroidism after long-term subclinical hyperthyroidism on glucose metabolism (Chapter 8). We found no effects of restoration to euthyroidism on several parameters of glucose- and lipid metabolism. The percentage of patients with impaired glucose tolerance assessed by the oral glucose tolerance test in our study (15.4%) was comparable with previous studies in the Netherlands and the USA (10.3% and 15.6%) (22)). Yavuz *et al*. (23) found a decreased insulin sensitivity index after 6 months of exogenous subclinical hyperthyroidism compared to matched controls, which is at odds with our study. These differences in outcome might be explained by differences in the duration of subclinical hyperthyroidism. We studied a population, that was treated for over 10 years with TSH suppressive therapy which might result in adaptation, whereas Yavuz *et al*. studied 20 patients with multinodular goitre who were treated for 6 months (24). It could also be that the "dose" (the extent of subclinical hyperthyroidism) in our study was not relevant to result in a "response" (glucose intolerance). However, this explanation is unlikely, because TSH values in our study were comparable to the values in the study of Yavuz *et al*.
In conclusion, we observed no effect of restoration of euthyroidism on glucose- or lipidmetabolsim in patients treated with long-term (>10 years) TSH suppressive therapy, which might imply that adaptation of glucose metabolism occurs in long-term TSH suppressive therapy.

VI. Autonomic nervous system

In the literature, the consequences of subclinical hyperthyroidism on the autonomic nervous system are not clear. In addition, most studies are performed in heterogeneous patient populations in which the duration and course of subclinical hyperthyroidism are not known. Furthermore, thyreostatic drugs and the use of β-blockers may influence the effects on the autonomic nervous system. Consequently, we performed the first prospective, placebo-controlled randomized study to investigate the effects of restoration of euthyroidism on the autonomic nervous system in patients with long-term exogenous subclinical hyperthyroidism (chapter 9).
We found that subclinical hyperthyroidism was associated with a lower degree of activation of the autonomic nervous system when compared to euthyroid controls, whereas activation was higher than in hyperthyroidism. Urinary catecholamine excretion was higher during subclinical hyperthyroidism compared to euthyroid controls, whereas it was lower compared to hyperthyroidism.
Restoration to euthyroidism in patients with long-term subclinical hyperthyroidism due to

TSH suppression had no effect on the autonomic nervous system. No differences were observed in urinary catecholamine excretion, heart rate variability parameters and the response to a mental stress test between the patients who remained on TSH suppression and those in whom biochemical euthyroidism was restored. Although, in all patients in the intervention-group (restoration to euthyroidism) TSH levels became within the normal range with a decrease in FT4 and FT3, restoration of euthyroidism in patients treated for more then 10 years with TSH suppressive therapy did not result in changes in the activity of the autonomic nervous system. An explanation could be that during long-term TSH suppressive therapy irreversible changes or adaptation occur, like we observed in glucose metabolism, or that restoration of the autonomic nervous system set-point takes longer than 6 months.

In our study, the LF component was significant higher in patients compared to controls, whereas there were no differences in the HF component and the LF/HF power ratio between the patients compared to healthy controls. Other studies reported sympathovagal imbalance with increased sympathetic activity and a decreased vagal tone during (subclinical) hyperthyroidism characterized by an increased LF component (expressed in normalized units) and a decreased HF component resulting in an increased LF/HF power ratio (25-31). Possible explanations for the fact that the HRV spectrum had characteristics of hyperthyroidism despite normal mean TSH levels could be that the patients in the present study were treated for a long period (5.0 ± 7.1 years) with TSH suppressive thyroxin replacement therapy preceding the present study and it is plausible that irreversible changes or adaptation of the autonomic nervous were present. This would concur with other recent findings of our group which showed that long-term subclinical hyperthyroidism affects the autonomic nervous system and that these changes persist even after a 6 months-period of restoration to euthyroidism (32).

We noticed however, a substantial difference between the patients and the controls in VLF, which could well be that this difference in the contribution of the VLF frequency band influenced our findings on the LF and HF component.

The response to a challenge of the autonomic nervous system, which seems to reveal the most prominent differences in the autonomic nervous system, was also investigated. We found no differences in the response to the mental stress test, which is a validated test to study the autonomic nervous system, during thyroxin replacement therapy as compared to the values mentioned in healthy controls.

