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

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01

General Introduction

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I. Introduction

Differentiated thyroid carcinoma (DTC) is a rare disease with an incidence varying from 2-10/100.000 (1-4). The prevalence of DTC is, however, high because of the good prognosis. In general, 80% of the newly diagnosed tumors are differentiated tumors originating from the epithelial follicular cells. Median age at diagnosis is between 45 and 50 year with a female to male predominance of 2:1 (5).
DTC is associated with an excellent prognosis, with reported 10-year survival rates reaching 90% (6). This is because of a combination of the favorable biological behaviour of the tumor as well as the availability of effective therapy, consisting of total thyroidectomy followed by radioiodine ablation. After initial therapy, all patients with DTC are initially treated with high doses of thyroxin aiming at significantly suppressing thyrotropin (TSH) levels, resulting in a subclinical hyperthyroid state. The rationale of this approach is based on the potential harmful effects of TSH on tumor recurrence (7;8). However, long-term TSH suppression may be associated with potential harmful effects on various systems, including bone metabolism (9-11), glucose metabolism (12-14), the autonomic nervous system (15-18) and quality of life (19-23).
According to protocollized follow up, thyroxin replacement therapy can be transiently stopped in these patients to detect residual or recurrent disease by TSH stimulated thyroglobulin levels. As a result of this standardized procedure, patients become overtly hypothyroid within 4-6 weeks. This may reversely affect the systems influenced by subclinical hyperthyroidism, mentioned above.
DTC patients are an unique model to study the metabolic effects of thyroid hormone, both depletion and excess, on physiological systems, because these DTC patients are treated with total thyroidectomy and therefore don't produce any endogenous thyroid hormones. Thyroid hormone levels are well documented in these patients and can be exactly regulated by changing the thyroxin dosages. During clinical follow-up, patients are sometimes withdrawn from thyroxin, which creates a state of controlled hypothyroidism, whereas many patients will be treated with TSH suppressive dosages of thyroxin, thereby creating a state of subclinical hyperthyroidism. Moreover, there is no interfering effect from thyroid disease, like in patients substituted with thyroxin for autoimmune thyroid disease.
In this introductory chapter a general overview of DTC, thyroid hormones and the clinical consequences of exogenous subclinical hyperthyroidism and thyroxin withdrawal will be provided and the questions addressed in this thesis will be introduced.

II. Differentiated thyroid carcinoma

Pathogenesis
Genetic alterations are involved in the pathogenesis of thyroid carcinoma. The analysis of these genetic alterations is important not only for the diagnosis of DTC, but also for the understanding of the pathophysiology of thyroid disorders(24-26). Mutations in one of the three RAS-genes are frequently found in follicular adenomas and carcinomas. Benign hyperfunctioning nodules or adenomas are associated with mutations in the GSP and TSH receptor genes.
The recent identification of mutations in B-RAF, which are present in 40-60 % of papillary thyroid carcinomas (PTC), has improved the understanding of the molecular pathogenesis of PTC. B-RAF is a component of the RET RAS RAF cascade that activates MAP kinase. Almost all patients with PTC have rearrangements and mutations of B-RAF, RAS, RAF and TRK (neutrotrophic tyrosine kinase receptor). Translocations of RET, that are found in DTC, give rise to a chimeric protein consisting of an activated RET tyrosine kinase domain (24;27-

42). Transcriptional and post-transcriptional mechanisms are thought to regulate MET overexpression as a secondary effects (43).
The genetic pathogenesis of follicular thyroid carcinoma (FTC) is less clear. However, it was found that FTC is related with rearrangements in PAX8 and PPAR-γ genes, which are traditionally associated with thyroid development (PAX 8) and cell differentiation and metabolism (PPAR-γ) (44). The chimeric protein acts as a dominant negative competitor for PPAR-γ. A downregulation of the PPAR-γ signaling route has been observed in experimental models of DTC (45).
The genetic alterations that are involved in the pathogenesis of DTC, result in proliferation by multiple pathways and the loss of thyroid specific proteins. Thyroid peroxidase (TPO) is believed to disappear in an early phase, followed by the disappearance of NIS.

Diagnosis
Fine needle aspiration (FNA) is the procedure of choice in patients presenting with thyroid nodules. The sensitivity of FNA is 90-95 %. The specificity of FNA is lower, 60-80%, when all patients with a non-benign FNA are referred for surgery (46). The distinction between benign and malignant follicular tumors is difficult to make by FNA, because the essential criterion for FTC is capsular invasion which can not be determined by cytology. Another problem is the differentiation between follicular adenoma and follicular variant of papillary thyroid carcinoma (FVPTC), because the essential criterion is the aspect of the nuclei. As a consequence, the frequency of FTC in hemi-thyroidectomies performed after suspicious outcome from FNA is only 20-30%.
The Tumor-Node-Metastases classification system is based on pathologic findings. This classification system divides patients into four stages, with progressively poorer survival with increasing stage. Recently, the 6th edition of the TNM system has become available (47). The most important difference with he 5th edition is the fact that the dimension of T1 has been extended to 1.5 cm and that tumors with limited extrathyroidal extension are designated T3 instead of T4, which has implications for the prognosis of DTC (48). Therefore, some experts propagate to continue the use of the 5th edition. In the studies in his thesis the 5th edition of the TNM staging system is used (49).

T0	No evidence of primary tumor
T1	Tumor 1 cm or less in greatest dimension
T2	Tumor > 1 cm, but nor more then 4 cm in greatest dimension, limited to the thyroid
T3	Tumor > 4 cm in greatest dimension limited to the thyroid
T4	Tumor of any size, beyond the thyroid capsule
Nx	Regional lymph nodes (cervical and upper mediastinum) cannot be assessed
N0	No regional lymph node metastases
N1	Regional lymph node metastases
N1a	Metastasis in ipsilateral cervical lymph node(s)
N1b	Metastasis in bilateral, midline or contralateral cervical or mediastinal lymph node(s)
Mx	Distant metastasis cannot be assessed
M0	No distant metastasis
M1	Distant metastasis

Figure 1. TNM classification system 5th edition, AJJC, Adapted from (50)

Initial therapy
Initial therapy for DTC consists of near-total thyroidectomy followed by radioiodine ablation. There is still some controversy about the extent of thyroid surgery. However, there are strong arguments in favor of total or near-total thyroidectomy in all patients (51). Only very low-risk patients (T1 (< 1 cm) N0M0 (5th edition) DTC, unifocal) may be treated by hemi-thyroidectomy.

In tumor stages of T2 and higher a total thyroidectomy is indicated (52-54). Near-total thyroidectomy results in lower recurrence rates than more limited thyroidectomy because many papillary tumors are multifocal and bilateral (55;56). In addition, total thyroidectomy facilitates total ablation with iodine-131 and reveals a higher specificity of thyroglobulin (Tg) as a tumor marker (52-55). Complications of total thyroidectomy are laryngeal nerves palsy in 2 % of DTC patients and hypoparathyroidism. The latter occurs in 1/3 of patients after total thyroidectomy, but persists longer then 3 months in only 2 % (50).
Controversy also exists about the routine use of iodide-131 ablation of thyroid remnants. However, many clinics give postoperative iodide-131 ablation for three reasons. First, iodide-131 destroys any remaining normal thyroid tissue thereby increasing the specificity of detectable serum Tg levels and positive whole-body scintigraphy indicating persistent or recurrent disease (5;54;57). Second, iodide-131 may destroy occult microscopic carcinomas, thereby decreasing the risk of recurrence thyroid carcinoma (8;54;58;59). Third, the use of large amounts of iodide-131 for therapy permits post ablative scanning to detect recurrent disease (60;61). A meta-analysis showed that the use of iodide-131 to prevent recurrence or death is uncertain (62). A beneficial effect is probably only present in patients with high risk or irradical surgery (8;53;63;64). Many authors are more careful advising I-131 ablation since various papers reported a relation between I-131 therapy and non-thyroid carcinoma (65-67).
In patients with a very low risk of recurrence/mortality (T1 (<1 cm) N0M0 unifocal) I-131 is not indicated. I-131 ablation is still the treatment of choice in patients with a high risk of recurrence/mortality 1) T3 or T4, 2) any T N1, and 3) Any T M1, and incomplete tumor resection (68;69). Controversy exists about patients with a low risk (T1 (>1 cm)N0M0, T2N0M0 or T1(<1 cm)N0M0 multifocal) of recurrence/mortality (50).
After initial therapy, all patients with DTC are treated with high doses of thyroxin aiming at significantly suppressing thyrotropin (TSH<01 mU/L) levels. The rationale of this approach is based on the potential harmful effects of TSH on tumor recurrence (7;8). One study demonstrated a preventive effect of TSH suppression on tumor recurrence or progression only in high risk DTC patients (70). However, long-term TSH suppression may be associated with potential harmful effects on various systems including bone metabolism, glucose metabolism, the autonomic nervous system and quality of life. The recent European Consensus on thyroid cancer (71), recommended that not all patients with DTC should be indiscriminately treated with TSH suppressive therapy because this represents in effect a state of subclinical hyperthyroidism, as defined by suppressed serum TSH levels (below 0.4 mU/l), in the presence of normal serum levels of (free) thyroxin. A recent analysis of our institution showed that TSH levels are positively associated to thyroid carcinoma related death and relapse (72). This effect became apparent at TSH levels above 2 mU/L and is in line with other studies (73).

Follow-up
The purpose of follow-up protocols in DTC is the early detection of tumor recurrence or metastatic disease in order to optimize additional treatment. Most patients during follow up have been cured definitely, and, as a consequence, have a low pre-test probability for recurrent disease. Therefore, the sensitivity of the diagnostic test must be adequate to detect the few patients with evident thyroid carcinoma, whereas specificity must also be high to avoid unnecessary treatments in patients without recurrent disease. In addition, the burden of diagnostic tests for the patient should be kept at a minimum.

a. Thyroglobulin
Thyroglobulin (Tg) is produced by normal or neoplastic thyroid follicular cells and Tg production is stimulated by TSH. In patients treated with a total thyroidectomy and I-131 ablation, Tg should be undetectable. The clinical interpretation of Tg is hampered by the

following analytical problems:
1. lack of universal standardization of the Tg assays, which results in considerable inter-assay variability (74),
2. a high intra-assay variability, which results in a poor comparability of results obtained within one patient during follow-up,
3. “hook” effects may be present, which affect IMA methods in particular and can lead to inappropriately low- or normal range Tg values in sera with very high serum Tg concentrations,
4. the presence of Tg auto-antibodies that can lead to lower or higher Tg levels.

Despite these analytical problems, Tg measurements are still the basis in the follow-up in DTC. Several studies have been performed on the diagnostic value of Tg measurements. The interpretation of these studies is difficult, because 1. heterogeneous patient groups with respect to initial therapy are included, 2. the time points of Tg measurements after diagnosis are not clearly indicated, and 3. fixed Tg cut-off levels are used, without receiver operator curve (ROC) analyses. The application of ROC data is essential, as a chosen cut-off level is a subjective choice based on the balance between a desired percentage of missed recurrences versus unnecessary therapies. Therefore, in the recent European consensus paper, it was recommended to define institutional Tg cut-off levels (71). In addition, most studies provide data on the diagnostic value of Tg for tumor presence, but do not give data on the *prognostic* significance for recurrence or death. The few studies that were published on the prognostic significance of Tg measurements used fixed cut-off levels, contained selected subgroups of patients, and included either Tg measurements at one time point or at undefined time points (75-79).
We, therefore, performed a study on the diagnostic and prognostic value of Tg in a homogeneous group of DTC patients with respect to initial therapy, using Tg measurements at 5 defined time-points after diagnosis, in combination with ROC analyses (chapter 2).

b. Thyroxin withdrawal versus rhTSH
Serum Tg measurements, I-131 ablation and diagnostic I-131 whole body scans are based on the responsiveness of DTC to TSH (80). TSH stimulated Tg measurements have superior diagnostic value in DTC compared to Tg measurements on thyroxin replacement therapy (81). High serum levels can be achieved by thyroxin withdrawal or injection with recombinant human TSH (rhTSH), which has less impact on quality of life (82). rhTSH is an adequate method to detect recurrence or metastases (78;83-85). A rhTSH stimulated Tg level greater than 2 mg/ml predicts persistent disease (78;83;86), whereas a rhTSH stimulated Tg level lower than 0.5 mg/dl has a 98 % likelihood of detecting patients free of tumor (78). Whole body scans performed after rthTSH-injections have a similar sensitivity and negative predictive value compared to thyroxin withdrawal (83-85). However, more negative whole body scans were found after rhTSH-injections compared to thyroxin withdrawal (83-85). The sensitivity and negative predictive value of Tg values after rhTSH-injections are 96.3 % and 99.5 % respectively by combining these measurements with a neck ultrasound (87).
Several studies have reported that radioiodine ablation of thyroid remnants after rhTSH-injections is as effective as ablation after thyroxin withdrawal (88;89). Radioiodine ablation after rhTSH-injections in patients with recurrence or distant metastases results in a beneficial effect in 75 % of patients (90;91). However, rhTSH has not been approved for this indication.

c. I-131 scintigraphy, Ultrasound, and FDG-PET
The result of iodine-131 whole body scanning depends on the presence and the ability of thyroid-cancer tissue to accumulate iodine-131 in the presence of high serum TSH concentrations. Diagnostic RaI whole body scintigrapies have a much lower sensitivity than ultrasound and Tg measurements. Therefore, the routine use of RaI scintigraphy in

the diagnostic follow-up of DTC patients is no longer recommended (87;92). Ultrasound combined with FNA had the highest sensitivity (even higher than Tg) for local recurrence and lymph node metastases in recent papers (87;93;94). Thus, ultrasound has an important place in he follow up of DTC. 18-F Fluorodeoxyglucose-positron emission tomography (FDG-PET) may be useful in patients with elevated serum Tg levels, in whom no RaI uptake is observed after diagnostic or post-therapeutic scintigraphy. The sensitivity of FDG-PET is increased with elevating serum Tg levels and after TSH stimulation (95). Robbins *et al* showed that FDG-PET positivity is associated with worse survival (96).

III. Thyroid hormones

The production of thyroid hormones by the thyroid is regulated by the hypothalamus-pituitary-thyroid axis. Thyrotropin releasing hormone (TRH), which is produced by the hypothalamus, stimulates the secretion of thyrotropin (TSH) by the anterior pituitary. TSH promotes the thyroid to synthesize the prohormone tetraiodothronine (T4) in the thyroid. Iodide is actively taken up by the thyroid gland by the sodium-iodide-symporter (NIS) at the basolateral plasma membrane. The expression and activity of NIS are controlled by TSH. Thyrogobulin, which is synthesized by the follicular cells, is then iodinated with one or two iodides to form monoiodotyrosine (MIT) or diiodotyrosine (DIT). This process is catalyzed by the enzyme thyroid peroxidase (TPO). Two DIT molecules are then coupled to form T4 and one DIT and one MIT molecule are coupled to form T3. The thyroid secretes approximately 90 % T4, 10 % triiodothyronine (T3) and less then 1 % reverse T3. The T3 molecule is the active form of thyroid hormone. The majority of the active form of thyroid hormone T3 is derived from conversion of T4 to T3 in peripheral tissues, such as the liver (see deiodinases). T4 and T3, in turn, have a negative effect on the TRH secretion by the hypothalamus and TSH secretion by the pituitary. Iodide is important for the synthesis of thyroid hormones.

Deiodinases

Peripheral thyroid metabolism is mainly regulated by the iodothyronine deiodinases D1, D2 and D3 (97;98). D1 convertes the prohormone T4 in T3, plays a role in the breakdown of rT3 (97;99) and is expressed in liver, kidney, thyroid and pituitary and at lower levels in other tissues as skeletal muscle, spleen and lung. D2 is essential for the production of T3 through outer ring deiodination of T4. It is present in brain, skeletal muscle, thyroid, pituitary, brown adipose tissue (BAT) and aortic smooth muscle cells (97;100-104). D3 inactivates T3 and prevents T4 activation by innerring deiodination (98) and is present in brain, skin, placenta and fetal tissues (97).

The deiodinases adjust the thyroid hormone levels of individual tissues in response to various conditions. The peripheral conversion of T4 to T3 is increased during *hypothyroidism* (97;105;106). Extrathyroidal T3 production changes from PTU sensitive to PTU insensitive during hypothyroidism in rats, representing an increase in the conversion of T4 to T3 by D2 and a decreased conversion by D1 (107). D1 gene transcription is decreased in liver and kidney during hypothyroidism (108), which is related to the presence of two T3 response elements in the human D1 gene (97;108-110). Thyroid status regulates D2 activity both at the pre- and posttranslational level. D2 activity is increased in different tissues predominantly during hypothyroidism by a decrease in substrate (T4)-induced degradation of D2 protein (97;111-113). Hypothyroidism elevates D2 mRNA in rat brain and BAT (97;100;114;115). D2 mRNA expression and activity were found in skeletal muscle samples from healthy subjects (103;116). This is fascinating, because D2 could therefore play a role in peripheral and intracellular T3 production (103). Maia *et al*. reported that D2 is a major source of T3 during euthyroidism and could therefore play an important role during hypothyroidism

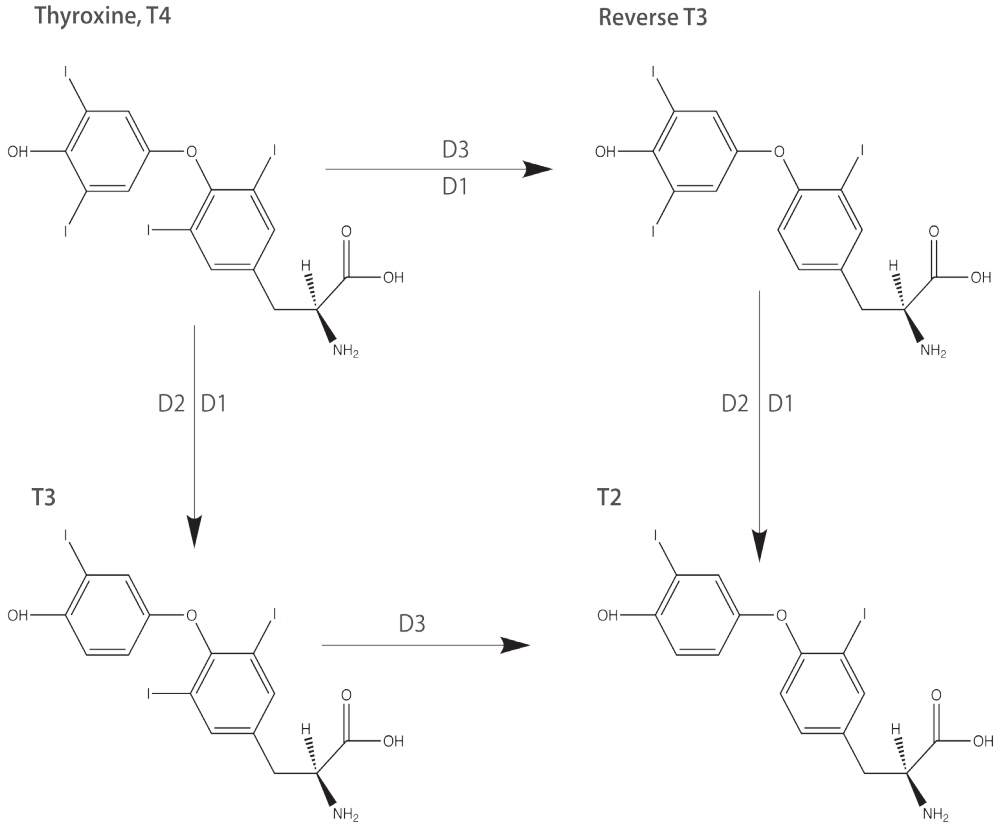

Figure 1. Structure of the iodothyronines and their activation and inactivation by iodothyronine deiodinase.

(117). As patients treated for DTC have no thyroid tissue left, we hypothesized that during hypothyroidism D2 in skeletal muscle could be essential in promoting the conversion of T4 to T3 (chapter 3).
Several polymorphisms in D2 have been described (118-120), with most studies investigating the consequences of the D2-Thr92Ala polymorphism. This D2-Thr92Ala polymorphism has been associated with BMI and insulin resistance in obese subjects and type 2 diabetes mellitus (118;119), although this was not confirmed in another study (121). The maximal velocity of D2 in vitro in thyroid and skeletal muscle of homozygous carriers of the Ala92 allele was decreased by 3–10-fold (118).

IV. Bone metabolism

Thyroid hormone impacts on bone metabolism, ranging from decreased skeletal development in childhood hypothyroidism to an increased risk for osteoporosis in hyperthyroidism (11;122;123). Thyroid hormone indirectly promotes osteoclast formation and activation by inducing the expression of cytokines, prostaglandins and the receptor activator of nuclear factor NF-κB ligand (RANKL) (124-126). RANKL, the key molecule in osteoclast differentiation, binds to its receptor, RANK, which is expressed on dendritic cells, T cells, osteoclast precursors and mature osteoclasts (127;128). RANKL promotes the survival of RANK positive T cells (127), stimulates osteoclast differentiation (129-133), increases

the activity of mature osteoclasts (130;134;135) and stimulates survival of osteoclasts by preventing apoptosis (135). Contact with stromal cells and M-CSF also promotes osteoclast differentiation (136;137). Thyroid hormone inhibits chondrocyte proliferation and promotes hypertrophic differentiation, mineralization, matrix synthesis but also apoptosis of chondrocytes in the growth plate.

Overt hyperthyroidism results in an increased risk for osteoporosis (123), the pathophysiology of which is multifactoral (124), including shortening of the bone remodelling cycle (138) and acceleration of bone turnover (139). The effects of *subclinical hyperthyroidism* on bone metabolism are not clear. Several studies have addressed this issue, but there is no consensus largely because of differences in study design, including patient groups, methodology used, follow-up time and choice of outcome parameters. To study the effects of subclinical hyperthyroidism on bone mineral density, we performed a systematic review including all clinical studies on TSH suppressive thyroxin therapy in thyroid cancer patients (chapter 4).

An interesting development has been the discovery of the *TSH* receptor (TSHR) in bone (140-142). TSHR knockout and haploinsufficient mice with normal thyroid hormone levels have decreased bone mass suggesting that TSH might directly influence bone remodeling (141;143;144). This is intriguing, because effects on bone metabolism that were previously ascribed to high thyroid hormone levels could also be attributed to suppressed TSH levels (143-145). Abe *et al*. suggested that TSH inhibits osteoclast formation and survival by attenuating JNK/c-jun and NFκB signaling in response to RANK-L and inhibits osteoblast differentiation and type 1 collagen expression as well by downregulating Wnt and VEGF signaling (141). The same group found also that TSH directly inhibits Tumour Necrosis Factor-α (TNF-α) production and that TNF-α is the critical cytokine mediating the downstream antiresorptive effects of TSH on the skeleton (146). Other studies suggest that serum TSH activates the type 2 deiodinase in osteoblasts, thereby linking TSH and increased local thyroid hormone availability (142). Furthermore, in animal studies, low doses of TSH increased bone volume and improved microarchitecture in ovariectomized rats (147), without increasing serum thyroid hormone levels.

It was recently reported that the TSHR-Asp727Glu polymorphism was associated with 2.3% higher BMD in elderly carriers (148). Although the functional consequences of this polymorphism are debated (149), the lower plasma TSH levels in patients carrying the polymorphism could point toward a higher sensitivity of the variant compared to the wild-type TSHR (150;151).

We, therefore, evaluated the independent relation between serum TSH levels and indicators of bone turnover in thyroidectomized patients for differentiated thyroid carcinoma receiving thyroid hormone substitution (chapter 5). In addition, we studied the relationship between the TSHR-Asp727Glu polymorphism and bone as these subjects are not expected to show compensatory lower serum TSH levels if they carry the TSHR-Asp727Glu polymorphism (150;151).

The consequences of *hypothyroidism* on bone metabolism are not clear. Various studies report decreased bone resorption (152-155) or bone formation (152), whereas other studies document no impact on bone turnover (156-158). Furthermore, it is not clear if the effects of hypothyroidism must be attributed to the increased TSH levels or decreased thyroid hormone levels. As mentioned above, TSHR knockout and haploinsufficient mice with normal thyroid hormone levels have decreased bone mass, suggesting that TSH might directly influence bone remodeling (141;143;144). However, other studies question the role of TSH in bone metabolism (159;160). Three studies in humans have investigated the effect of TSH on bone metabolism, but their results were not consistent showing either no impact on bone turnover (161), increased bone formation (162;163) or decreased bone resorption (163).