The impact of hypothyroidism on the autonomic nervous system is inconclusive. Current literature shows conflicting results with either increased sympathetic activity, decreased sympathetic modulation or increased vagal tone. For that reason, we investigated the effect of controlled hypothyroidism and reinstitution of thyroxin replacement therapy on the autonomic nervous system in DTC patients by measuring heart rate variability at rest and in response to a mental stress test. (chapter 10). The LF/HF power ratio, representing sympathovagal balance with lower levels suggesting sympathetic withdrawal (33), was significant lower during hypothyroidism compared to thyroxin replacement therapy, indicating sympathovagal imbalance with sympathetic withdrawal. The LF component tended to be lower during hypothyroidism compared to thyroxin replacement therapy. Although the LF component is associated with both sympathetic and vagal activity (33), other studies report that the LF component, especially when expressed in normalized units, reflects sympathetic activity (27;33;34). This is in agreement with the literature. No differences were observed in the response to a mental stress test during hypothyroidism compared to values in healthy controls.

In summary, long-term exogenous subclinical hyperthyroidism affects the autonomic nervous system as measured by heart rate variability and urinary catecholamine excretion, whereas 6 months after restoration of euthyroidism no differences were observed which points out that

long-term TSH suppressive therapy may result in irreversible changes or adaptation. Short-term hypothyroidism in thyreoidectomized patients results in a sympathovagal imbalance with sympathetic withdrawal during hypothyroidism. The cardiovascular reflexes to (mental) stress were however preserved

Clinical implications
Our findings indicate an adaptation of the autonomic nervous system to long-term TSH suppressive therapy, which is not repaired within 6 months of restoration to euthyroidism. We therefore believe that the need for long-term TSH suppressive therapy should be reconsidered carefully in DTC patients.

VII. Quality of life

Quality of life may be affected in DTC patients by either the diagnosis with initial therapy or TSH suppressive therapy. Several studies have investigated this subject, but these results are inconclusive. For that reason, we studied quality of life (QOL) in a large cohort of cured DTC patients using multiple quality of life questionnaires and compared the results to those found in healthy matched controls (chapter 11). Our findings indicate decreased QOL in DTC patients, which may be restored after years of follow-up. Longer duration of cure was associated with better scores on different quality of life items. Advantages of our study are the large number of patients included in the study, the use of multiple quality of life questionnaires and comparison with matched health controls.
The consequences of subclinical hyperthyroidism on QOL are less clear (35-37). Studies investigating this subject included selected groups of DTC patients or patients with endogenous subclinical hyperthyroidism in which the duration and course of subclinical hyperthyroidism are not known. In our study, QOL was not affected by alterations in TSH levels at time of the survey or over time since initial therapy.
In summary, QOL is affected in a large cohort of cured DTC patients compared to healthy matched controls. Only long duration of cure could normalize these effects.

Clinical implications
Clinicians should be aware that despite the good prognosis psychological well-being of cured DTC patients can be affected for years and should provide professional support when necessary.

VIII. D2-Thr92Ala and thyroxin dose

Controversy exists on the functional implications of the D2-Thr92Alal polymorphism. We investigated the association between this D2-Thr92Ala polymorphism, thyroid hormone levels and thyroxin dose in 2 separate groups of patients: athyroid patients after treatment for DTC or patients with Hashimoto thyroiditis (chapter 12). The D2-Thr92Ala polymorphism was not associated with thyroid hormone parameters or thyroxin dosages in the 2 separate groups of patients included in our analyses, which is in agreement with previous studies. However, Torlontano *et al*. reported that homozygous DTC carriers of the D2-Ala92 allele need higher thyroxin dosages (38), which was present in the near-suppressed TSH group,

but not in the suppressed group. Limitations of their study were the absence of data on TSH levels in the near-suppressed group and the analysis strategy, which should be primarily based on regression analysis, rather than TSH categories. It is noteworthy, that Torlontano *et al*. did not find any differences in thyroid hormone levels suggesting that patients with D2-Ala92 alleles need a higher thyroxin dose to reach the same serum FT4 level indicating that the Ala allele would affect T4 resorption instead of T4 feedback. Furthermore, the association between the D2-Thr92Ala polymorphism and thyroxin dose was only found in the TSH suppressive group, which is an ill-defined group with a wide plasma TSH range including patients with normal TSH levels.

In conclusion, we found no association between the D2-Thr92Ala polymorphism and thyroxin dose in athyroid patients and Hashimoto thyroiditis patients.

13 Summary and general discussion

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