To document the effects of hypothyroidism on bone metabolism and to discriminate between effects mediated by decreased thyroid hormone levels *versus* those mediated by increased TSH levels, we studied bone metabolism in eleven patients with differentiated thyroid carcinoma (DTC) during short-term thyroxin withdrawal and compared with eleven age-, gender- and BMI-matched DTC patients with increased TSH levels and normal thyroid hormone levels due to rhTSH injections (chapter 6).
Although earlier studies on the role and functional expression of iodothyronine deiodinase enzymes in the skeleton have not revealed unequivocal answers (142;164-167), a recent study reported normal growth in mice with deficiencies in D1 and D2 indicating that D2 may not be critical in skeletal development (168). This was supported by another study, which found that D2 activity is restricted to mature osteoblasts, suggesting a possible role for D2 in mature osteoblast function (169). Because it is difficult to study the role of D2 *per se* on skeletal metabolism in humans, we choose to study the effects of functional D2 polymorphisms on BMD and indicators of bone turnover. Canani *et al*. (118) reported that the maximal velocity of D2 *in vitro* in thyroid and skeletal muscle of homozygous carriers of the Ala^{92} allele was decreased by 3–10-fold. We, therefore, studied the relationship between the functional D2-Thr92Ala polymorphism, BMD and indicators of bone turnover (chapter 7).

V. Glucose metabolism

Thyroid hormone has effects on glucose- and lipid metabolism (13;170). There is a relation between serum thyroid hormone levels and basal and insulin-mediated glucose metabolism in euthyroid subjects with preserved thyroid function (171-173). It has been suggested that T3 regulates insulin response after glucose ingestion in humans (174).
Hyperthyroidism has been associated with impaired glucose tolerance and increased insulin resistance (175-181), predominantly at the level of the liver (182). The pathophysiology has not been completely elucidated, but it has been ascribed to a combination of multiple factors, including diminished pancreatic secretion of insulin (183;184), diminished suppression of glucagon by glucose (185) and increased adrenergic activity (186).
Limited data are available on the consequences of *subclinical hyperthyroidism* on glucose- and lipid metabolism. This issue has been studied only by Yavuz *et al*., who reported a decreased insulin sensitivity index by oral glucose tolerance test in patients with exogenous subclinical hyperthyroidism compared to values after restoration of euthyroidism and compared to controls (187). Regarding lipid metabolism, most studies report no differences in lipid profiles during subclinical hyperthyroidism (188-190), with the exception of 2 studies, that observed decreased total and LDL cholesterol levels (191;192). Franklyn *et al.* reported decreased total cholesterol concentrations only in patients older than 55 years and LDL cholesterol levels were decreased only in patients older than 65 years (193). We therefore performed a prospective placebo-controlled randomized trial to investigate the effects of restoration of exogenous subclinical hyperthyroidism to euthyroidism on glucose- and lipid metabolism (chapter 8).

VI. Autonomic nervous system

The consequences of *hyperthyroidism* on the heart are profound, including tachycardia and/or arrhythmias, increased systolic pressure, increased systolic function, left ventricular hypertrophy and diastolic dysfunction (194-196). It is suggested that these effects are the

Result of direct effects of thyroid hormone on the cardiovascular system and the interaction of thyroid hormones with the sympathetic nervous system (195;197). Hyperthyroidism is associated with a sympathicovagal imbalance, characterized by increased sympathetic activity in the presence of reduced vagal tone, which corresponds with increased urinary excretion of catecholamines (15;16;198). Therefore, the current consensus is that manifestations of altered autonomic nervous system function play a role in the pathophysiology and clinical presentation of thyrotoxicosis.
During *subclinical hyperthyroidism*, cardiovascular effects may also occur, but these are less well known and seemingly less severe. Regular findings during subclinical hyperthyroidism include increased heart rate, supraventricular arrhythmias and abnormalities of LV morphology and function (195;199-201). The consequences of subclinical hyperthyroidism on the autonomic nervous system function are less well defined. Several studies, using measures of heart rate variability, found evidence that in patients with endogenous subclinical hyperthyroidism a reduction of cardiac parasympathetic control is present (18), (200), (202). This is supported by findings on heart rate turbulence by Osman *et al* (203). However, in the study of Goichot (18) no differences in the ratio of low frequency power over high frequency power (LF/HF) were reported in these patients. The LF/HF ratio is commonly used to characterize the balance between vagal and sympathetic influences. To further clarify this issue, we performed a prospective, randomized, placebo-controlled study using heart rate variability to assess the autonomic nervous system in patients with DTC with longer than 10 years exogenous subclinical hyperthyroidism and investigated whether restoration to euthyroidism affects autonomic nervous function (chapter 9).
Hypothyroidism is associated with bradycardia, mild diastolic hypertension, increased peripheral cardiovascular resistance (194;204;205), decreased cardiac output and diastolic dysfunction (194;204;206;207). Hypothyroidism also induces sympathovagal imbalance (17;208;209). Nevertheless, current literature shows inconsistent results with either an increased sympathetic activity (17), a decreased sympathethic modulation (208) or an increased vagal tone (209). We therefore investigated the effects of short-term overt hypothyroidism, 4 weeks after thyroxin withdrawal, and restoration to subclinical hyperthyroidism on the autonomic nerves system (chapter 10).

VII. Quality of life

DTC is associated with an excellent prognosis. This may imply that quality of life in cured DTC patients may be quite normal. However, patients are treated long-term with TSH suppressive thyroxin replacement therapy, reflecting in effect a state of *subclinical hyperthyroidism*, which may impact quality of life (210-212).
Quality of life in cured DTC patients is investigated in a few studies (20;21;213-215). However, these studies are limited by small patient numbers(21;213), limited number of quality of life questionnaires (20;215) or the absence of a healthy control group (20;213;214).
Studies reporting the relation between the level of TSH suppression and quality of life in DTC patients are inconclusive because of small patient numbers, selection of patients with symptoms of hyperthyroidism or selection of patients with a long duration of cure (210;216). For that reason, we investigated quality of life in a large cohort of cured DTC patients compared to controls matched for age, gender and socioeconomic status. In addition, the determinants of quality of life, including serum TSH levels were investigated (chapter 11).
Thyroxin withdrawal resulting in overt *hypothyroidism* may also impact quality of life. It results in fatigue, anorexia, constipation, problems with motor skills and fluid retention. Quality of life during thyroxin withdrawal is also affected by a decreased motivation, productivity and quality of work and by interfering with family and social life (22). In addition, a decreased psychomotor function and an increased fear are reported during thyroxin withdrawal (19;217).

VIII. D2-Thr-92-Ala and thyroxin dose

Several polymorphisms in D2 have been described (118;119;218;219). The functional implications of the *D2-Thr92Ala* polymorphism are inconclusive. One in vitro study found an association with a decreased D2 activity (118) whereas another study found no difference (219). So far no associations between the D2-Thr92Ala polymorphism and serum thyroid hormone levels were documented (151;218;220). A study of Torlontano *et al*. documented that homozygous carriers of the D2-Ala92 allele needed higher dosages of thyroxin in thyroidectomized differentiated thyroid carcinoma (DTC) patients, particular in the group with near-suppressed TSH levels (TSH values between 0.1 and 0.5 mU/L)(221). However, this study had limitations, because actual values of serum TSH levels for wild-type and homozygous groups within the near-suppressed TSH group were not given. It is, therefore, unclear whether TSH levels in both groups were indeed identical, which would be a key finding to ascribe the slight differences in thyroxin dose indeed to the polymorphism. The fact that serum T4 and T3 levels did not differ between the wild-type group and D2-Thr92Ala homozygotes is also remarkable. We, therefore, studied the association between the D2-Thr92Ala polymorphism, thyroid hormone levels and thyroxin dosage (chapter 12).

IX. Outline of this thesis

In chapter 2, we describe the diagnostic and prognostic value of thyroglobulin (Tg) in a homogeneous group of differentiated thyroid carcinoma (DTC) patients with respect to initial therapy, using Tg measurements at 5 defined time-points after diagnosis, in combination with ROC analyses.
In the continuation this thesis, questions about the clinical consequences of exogenous subclinical hyperthyroidism and hypothyroidism on bone metabolism, glucose metabolism, the autonomic nervous system and quality of life in patients with DTC are addressed.
Chapter 3 evaluates the D2 activity and expression of deiodinases 1, 2 and 3 in skeletal muscle samples in DTC patients both during hypothyroidism and thyroxin replacement therapy.
Chapter 4 shows the results of a systematic review describing the effects of TSH suppressive thyroxin therapy on bone mineral density in DTC patients.
In chapter 5, we evaluate the independent relation between serum TSH levels and indicators of bone turnover in DTC patients receiving thyroid hormone substitution.
In chapter 6 we describe a prospective study to investigate the effects of hypothyroidism on bone metabolism and to discriminate between potential effects mediated by decreased thyroid hormone levels versus those mediated by increased TSH levels.
Chapter 7 presents the relationship between the functional D2-Thr92Ala polymorphism, BMD and indicators of bone turnover.
In chapter 8, we investigate the effects of restoration of exogenous subclinical hyperthyroidism to euthyroidism on glucose- and lipid metabolism in a prospective, randomised, placebo-controlled trial.
Chapter 9 describes a prospective, randomized, placebo-controlled study to assess autonomic nervous function in patients with DTC with longer than 10 years exogenous subclinical hyperthyroidism and to investigate whether restoration to euthyroidism affects autonomic nervous function.
In chapter 10 we show the effects of short-term overt hypothyroidism, 4 weeks after thyroxin withdrawal, and restoration to subclinical hyperthyroidism on the autonomic nervous system.
Chapter 11 describes quality of life in a large cohort of cured DTC patients compared to

controls matched for age, gender and socioeconomic status. In addition, the determinants of quality of life, including serum TSH levels were investigated.
In chapter 12, we studied the association between the D2-Thr92Ala polymorphism and thyroid hormone levels and thyroxin dosage

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02

Serum Thyroglobulin Concentrations Predict Disease-free remission and Death in Differentiated Thyroid Carcinoma

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Abstract

Objective: Most studies on the diagnostic value of serum thyroglobulin (Tg) concentrations in differentiated thyroid carcinoma (DTC) use fixed cut-off levels in heterogeneous groups of patients with respect to initial therapy and do not provide prognostic data. The objective was to investigate the prognostic values of serum Tg for disease-free remission and death, measured at fixed time points after initial therapy using receiver operator characteristic (ROC) curve analyses.
Design: Single-centre observational study with 366 consecutive patients with DTC, who had all been treated according to the same protocol for initial therapy and follow-up.
Methods: Tg concentrations were measured at five fixed time points after initial surgery. Tg cut-off values with highest accuracy were calculated with ROC analyses.
Results: During follow-up of 8.3±4.6 years, 84% of the patients were cured. Pre-ablative Tg levels were an independent prognostic indicator for cure (Tg cut off value 27.5 µg/L, positive predictive value 98%). Highest diagnostic accuracies of serum Tg for tumor presence were found during TSH stimulated Tg measurements, 6 months after initial therapy (Tg cut-off value 10 µg/L: sensitivity 100%, specificity 93%).
DTC related mortality was 14%. TSH stimulated Tg levels before ablation and 6 months after initial therapy were independent prognostic indicators for death.
Conclusion: Optimal institutional Tg cut-off levels for diagnosis and prognosis should be defined using ROC analyses for each condition and time-point. Tg measurements 6 months after initial therapy during TSH stimulation had an excellent diagnostic value. Tg levels are independent prognostic indicators for disease-free remission and death. Using this strategy, high-risk patient groups can be selected based on Tg levels, in addition to conventionally used prognostic indicators.

Introduction

Differentiated thyroid carcinoma (DTC) has an excellent prognosis with 10-year survival rates of 85-93 % (1). The purpose of follow-up protocols in DTC is the early detection of tumor recurrence or metastatic disease in order to optimize additional treatment. Most patients during follow up have been cured definitely, and consequently have a low pre-test probability for recurrent disease. Therefore, the sensitivity of the diagnostic test must be sufficient to detect the few patients with evident thyroid carcinoma, whereas specificity must also be high to avoid unnecessary treatments in patients without recurrent disease. In addition, the burden of diagnostic tests for the patient should be kept at a minimum.

Serum thyroglobulin (Tg) measurements are the cornerstone in the follow-up in DTC. Numerous studies have been performed on the diagnostic value of Tg measurements. We recently published a structured meta-analysis on the diagnostic value of Tg including 46 articles (2). The interpretation of many studies on Tg performed so far is difficult, because in most studies 1) heterogeneous patient groups with respect to initial therapy are included, 2) the time points of Tg measurements after diagnosis are not clearly indicated, and 3) fixed Tg cut-off levels are used, without receiver operator characteristic (ROC) curve analyses. The application of ROC data is essential, as a chosen cut-off level is a subjective choice based on the balance between a desired percentage of missed recurrences vs. unnecessary therapies. A recent European consensus paper recommended that there should be defined institutional Tg cut-off levels (3). Only a few studies have been published on the interpretation of Tg levels during follow up of DTC using ROC analyses. However, in those studies, heterogeneous patient groups were included and the time-points of Tg measurements were not clearly indicated (4-6). In addition, most studies provide data on the diagnostic value of Tg for tumor presence, but do not give data on the prognostic significance for disease-free remission or death. One large study (7) investigated the prognostic significance of 1-month post-surgical Tg levels and found a significant prognostic cut-off level of
10 µg/L. The few studies that were published on the prognostic significance of Tg measurements used fixed cut-off levels, contained selected subgroups of patients and included Tg measurements either at one time point or at undefined time points (8-12).

We therefore performed a study on the diagnostic and prognostic value of Tg in a homogeneous group of DTC patients with respect to initial therapy, using Tg measurements at 5 defined time-points after diagnosis, in combination with ROC analyses. In addition, we studied the diagnostic and prognostic value of Tg antibodies for tumor presence or death.

Patients and methods

Three-hundred-and-sixty-six consecutive patients were included in the study. These patients had received initial therapy for DTC between January 1986 and January 2000. All follow up data were collected until January 1, 2003. January 1986 was chosen as a starting date, because from that date forward, all relevant patient data were registered in a computerized database. Initial surgery and radioiodine ablation therapy were performed at the Leiden University Medical Centre or at one of the connected general hospitals. All hospitals are affiliated in the Regional Comprehensive Cancer Centre and use the same standardized protocol for the treatment and follow-up of DTC.

All patients were treated by near-total thyroidectomy, followed by routine radioiodine ablative therapy with 2800 MBq I-131.

Follow-up was performed according to a standard protocol. Serum Tg levels were measured at the following time-points: 1) after initial surgery during thyroxin withdrawal just before radioiodine ablation, 2) during T4 therapy, 3) 6 months after initial surgical therapy after T4 withdrawal (‘off’) and 4) annually during T4 therapy.

Although additional TSH stimulated Tg measurements were performed in selected subgroups of patients at other time points after initial therapy, we did not include those data as these tests were not uniformly done in all patients, and calculations of diagnostic values would have been biased. T4 therapy was aimed at suppressing TSH levels (below 0.1 mU/L). Six months after initial therapy a diagnostic 185 MBq I-131 scintigraphy was performed after T4 withdrawal.

Tumor presence during follow-up was defined as histologically or radiologically (X-ray, computed tomography (CT) scan, magnetic resonance imaging (MRI) scan, 18F-fluorodeoxyglucose positron emission tomography (FDG-PET) scan or I-131 scintigraphy) within a 1-year interval before or after the time of Tg measurements. Although we realize that Tg is considered the best parameter for tumor presence, Tg was not used as the 'gold standard' for tumor presence, as the diagnostic value of Tg was the subject of this study.

Disease-free remission was defined as the absence of thyroid carcinoma for a minimum of 3 years according to the above mentioned parameters.

The following data were registered: age at diagnosis, sex, date of diagnosis, histology, TNM stage, date of cure, date of recurrence, tumor localization, cause of death, Tg levels, TSH levels, Tg antibody levels and date of last follow up or death. TNM stage was registered according to the 5th edition of the *TNM Classification of Malignant Tumors* (13) because most patients were analysed before the latest edition of the TNM classification had been published. We used the following end-points of follow-up: date of death (82 patients), date of emigration (12 patients) and date of most recent contact (272 patients).

Causes of death were analysed in all 82 patients who had died during follow-up. Cause of death was investigated using medical records, death certificates, enquiries with physicians involved in the treatment of each patient, enquiries in other hospitals, enquiries of general practitioners and autopsy findings. Causes were divided into thyroid cancer related death and other causes. Analyses were performed in evaluable patients, defined as patients in whom all of four conditions were fulfilled: alive at time-point of Tg measurement, documented serum Tg measurements, documented serum Tg antibody measurements and documented gold standard parameters for the presence or absence of disease. If Tg antibodies were present, the Tg measurement at this time-point was excluded from the calculations because of possible interference with the Tg assay. The numbers of these patients are given in table 2.

Measurements of Tg and Tg-Ab

Until January 1997 serum Tg was measured using an immunoradiometric assay (IRMA), the Dynotest TG (Brahms Diagnostica GmbH, Germany) was used with a sensitivity of 0.03 µg/l. From January 1997, the Dynotest TG-s (Brahms Diagnostica GmbH) was used, with a sensitivity of 0.05 µg/l. Inter-assay variability was 0.3 µg/l. The comparability of the two methods is excellent: R2=0.99, slope 0.99, intercept 0.09 (14). Serum Tg-antibodies were also measured at these specific time points by the Ab-HTGK-3 IRMA (DiaSorin Biomedics, Italy).

Statistical analyses

Data are presented as mean ± SD. All statistical analyses were performed using SPSS version 12.0 (SPSS Inc., Chicago, IL, USA). Data are expressed as number of patients (percentages), as mean ± Standard Deviation (SD) or as median (range). ROC analyses were used to find the cut-off value with highest accuracy. Prognostic indicators for recurrence or death were calculated using univariate- and multivariate Cox-regression analyses; indicators that were identified as significant for survival in univariate analysis were entered into a stepwise multivariate model. A p-value of < 0.05 was considered significant.

Table 2. Diagnostic Values of Serum Tg Measurements for Active Tumor Calculated with ROC Analysis. Patients with Tg antibodies were excluded.

	Evaluable patients (N)*	Patients with positive TgAb (N, % of evaluable patients)	Tumor Location Patients with tumor (N, % of patients without Tg antibodies)		Tg Cut-Off (µg/L)	Sensitivity ±SE (%)	Specificity ±SE (%)	PPV (%)	NPV (%)
Pre-ablation	304	82 (27.0)	All	33 (15.1)	27.5	87.9 ± 5.7	90.3 ± 2.2	61.7	97.7
			Distant Metastases	21 (9.6)	27.5	85.7 ± 7.6	85.3 ± 2.5	38.3	98.2
Six months after initial therapy, suppressed TSH	287	79 (27.5)	All	37 (18.0)	2.5	89.2 ± 5.1	93.5 ± 2.0	75.0	97.5
			Distant Metastases	24 (11.7)	2.5	87.5 ± 6.8	87.3 ± 2.5	47.7	98.1
Six months after initial therapy, stimulated TSH	287	79 (27.5)	All	37 (18.0)	10.0	100.0 ± 0.0	93.1 ± 2.1	76.7	100.0
			Distant Metastases	24 (11.7)	10.0	100.0 ± 0.0	86.0 ± 2.8	48.8	100.0
Two years after initial therapy, suppressed TSH	244	32 (13.1)	All	43 (20.6)	2.0	85.0 ± 5.4	85.7 ± 2.7	60.6	95.7
			Distant Metastases	33 (15.8)	2.0	72.7 ± 7.8	88.6 ± 2.4	54.5	
Five years after initial therapy, suppressed TSH	182	23 (12.6)	All	35 (22.6)	2.5	82.9 ± 6.4	96.7 ± 1.6	87.9	95.1
			Distant Metastases	30 (19.4)	2.5	83.3 ± 6.8	93.6 ± 2.2	75.8	95.9

ROC=receiver operator characteristic, TgAb=thyroglobulin antibodies, PPV= positive predicted value, NVP = negative predicted value

* = Patients who were alive at the time-points of measurement and in whom Tg, TgAb and documentation of disease state according to the criteria for the 'gold standard' (see methods) could be evaluated.

Results

Characteristics of the patients are shown in Table 1. Mean age at time of surgery was 48 ± 18 years. Mean follow-up was 8.3 ± 4.6 years. Significant prognostic factors for cure and death are given in Table 4.

Table 1. Patient characteristics

Parameter	N	Cured Patients N (%)	Patients with Relapse after Cure (N, (%))	Thyroid Carcinoma Deaths (N (%))
Total	366	305 (84)	46 (13)	52 (14)
Gender (Male/ Female)	91/ 275	72 (80)/ 233 (85)	13 (14)/ 33 (13)	13 (14)/ 39 (14)
Stages				
T1	22	21 (96)	1 (5)	0 (0)
T2	188	176 (94)	17 (9)	10 (5)
T3	56	51 (91)	9 (16)	8 (14)
T4	96	53 (55) * #	17 (18)	32 (33) * #
T unknown	4	0 (0)	0 (0)	2 (50)
N1	107	76 (71) *	15 (14)	22 (21) *
M1	52	19 (36) * #	6 (11)	27 (54) * #
Histology				
Papillary	203	173 (86)	28 (14)	25 (12)
Follicular	72	58 (81) *	11 (15)	17 (24) *
Follicular variant papillary carcinoma	68	56 (82)	5 (7)	6 (9)
Hürthle Cell	23	18 (78)	2 (9)	4 (17)
Age (continuous)		* #		* #
< 55 yr	210	221 (95)	18 (8)	3 (1)
> 55 yr	156	84 (64)	28 (21)	49 (31)

** Significant at univariate analysis, # Significant at multivariate analysis (see Table 4)*

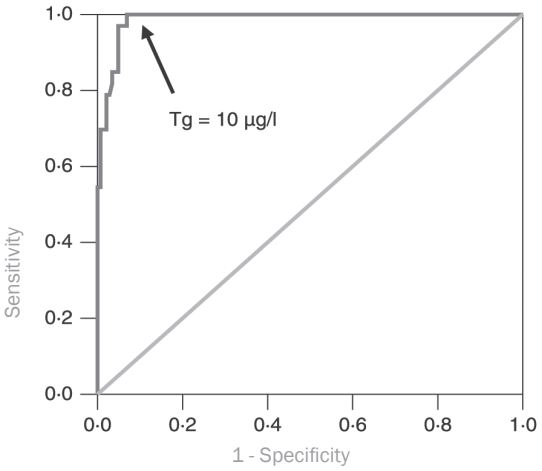
Diagnostic value of Tg.

The diagnostic values of Tg measurements at the different time points are given in Tables 2. The diagnostic value of Tg before ablation therapy was reasonable in our analysis, with a sensitivity of 87.9% and a specificity of 90.3% at a cut-off value of 27.5 µg/L. When a cut-off level of 2 µg/L was used, sensitivity increased to 93.9%, whereas the specificity fell to 45% with a positive predictive value of only 23% instead of 62%, and with similar negative predictive value.

The highest diagnostic value of Tg was found during TSH stimulated Tg measurements 6 months after initial therapy (see Fig. 1). The Tg cut-off value with highest accuracy was 10.0 µg/L, with sensitivity and specificity of 100.0 and 93.1%, respectively. When the more commonly used cut-off value of 2 µg/L was used, the sensitivity remained similar, but the specificity fell to 82% with a positive predictive value of only 54%, instead of 77% (Fig. 1). We analysed the course of 9 patients with Tg values > 10 µg/L, 6 months after initial therapy during TSH stimulation: in three patients, tumors were detected 2-5 years after initial therapy. In four patients Tg became undetectable and they were considered cured. Two patients had

persistent measurable Tg, but no tumor was detectable up to 15 years after initial therapy. Tg measurements on T4, 2 and 5 years after initial therapy had lower sensitivities, but comparable specificities and negative predictive values, albeit at lower Tg cut-off values.

Figure 1 Receiver Operator Curves six months after initial therapy, during TSH stimulation to obtain optimal cut-off levels of serum Tg measurements for the diagnosis of active tumor in patients with differentiated thyroid carcinoma.



Prognostic value of Tg

Disease-free remission. The prognostic value of Tg for disease-free remission is given in Tables 3 and 4. Tg before ablation had a high predictive value of 97.8% for disease-free remission at a cut-off value of 27.5 µg/L. Tg appeared to be an independent prognostic marker for disease-free remission (likelihood ratio for disease-free remission 43.2 for Tg < 27.5 µg/L, $p<0.001$), irrespective of TNM stages T4, M1 and age.

Table 3. Prognostic Value of Serum Tg Measurements for disease-free remission and thyroid carcinoma-related death. Patients with Tg antibodies were excluded.

	Outcome	Tg Cut-Off (µg/l)	Sensitivity ± SE (%)	Specificity ± SE (%)	PPV (%)	NPV (%)
Pre-ablation	Disease-free remission	27.5	84.4 ± 2.6	88.9 ± 5.6	97.8	49.1
	Death	21.5	66.7 ± 9.6	81.3 ± 2.8	30.2	95.3
Six months after initial therapy, suppressed TSH	Death	2.5	72.0 ± 9.0	85.7 ± 2.6	40.9	95.7
Six months after initial therapy, stimulated TSH	Death	10.0	85.0 ± 8.0	83.5 ± 2.9	39.5	97.8
Two years after initial therapy, suppressed TSH	Death	2.0	85.0 ± 8.0	85.7 ± 2.5	38.6	98.2
Five years after initial therapy, suppressed TSH	Death	2.0	82.4 ± 9.2	92.8 ± 2.2	58.3	97.7

PPV= positive predictive value, NVP = negative predictive value, ROC=Receiver Operator Characteristics Curve.

Table 4. Likelihood Ratios for Serum Tg values for Outcome (disease-free remission or thyroid carcinoma-related death) as calculated with Cox-survival Analysis. Patients with Tg antibodies were excluded. Tg Cut Off values are given in Table 3.

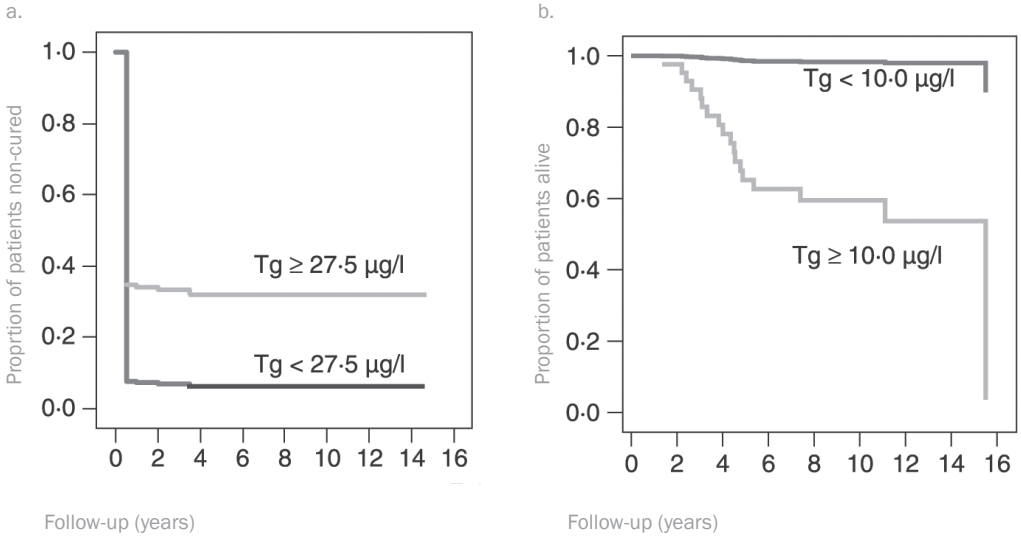
	Outcome	Univariate Analysis p	Likelihood Ratio (CI)	Multivariate Analysis p	Likelihood Ratio (CI)	Other Significant Parameters
Pre-ablation	Disease-free remission	<0.001	43.2 (15.0–124.3) #	<0.001	29.9(5.2–171.5) #	Age, M1, T4
	Death	<0.001	8.0 (3.4–18.7)*	Ns	–	Age, M1, T4
Six months after initial therapy, suppressed TSH	Death	<0.001	14.4 (5.7 – 36.7) *	ns.	–	Age, T4
Six months after initial therapy, stimulated TSH	Death	<0.001	31.2 (7.1–136.7) *	0.008	10.9 (1.9–63.5) *	Age, T4
Two years after initial therapy, suppressed TSH	Death	<0.001	30.9 (9.0–105.7) *	<0.001	12.9 (3.4–49.2) *	Age, M1
Five years after initial therapy, suppressed TSH	Death	<0.001	24.2 (5.0–116.2) *	0.001	29.1 (3.6–232.2) *	Age

CI=confidence interval. M1= metastases, T4=extra thyroidal extension

*# Likelihood Ratio for Tg value < Cut-Off, * Likelihood Ratio for Tg value > Cut-Off*

Thyroid-Specific Death. The prognostic values for Tg measurements for DTC-related death are given in Tables 3 and 4 and Fig. 2. The negative predictive value was high for all time-points of Tg measurements. Tg was an independent predictor for thyroid-related death during TSH stimulation, 6 months after initial therapy (hazard ratio 10.9 for Tg > 10.0 µg/L, p=0.008, Table 4, Fig. 2), 2 years after initial therapy (hazard ratio 12.9 for Tg > 2.0 µg/L, p<0.001) and 5 years after initial therapy (hazard ratio 29.1 for Tg > 2.0 µg/L, p=0.001).

Figure 2 Prognostic value of Tg measurements for Differentiated Thyroid Carcinoma a) Proportion of patients non-cured, Tg levels pre-ablation, four weeks after surgery; b) Survival according to TSH stimulated Tg, 6 months after initial therapy;



Tg antibodies. The percentage of patients with Tg antibodies decreases from 27 % immediately after initial surgery to 12 % 5 years after initial therapy (see Table 2). There were no significant differences in tumor presence between patients with and without Tg antibodies: 15 – 23% in patients without Tg antibodies and 16 – 33% in patients with Tg antibodies. The presence of Tg antibodies did not have a significant prognostic for disease-free remission or death.

Discussion

In the present study we investigated the diagnostic and prognostic value of serum Tg measurements for tumor presence, disease-free remission and death in the follow-up of DTC by ROC analysis in a homogeneous group of patients with respect to initial therapy. The study differed from earlier investigations with respect to the homogeneity of the patient group with respect to initial therapy, the fact that multiple Tg measurements were analysed at fixed time points during follow-up and the use of ROC.

We found 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 (2;8;11;15;16). When we used the more commonly used cut-off value of 2 µg/L, the specificity and positive predictive value decreased considerably. We also found that Tg cut-off levels are dependent on the time-point of follow-up, which is an important finding, as in most papers on Tg, the time after diagnosis is not considered.

Tg levels are not only diagnostic indicators of tumor presence, but also predict disease-free remission or death. We found that serum Tg levels before radioiodine ablation are an independent predictor for disease-free remission, irrespective of the classical prognostic indicators. In our series a patient with Tg level pre-ablation of < 27.5 µg/L has an almost 98% chance of being cured irrespective of the prognostic indicators 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. Negative predictive values for DTC-related death were high (95.3 – 98.2%) at all 5 time points of follow up, albeit with different Tg cut-off values.

In the discussion about the diagnostic value of Tg, specificity is a controversial issue. It has been argued that the specificity of Tg is, by definition, 100%. Although from a biological point of view it is undoubtedly correct that Tg is only synthesized by thyroid cells, in clinical practice the meaning of measurable Tg levels is not always clear, and even less so with the advent of high sensitive Tg assays. A less than 100% specificity of Tg for thyroid carcinoma can be explained by the limitations of current imaging techniques to detect thyroid carcinoma. In this respect, administering a high dose of radioiodine to patients with elevated Tg levels has been advocated, a policy that we agree with (17-20). However, we also observed that in only three of the nine patients with TSH-stimulated Tg levels > 10 µg/L without detectable tumor, did a tumor become apparent during follow up, which is in line with the observations of Baudin *et al*. (8). Therefore, in our opinion, a potential solution to circumvent the debate about specificity of Tg is to consider Tg a risk indicator. The independent prognostic value of serum Tg values for disease-free remission and death are arguments to include Tg in the conventional panel of risk factors. A potential consequence could be to administer higher dosages of radioiodine for ablation in patients with Tg levels above the above-mentioned thresholds. As such we do not advocate that patients with Tg levels below institutionally defined cut-off levels should not be followed up carefully, but we belief that the elimination of Tg should not be a treatment goal in itself.

Tg cut-off levels are influenced not only by clinical considerations, but also by analytical aspects. Analytical problems include the lack of universal standardisation of the Tg assays, (21), intra-assay variability, "Hook" effects and the presence of Tg auto-antibodies (22;23). Another important point, not addressed in this study, is the observation that Tg increases may be more informative than absolute Tg levels (8;24).

The percentage of patients with Tg antibodies (initially 27%) is in line with previous studies (23;25;26). The percentages of active tumor in patients with and without Tg antibodies were comparable, confirming the lack of diagnostic value of Tg antibodies.

Because our study involved a large cohort of patients studied before the introduction of rhTSH, we did not include rhTSH stimulated Tg measurements in our series. However, recent reports indicate that the diagnostic accuracy is comparable (2;3;15). It has been suggested that Tg cut-off levels for rhTSH should be lower than for thyroid hormone withdrawal (27). However, no systematic analyses have been published comparing optimal Tg cut-off levels for both strategies. Furthermore, in a large study, similar Tg cut-off values were used for rhTSH and T4 withdrawal (16).

Because our analysis is based on retrospective data, we believe that the prognostic Tg cut-off values as found in our study should be interpreted with some caution, and they should be confirmed in a prospective study. We maintain, however, that the main message is valid-that Tg cut-off levels should not be adopted from the literature, that Tg cut-off levels are dependent on the time-points, and that Tg has a prognostic value.

In conclusion, our studies illustrate the importance of the definition of institutional Tg cut-off levels. We analysed the diagnostic value of Tg at specific time-points and detected an excellent prognostic value 6 months after initial therapy during TSH stimulation. Our analyses allow the definition of groups of patients with an increased risk for residual disease or mortality, in addition to conventionally used prognostic indicators. Based on our analysis we recommend subjecting every patient, who has undergone thyroid surgery and thyroid remnant ablation, at least once to TSH-stimulated Tg measurements.

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03

Type 2 Iodothyronine Deiodinase in Human Skeletal Muscle: Effects of Hypothyroidism and Fasting

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Abstract

Context: The iodothyronine deiodinases D1, D2 and D3 enable tissue-specific adaptation of thyroid hormone levels in response to various conditions, such as hypothyroidism or fasting. The possible expression of D2 mRNA in skeletal muscle is intriguing as this enzyme could play a role in systemic as well as local T3 production.
Objective: We determined D2 activity and D2 mRNA expression in human skeletal muscle biopsies under control conditions and during hypothyroidism, fasting and hyperinsulinemia.
Design: Prospective study.
Setting: University hospital.
Patients: We studied 11 thyroidectomized patients with differentiated thyroid carcinoma (DTC) on and after 4 weeks off thyroxine replacement, and 6 healthy lean subjects in the fasting state and during hyperinsulinemia after both 14 and 62 h of fasting.
Mean outcome measures: D2 activity and D2 mRNA levels were measured in skeletal muscle samples.
Results: No differences were observed in muscle D2 mRNA levels in DTC patients on and off thyroxine replacement therapy. In healthy subjects, muscle D2 mRNA levels were lower after 62 h compared to 14 h of fasting. Insulin increased mRNA expression after 62 h, but not after 14 h of fasting. Skeletal muscle D2 activities were very low and not influenced by hypothyroidism and fasting.
Conclusion: Human skeletal muscle D2 mRNA expression is modulated by fasting and insulin, but not by hypothyroidism. The lack of a clear effect of D2 mRNA modulation on the observed low D2 activities questions the physiological relevance of D2 activity in human skeletal muscle.

Introduction

Peripheral thyroid hormone metabolism is mainly regulated by the iodothyronine deiodinases D1, D2 and D3 (1;2). D1 is expressed in liver, kidney, thyroid and at a lower level in the pituitary. This enzyme converts the prohormone T4 to active T3 and is very active in the breakdown of rT3 (1;3). Although D1 contributes significantly to peripheral T4 to T3 conversion, it is probably not the major source of extrathyroidal T3 production in humans (1;2;4;5). D2 also catalyzes the production of T3 through outer ring deiodination of T4, and is present in brain, pituitary, thyroid, brown adipose tissue (BAT) and, perhaps, skeletal muscle (1;6-10). In brain, pituitary and BAT, D2 is very important for local T3 production. D3 inactivates T3 and T4 by inner ring deiodination (2) and is present in brain, skin, placenta and fetal tissues (1). These deiodinases allow the adaptation of thyroid hormone levels of individual tissues in response to various conditions.

During hypothyroidism, the conversion of T4 to T3 by D2 is increased, whereas the activities of D1 and D3 are decreased (1;11;12). D2 mRNA was found to be expressed in skeletal muscle samples from healthy subjects (9;13). Since skeletal mass is a major body compartment, muscle could therefore play a role in systemic and local T3 production (9). Maia *et al*. proposed that D2 is a major source of circulating T3 in euthyroid subjects and even more so during hypothyroidism (14). In line with this assumption, we hypothesized that during hypothyroidism caused by withdrawal from thyroxine substitution therapy in thyroidectomised patients treated for differentiated thyroid cancer (DTC), D2 activities might be up-regulated in skeletal muscle.

Short-term fasting induces a decrease in plasma T3 that is most probably due to a decreased activity of D1 and/or D2 and/or an increased activity of D3 (1;1;15;16). Indeed, the fasting-induced decrease in serum T3 levels has been attributed to lower peripheral conversion of T4 to T3 (17;18). Since the fall in T3 levels (50%) may be larger than can be accounted for by a drop in D1 activity, and because D2 has an extremely short half-life, D2 activity may have an important role in the reduction in serum T3 as well (1). In contrast to fasting, insulin has been shown to increase both D2 activity and mRNA expression in BAT in animal studies (19-21). Moreover, a recent study demonstrated that incubation of human myoblasts and myotubes with peroxisome proliferator-activated receptor (PPAR)-γ-agonists resulted in increased D2 activity and also suggested a possible role for D2 in insulin signalling (22). We therefore hypothesized that conditions of fasting as well as hyperinsulinemia would affect skeletal muscle D2 expression and activity in vivo since these conditions affect insulin signaling (23).

To our knowledge, no studies in human skeletal muscle samples have been performed to investigate the effect of hypothyroidism or fasting and insulin on skeletal muscle deiodinase mRNA expression or D2 activity. To address this issue, we analyzed D2 activity and mRNA expression of D2 and D3 in skeletal muscle samples in thyroidectomised patients with differentiated thyroid carcinoma (DTC) on and after 4 weeks off thyroxine replacement therapy, and in healthy subjects in the fasting state and during hyperinsulinemia after both 14 and 62 h of fasting.

Material and methods

Subjects with Differentiated Thyroid Carcinoma

Patients were recruited from the outpatient clinic of the Department of Endocrinology of Leiden University Medical Center, which is a tertiary referral center for differentiated thyroid carcinoma. Patients were included who had been diagnosed with DTC and had received initial therapy consisting of near-total thyroidectomy and radioiodine ablation therapy.

Additional therapies were allowed, as long as they resulted in cure. Cure was documented by the absence of measurable serum thyroglobulin (Tg) during TSH stimulation as well as by a negative total-body scintigraphy with 4 mCi 131I. The patients had to be on TSH suppressive therapy, defined as TSH levels below the lower reference value for TSH (0.4 mU/l). The adequacy of the TSH suppressive therapy was documented by yearly TSH measurements.
14 and 62 h of fasting. Patients who had diabetes mellitus or other endocrine diseases or had a BMI >30 kg/m^2 were excluded. Patients who used any drugs known to influence thyroid hormone metabolism were also excluded. The ethics committee of Leiden University Medical Center approved the study, and written informed consent was obtained from all subjects.

Study design in DTC patients

Patients with DTC undergoing TSH-stimulated ^{131}I scintigraphy were asked to participate in the study. Four weeks after thyroxine withdrawal and 8 weeks after subsequent thyroxine replacement, patients were admitted to the clinical research unit at 8 a.m.. All subjects fasted from the preceding evening (6 p.m.) until the end of the study day. Length (m), weight (kg) and BMI (weight/length^2 [kg/m^2]) were measured. Patients were studied in a semi-recumbent position. A catheter was inserted in a dorsal hand vein to collect plasma samples for measurement of TSH, FT4, T3 and rT3. Muscles biopsies were taken from the quadriceps muscle (vastus lateralis) under local anesthesia (Lidocaine 20 mg/ml; Fresenius, Kabi, Den Bosch, The Netherlands) as described earlier (23). One skeletal muscle biopsy obtained during hypothyroidism was lost. Biopsies were quickly washed in HEPES-buffered saline to remove blood, inspected for fat or fascia content, dried on gauze swabs, and subsequently stored in liquid nitrogen until analysis. Serum samples were handled immediately and stored at –20 C.

Fasting subjects

Six lean healthy men with a normal thyroid status who participated in a study on fasting-induced peripheral insulin resistance were included in this study (23). Written informed consent was obtained from all subjects after explanation of purpose, nature, and potential risks of the study. The study was approved by the Medical Ethical Committee of the Academic Medical Center of the University of Amsterdam.

Study design in fasting subjects

The experimental protocol has been briefly described earlier (23). In short, subjects were studied twice: after 14 and 62 h of fasting. Study days were separated by at least a week. Subjects were fasting from 8 p.m. until 10 a.m. the next day or 3 days later. They were allowed to drink water ad libitum.
After admission at the metabolic research unit, a catheter was inserted into an antecubital vein for sampling of venous blood for determination of plasma TSH, T4, FT4, T3 and rT3. Hereafter, a muscle biopsy (vastus lateralis of the quadriceps muscle) was performed as described above. Thereafter a continuous infusions of insulin (60 mU/m^2/min) (Actrapid 100 IU/ml; Novo Nordisk Farma B.V., Alphen aan den Rijn, The Netherlands) and glucose 20% (to maintain a plasma glucose level of 5 mmol/L) were started. Plasma glucose levels were measured every 5 min at the bedside. After 5 h of insulin infusion, muscle biopsies were repeated.

Thyroid parameters

Plasma and serum thyroid hormone levels of DTC patients and fasting volunteers were determined as described previously (reference (24) and (25) respectively).

D2 activity

Skeletal muscle samples were homogenized on ice in 10 volumes of PED10 buffer (0.1 M phosphate, pH 7.2, 2 mM EDTA, and 10 mM dithiothreitol) using a polytron (Kinematica AG, Lucerne, Switzerland). Protein concentrations were measured with the Bio-Rad protein assay (Bio-Rad, Veenendaal, The Netherlands) using BSA as the standard according to the manufacturer's protocol.

Skeletal muscle D2 activities were measured as previously described (9). Duplicates of 200 µg homogenate protein were incubated for 60 min at 37 C with 1 nM (1-2x10^5 cpm) [3',5'-^{125}I]T4 in a final volume of 0.1 ml PED10 buffer. The incubations were done in the absence or presence of 0.1 µM unlabeled T3, to prevent inner ring deiodination of the labeled T4 substrate by D3, if present, and in the absence or presence of 0.1 µM unlabeled T4, which is sufficient to saturate D2. Deiodination of labeled T4 in the absence minus that in the presence of excess labeled T4 represents D2 activity. Reaction products were analyzed by determination of the [^{125}I]T3 generated by HPLC analysis of ethanol extracts of the reaction mixtures as previously described (26). The samples from the DTC patients were also analyzed by isolation of the released ^{125}I- from the supernatant after addition of albumin and protein precipitation with 10% TCA.

To rule out interfering effects of local anesthesia on D2 activity in the human muscle samples, we analyzed the effects of increasing lidocaine concentrations on D2 activity expressed in COS1 cells transfected with a human D2 construct (D2-COS1 cells) in pcDNA3 as previously described (27). To rule out the presence of factors in skeletal muscle homogenates that could inhibit D2 activity, we measured D2 activity in D2-COS1 cell lysates with addition of increasing volumes (12.5-50 µl) of homogenate (50-200 µg of protein).

Quantitative mRNA analysis

RNA was isolated from skeletal muscle samples using High Pure RNA kit (Roche Diagnostics, Almere, The Netherlands) following the manufacturer's instructions. RNA concentrations were determined using the RiboGreen RNA quantification kit (molecular Probes, Leiden, The Netherlands). All samples were diluted to 0.1 µg/µl, and 1 µg was used for cDNA synthesis using TaqMan RT kit (Roche Diagnostics, Almere, The Netherlands). D2 and D3 cDNA were analyzed on an ABO PRISM 7700 sequence detection system (Applied Biosystems, Nieuwerkerk aan den IJssel, The Netherlands), which uses TaqMan chemistry for highly accurate quantitation of mRNA levels. Sequences and concentrations of the primers are given in Table 1. The D2 and D3 mRNA levels are expressed relative to those of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) or cyclophilin A. Probes and primers for these housekeeping genes were provided as preoptimized control system (Applied Biosystems).

Table 1. Primers and probes used for the determination of D2 and D3 mRNA levels by quantitative real-time RT-PCR

Primers and probes	Sequence
D2 forward	CAAGTCCACTCGCGGAGA
D2 reverse	GACATGCACCACACTGGA
D2 probe	ACGCAGGCGCAGTCCCTCT
D3 forward	TTCCAGAGCCAGCACATC
D3 reverse	ACGTCGCGCTGGTACTTA
D3 probe	TGCACCTGACCACCGTTC

Reactions were done for 2 min minimal at 50 C and for 10 min at 95 C, followed by 40 cycles of 15 sec at 95 C and 1 min at 60 C. Following the manufacturer's guidelines, the cycle threshold (Ct) was determined, which represents the cycle number at which probe-derived dye absorbance reaches the calculated threshold value. Data were expressed as $2\Delta Ct$, where ΔCt represents the Ct value of the housekeeping gene minus the Ct value of the target gene.

Statistical Analysis

Statistical comparisons were performed with the Wilcoxon Signed Rank test. Differences were considered statistically significant at $P<0.05$. The SPSS statistical software program version 12.0.2 (SPSS Inc, Chicago, IL) was used for statistical analysis. Data are presented as mean ± SE.

Results

DTC patients characteristics and thyroid hormone levels

Eleven patients were included in the DTC group (4 males/7 females). Mean ± SE age was 45.5 ± 2.1 years. Mean ± SE duration of TSH suppressive therapy was 5.0 ± 2.1 years (range 0.6-24.3 years). Mean ± SE thyroxine dosage was 197 ± 13 µg/day. Thyroid parameters are presented in Table 2.

Table 2 Thyroid hormone parameters in DTC patients

	Hypothyroidism (n=11)	Thyroxine treatment (n=11)	P-value
FT4 (pmol/L)	1.4 ± 0.2	24.8 ± 1.2	0.003
TSH (mU/L)	142.4 ± 10.4	0.8 ± 0.3	0.003
T3 (nmol/L)	0.3 ± 0.1	1.3 ± 0.1	0.005
rT3 (nmol/L)	0.02 ± 0.00	0.29 ± 0.02	0.003

Wilcoxon signed rank test. Data are presented as mean ± SE

After 4 weeks of thyroxine withdrawal, all patients were overtly hypothyroid. After 8 weeks of thyroxine replacement therapy, FT4, T3 and rT3 increased significantly ($p=0.003$), whereas TSH decreased significantly ($p=0.003$). Six patients had thyroid parameters within the reference range, whereas 5 patients had a TSH below the reference range with normal plasma T3 and T4 levels.

Skeletal muscle deiodinase expression and D2 activity in DTC patients

Results of the quantitative RT-PCR analysis of deiodinase mRNA levels in skeletal muscle biopsies are presented in Fig. 1A. D2 and D3 mRNA levels were present in all muscle biopsies, but there was no significant difference between the hypothyroid and thyroxine replacement states.

Very little D2 activity was detected in the muscle biopsies and it was not different between the thyroxine replacement and hypothyroid states (Fig. 2A). Similar results were obtained by HPLC analysis of T3 formation (Fig. 2A) and using the iodide release assay (data not shown). Little D3-catalyzed conversion of T4 to rT3 was observed in the skeletal muscle biopsies, and this was also not different between the hypothyroid and thyroxine replacement states (data not shown).

Figure 1a

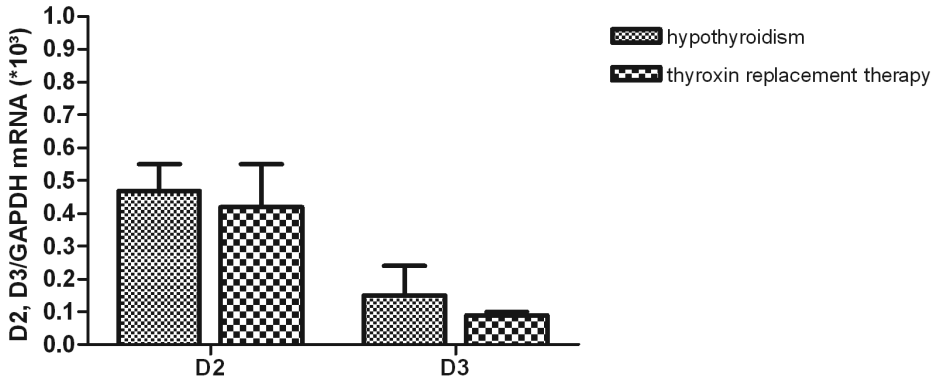

Figure 1b

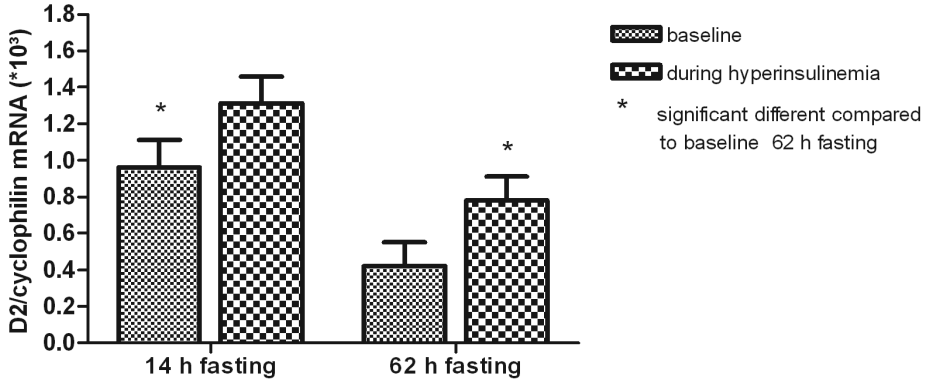

Figure 1. mRNA levels of deiodinase 2 and 3 vs. the household gene GAPDH or cyclophilin (*10^{-3}) in skeletal muscle samples (mean ± SE).

A. In DTC patients (n=11) during hypothyroidism and during thyroxine replacement treatment.

B. In healthy subjects (n=6) after 14 and 62 h of fasting before and after insulin infusion. P < 0.05 for D2 mRNA expression in the basal state after 62 h of fasting compared to the basal state after 14 h of fasting and D2 mRNA expression after insulin infusion after 62 h of fasting compared to the basal state after 62 h of fasting.

Fasting subjects characteristics and thyroid hormone levels

Six lean healthy men were included. Subject characteristics were: age: 23 ± 1.6 yrs; weight 69.4 ± 2.2 kg after 14 h and 67.5 ± 2.2 kg after 62 h of fasting, P = 0.002; BMI 21.2 ± 0.7 Kg/m^2 after 14 h and 20.5 ± 0.7 kg/m2 after 62 h of fasting, P = 0.001 (23).

Plasma FT4 and TSH levels were not different between 14 and 62 h of fasting (Table 3). T3 levels were significantly higher and rT3 levels were significantly lower after 14 h of fasting compared to 62 h of fasting. The T3/T4 (not shown) and T3/rT3 ratio's were significantly higher after 14 h of fasting compared to 62 h of fasting.

Skeletal muscle deiodinase expression and D2 activity after 14 and 62 h of fasting

D2 mRNA levels in skeletal muscle biopsies were significantly lower after 62 h of fasting compared to 14 h of fasting in the basal state (p=0.028). (Fig. 1B). No differences in D2 mRNA levels were observed during hyperinsulinemia after 62 h compared to 14 h of fasting.

Figure 2a

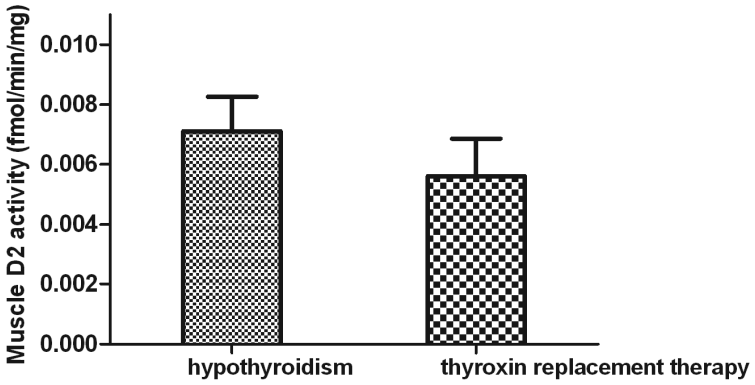

Figure 2b

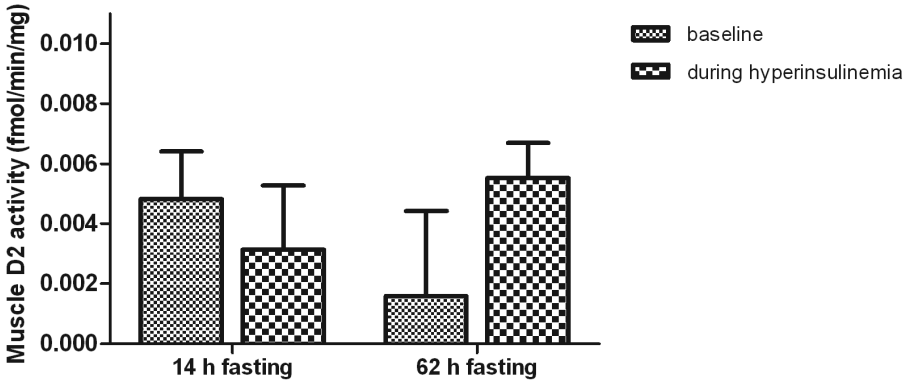

Figure 2. D2 activity in skeletal muscle samples (mean ± SE)

A. In DTC patients during hypothyroidism and thyroxine treatment.

B. In healthy subjects after 14 and 62 h of fasting.

Insulin infusion did not significantly increase D2 mRNA expression after 14 h of fasting, whereas insulin induced a significant increase in D2 mRNA levels after 62 h of fasting (p=0.028). D3 mRNA measurements were not reliable due to contamination with genomic DNA. D2 activity was low, but detectable in the muscle biopsies in the basal state and after 5 h of hyperinsulinemia both after 14 and 62 h of fasting. However, no significant differences were found (Fig. 2B). Very little D3 activity was demonstrated, and also here no differences were found (data not shown).

Different household genes were used for standardization of mRNA mesurements in DTC patients and healthy fasting subjects. However, their expression levels were constant in the patients and healthy subjects during the different conditions.

D2 activity, lidocaine, and possible inhibitors in muscle homogenate

Addition of increasing concentrations up to 1 mM of lidocaine did not inhibit D2 activity expressed in COS1 cells transfected with human D2 cDNA (Fig. 3A). Although a dose-dependent inhibition of D2 activity was observed after addition of increasing volumes of muscle homogenate up to of 50% of the total incubation volume, remaining activity still amounted to 60% of that expressed in D2-COS1 lysates (Fig. 3B).

Table 3 Thyroid hormone parameters in healthy subjects

	At baseline (n=6)			During hyperinsulinemia (n=6)		
	14h fasting	62h fasting	p-value	14h fasting	62h fasting	p-value
FT4 (pmol/L)	14.6 ± 1.0	14.6 ± 1.3	0.985	13.7 ± 0.8	14.4 ± 1.0	0.279
TSH (mU/L)	1.0 ± 0.2	0.5 ± 0.1	0.102	0.8 ± 0.1	0.5 ± 0.1	0.116
T3 (nmol/L)	1.6 ± 0.1	1.1 ± 0.1	0.027	1.4 ± 0.1	0.9 ± 0.05	0.027
rT3(nmol/L)	0.24 ± 0.02	0.44 ± 0.02	0.027	0.26 ± 0.01	0.38 ± 0.01	0.027
T3/rT3 ratio	6.9 ± 0.7	2.5 ± 0.2	0.028	5.3 ± 0.5	2.5 ± 0.2	0.028

Wilcoxon signed rank test. Data are presented as mean ± SE

Discussion

In this study, we investigated the D2 activity and expression of D2 and D3 mRNA in skeletal muscle samples in DTC patients on and off thyroxine replacement therapy and in healthy subjects after 14 and 62 h of fasting and during hyperinsulinemia. Hypothyroidism induced by withdrawal of thyroxine substitution in thyroidectomised patients did not affect muscle D2 mRNA expression, whereas fasting for 62 h reduced muscle D2 mRNA levels compared to fasting for 14 h.

Figure 3a

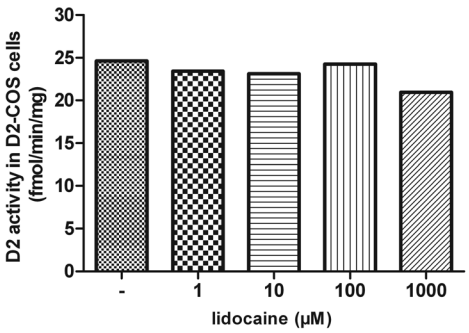

Figure 3b

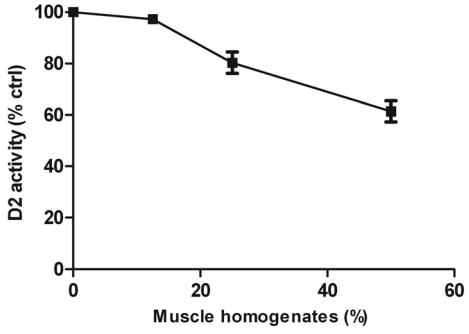

Figure 3

D2 activity in D2-COS cells with increasing levels of lidocaine.

Conversely, insulin increased mRNA expression after 62 h, but not after 14h of fasting. Nonetheless, skeletal muscle D2 activities were very low, and not influenced by hypothyroidism, fasting or insulin.

D2 activity is regulated by thyroid status both at the pre- and posttranslational level. In hypothyroidism, D2 activity is increased in different tissues predominantly by a decrease in substrate (T4)-induced degradation of D2 protein (1;28-30). Hypothyroidism also elevates D2 mRNA in rat brain and BAT (1;6;31;32). We found no differences in D2 mRNA expression in skeletal muscle of DTC patients between hypothyroid and thyroxine replacement states.

We measured D2 activity with a highly specific and sensitive D2 assay based on the measurement of radiolabeled T3 production (9;33), but found little D2 activity present in skeletal muscle samples of the DTC patients.

Furthermore, no differences were observed between the hypothyroid and thyroxine replacement states. The peripheral conversion of T4 to T3 is increased during hypothyroidism (1;11;12). In rats, extrathyroidal T3 production changes from PTU sensitive to PTU insensitive during hypothyroidism, representing an increase in the conversion of T4 to T3 by D2 and a decreased conversion by D1 (34). There is, however, no D2 expression and activity in rat skeletal muscle (6), whereas D2 activity has been reported in human skeletal muscle (14;35). Animal studies have shown significantly increased D2 activity in the cerebral cortex and pituitary during hypothyroidism (36-38). Collectively, our data on D2 mRNA expression and D2 activity in hypothyroid and thyroxine treated patients point out that changes in circulating plasma levels of thyroid hormones do not regulate muscle D2 activity or mRNA expression. Consequently, increased D2 activity in other tissues must be responsible for the increased conversion of T4 to T3 in hypothyroid subjects.

Since it has been shown that skeletal muscle expresses D3 mRNA and activity, we assessed D3 mRNA expression and activity as well. Furthermore, in the rat brain D3 mRNA was found to be decreased during hypothyroidism (1;39). However, there were no differences in D3 mRNA expression or activity during hypothyroidism and thyroxine replacement.

Between 14 and 62 h of fasting, plasma T3 levels decreased, whereas rT3 levels increased as has been shown earlier (15;40). Both the decrease in serum T3 and increase in serum rT3 may be explained by decreased D1 activity (in liver/kidney), decreased D2 activity (in muscle), or increased D3 activity (in the central nervous system) (2).

D2 mRNA levels in skeletal muscle samples were significantly lower after 62 h of fasting compared to 14 h of fasting. In mice, fasting decreased the expression of D2 in the pituitary (36). In other conditions of insulin deprivation, such as streptozotocin-induced diabetes in rats, the increase of D2 activity in BAT after insulin was exaggerated compared to the normal response (19).

A significant increase in D2 expression was found after 5 hours of hyperinsulinemia in healthy subjects after 62 h of fasting, whereas this was not the case after 14 h of fasting. It had been shown in rats D2 activity and expression are upregulated by insulin in BAT (17-19). Injections with insulin resulted in an increased D2 activity in BAT in diabetic and non-diabetic rats (19) and addition of insulin to rat brown adipocytes in vitro leads to an increase in V_{max} of D2 (18). Martinez-deMena *et al.* found that this induction is not a direct effect of insulin, but that insulin improves the adrenergic stimulation of D2 activity (17). A role of insulin in regulating D2 in skeletal muscle is conceivable. Moreover, the lack of a significant increase after 14 h of fasting suggests that minor increments of insulin (i.e. postabsorptive plasma insulin levels) are sufficient to induce D2 mRNA expression.

Little D2 activity was present in skeletal muscle samples after 14 and 62 h of fasting and no difference was observed between the two conditions, in contrast with the observed changes in D2 mRNA expression. There are several possible explanations for this. The particular level of D2 mRNA expression in skeletal muscle may not result in significant D2 activity. Therefore, D2 activity in other tissues may be responsible for the decrease in T3 levels. In rats, D1 activity in the thyroid and liver, and D2 activity in the thyroid were decreased after fasting (37;38), whereas D2 activity in the hypothalamus was increased (39). In other conditions where T3 levels decrease significantly, such as acute critical illness, no D2 activity could be measured in liver and skeletal muscle biopsies (30). However, Mebis *et al.* found low but significant skeletal muscle D2 activity during prolonged critical illness, indicating an adaptation to the low T3 levels (11). This may suggest that our volunteers had not been fasting long enough.

It is not likely that the local anaesthetics used for the sample collection could have influenced D2 activity, since we found no effect on D2 activity in D2-COS1 cells with increasing lidocaine concentrations. However, we cannot exclude a local effect of lidocaine resulting

in downregulation of D2 activity. On the other hand, Mebis *et al*. reported no differences in D2 expression and D2 activity in muscle samples taken under local anaesthetics or during laparotomy (11).
Recent findings show that D3 mRNA and activity (catalyzing T4 to rT3 deiodination and T3 degradation) may be increased in muscle and liver of patients hospitalized in the intensive care unit (40). However, we found no change in D3 activity in skeletal muscle during fasting or hyperinsulinemia.
In summary, no differences were observed in the expression of skeletal muscle D2 mRNA between hypothyroidism and thyroxine treatment, although a robust decrease was observed after 62 h of fasting. Moreover, insulin restored D2 mRNA expression after 62 h of fasting. Little D2 activity was measured in skeletal muscle samples and no differences were observed between hypothyroidism and thyroxine treatment or after 14 and 62 h of fasting. Our results therefore imply that skeletal muscle D2 mRNA expression is modulated by fasting and insulin, but not by hypothyroidism or thyroxine treatment. The lack of effect of changes in D2 mRNA on already low D2 activity questions the importance of a role for D2 activity in human skeletal muscle.

Acknowledgments

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04

The Effects of Thyrotropin Suppressive Therapy on Bone Metabolism in Patients with Well-Differentiated Thyroid Carcinoma

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Thyroid. 2006;16(6):583-91

Abstract

Patients with differentiated thyroid carcinoma (DTC) are commonly treated long-term with thyrotropin (TSH)-suppressive thyroxin replacement therapy resolving in a state of subclinical hyperthyroidism. The relationship between subclinical hyperthyroidism and osteoporosis is not clear. In this review, we systematically selected and analyzed 21 studies addressing this issue. Although multiple methodological differences between studies prevented a structured meta- analysis, our data suggest that postmenopausal women with subclinical hyperthyroidism are most at risk, whereas no increased risk was observed in men and premenopausal women. Based on these findings we believe that measurement of bone mineral density is recommended in postmenopausal women with DTC starting TSH suppressive therapy. This should be subsequently regularly measured to enable timely intervention with bone protective agents.

Background

Differentiated thyroid carcinoma (DTC) is associated with an excellent prognosis, with reported 10-year survival rates reaching 90% (1). This is because of a combination of the favorable biological behaviour of the tumor as well as the availability of effective therapy in the form of total thyroidectomy followed by radioiodine ablation. After initial therapy, all patients with DTC are treated with high doses of thyroxin aiming at significantly suppressing thyrotropin (TSH) levels, the rationale of this approach is based on the potential harmful effects of TSH on tumor recurrence (2;3). One study demonstrated a preventive effect of TSH suppression on tumor recurrence or progression only in high risk DTC patients (4). However long-term TSH suppression may be associated with potential harmful effects, so a recent European Consensus Meeting on thyroid cancer (5), recommended that not all patients with DTC should be indiscriminately treated with TSH suppressive therapy because this represents in effect a state of subclinical hyperthyroidism, as defined by suppressed serum TSH levels in the presence of normal serum levels of (free) thyroxin. The question which clearly is to be answered is whether long-term TSH suppressive therapy is beneficial and safe in patients with DTC.

Subclinical hyperthyroidism has effects on carbohydrate metabolism (6), the cardiovascular system (7-11) and psychological well being (7;12). Whether subclinical hyperthyroidism also deleteriously affects the skeleton remains the subject of discussion (13;14).

Overt hyperthyroidism is associated with an increased risk for osteoporosis (15), the pathophysiology of which is multifactoral (16), including shortening of the bone remodelling cycle (17) and acceleration of bone turnover (18). Thyroid hormone indirectly promotes osteoclast formation and activation by inducing the expression of cytokines, prostaglandins and the receptor activator of nuclear factor NF-κB ligand (RANKL) (16;19;20). RANKL is the key molecule in osteoclast differentiation. It binds to its receptor, RANK, which is expressed on dendritic cells, T cells, osteoclast precursors and mature osteoclasts (21;22). RANKL increases the survival of RANK positive T cells (21), promotes osteoclast differentiation (23-27), stimulates the activity of mature osteoclasts (24;28;29) and promotes survival of osteoclasts by preventing apoptosis (29). Osteoclast differentiation is also stimulated by contact with stromal cells and M-CSF (30;31). Thyroid hormone also inhibits chondrocyte proliferation and promotes hypertrophic differentiation, mineralization, matrix synthesis but also apoptosis of chondrocytes in the growth plate. An exciting new development has been the discovery of functional TSH receptors in bone (32;33) because of the implication that effects that traditionally have been attributed to high thyroid hormone levels may be in effect related to low TSH levels.

The relationship between subclinical hyperthyroidism and osteoporosis is not clear. Several studies have addressed this issue, but there is no consensus largely because of differences in study design, included patient groups, methodology used, follow-up time and choice of outcome parameters. It is of note that although the role of subclinical hyperthyroidism in the pathogenesis of osteoporosis has been the topic of several reviews, no attempt has been made to categorize the original studies so far published according to the various parameters above mentioned. In this review, all clinical studies on TSH suppressive thyroxin therapy in thyroid cancer patients have been systematically selected for analysis.

Methods

Searches of Medline, Cochrane and EMBASE were conducted using the keywords: “thyroid cancer AND bone mineral density”, “thyroid cancer AND osteoporosis” and “thyroid cancer AND bone metabolism”. Our aim was to include all studies in which patients with DTC were treated with TSH suppressive thyroxin therapy. We restricted our search to publications in “English language”, on “Human subjects” and “articles containing Abstracts”. The last search was conducted on January 17, 2006. Of the initial 230 publications found, 32 publications fulfilled these criteria and were selected for detailed analysis.

Each study included was scored semi-quantitatively by assessing the following: whether hormonal state of female patients was mentioned; whether they were estrogen-replete or -deplete; whether additional risk factors for osteoporosis were reported; whether a control group was included; whether duration of follow-up was shorter or longer than 5 years; and whether TSH concentrations were adequately suppressed. Eleven studies were excluded on the basis of insufficient data: Mikosch *et al.* (34) and Rosen *et al.* (35) did not report the duration of thyroxin therapy. Rosen and coworkers included patients who were taking thyroid hormone for at least 6 months. In one study patients did not have sufficiently suppressed serum TSH concentrations (36). Guo *et al.* (37) and Gonzalez *et al.* (38) did not report serum TSH levels. Mikosch *et al.* (34;39) and Taimelia *et al.* (40) did not measure bone mineral density. Subanalysis according to gender and menopausal state were not performed in two studies (35;41). In an additional 4 studies (40-43), it was not indicated if other risk factors for osteoporosis were investigated. The last 4 studies (38;40;42;44) lacked control groups.
Twenty-one studies fulfilling all criteria were finally included in the analysis, the following parameters were documented: study design, number of patients included, age, gender, hormonal status of female patients, additional risk factors for osteoporosis, dose of thyroxin prescribed, serum level of TSH, duration of TSH-suppressive therapy, the presence of a control group and the final outcome based on differences in bone mineral density (BMD). The studies were categorized according to gender and menopausal state and subgroup analyses undertaken accordingly.
Although we set out to conduct a structural meta-analysis, the heterogeneity of the available data did not allow us to do so.

Results

The results of the analyses are shown in Tables 1-5. Almost all studies excluded patients with diseases and those using glucocorticoids or other drugs potentially affecting bone metabolism. Of the 21 studies included, 4 of 6 prospective studies reported a significant decrease in BMD with time on treatment, as in 4 of 17 cross-sectional studies there was a significant difference in BMD between DTC patients and controls. The results of the subgroup analyses according to gender and menopausal state were as follows:

Premenopausal women
The effect of TSH suppressive therapy in premenopausal women is described in 15 studies, of which 12 had a cross-sectional design and 4 were prospective studies (Table 1 and 2). Two cross-sectional studies found a significant decrease in BMD in DTC patients receiving TSH suppressive therapy compared to controls: Jodar *et al*. (45) evaluated 37 DTC patients, significantly lower BMD of the distal radius was found in DTC patients compared to controls; although, this was still within the normal range for age and gender. There was a significantly positive relationship between thyroxin dose and lumbar spine and distal radius BMD. Diamond *et al*. (46) evaluated 14 DTC patients. BMD of the femoral neck in DTC patients was significantly lower (-10.6%) than in age-, gender- and menopausal state-matched controls. There were no significant differences between patients and controls in the other cross-sectional studies analyzed.
Two of the prospective studies found a significant effect of TSH suppressive therapy on BMD. Jodar *et al*. (45) studied 14 DTC patients for 18 months. He reported that BMD of the femoral neck was significantly lower in DTC patients than in age-, gender-, body- weight- and menopausal state-matched controls. There were no differences observed between premenopausal and postmenopausal women. Sijanovic *et al.* (47) studied 19 premenopausal women. There was a significant reduction in BMD of the distal radius after 4 years of follow-up.

Table 1. Premenopausal women Cross-sectional studies

Author	Patients (N)	Duration (Years)	L-thyroxin Dose (µg/day)	TSH (mU/L)	Controls	Outcome (BMD (z-scores), unless otherwise indicated)
Franklyn (13)	18	7.7 (1-19)	217 (100-300)	0.67 ± 2.20	Yes	NS
Jodar (45)	37	5.4 ± 2.8	177 ± 43	0.61 ± 1.18	Yes	BMD (z-score): DTR : -0.84 ± 1.00 (significant below 0)
Toivonen (64)	15	9-11	215 ± 53	<0.05	Yes	NS
Marcocci (65)	47 (38 DTC)	10.1 Median: 9.2 (5-28)	154.3 ± 5	No quantitative data	Yes	NS
Stepan (50)	20	6.0 ± 5.2	151.1 ± 47.1	0.10 (0.01-3.80)	Yes	NS
Goerres (66)	7	5.7 ± 6.8	Cumulative dose: 7124.5 ± 9448.6 µg/kg	0.019 ± 0.056	Yes	NS
Gianinni (67)	12	9.25 ± 0.9	152.1 ± 3.72	<0.1	Yes	NS
Diamond (46)	14	10.7 ± 1.7	Cumulative dose: 816 ± 159 mg	No quantitative data	Yes	FN 0.98 ± 0.03 vs. 1.03 ± 0.01 (p=0.01)
Florkowski (68)	20	Median: 9.6 (3-42)	167 (125-300)	< 0.2 mU/l	No	NS
Chen (69)	44	7.2 ± 2.8	No quantitative data	1.98 ± 0.44 n=22 partly suppressive n=22 suppressive	Yes	NS
Heijckmann (70)	26	Median: 4 (1-14)	2.2 ± 0.5 µg/kg/day	0.06 (< 0.05-0.35)	No	NS
Reverter (71)	44	12 ± 5	195 ± 43	0.03 ± 0.03	Yes	NS

All values expressed as mean±SD unless indicated otherwise, DTR: Distal Third of the Radius; FN: Femoral Neck, NS=Not significant

Table 2. Premenopausal women Longitudinal studies

Author	Patients (N)	Duration (Years)	L-thyroxin Dose (µg/day)	TSH (mU/L)	Controls	Outcome (BMD (z-scores) unless otherwise indicated)
Jodar (45)	14	5.4 ± 2.8	177 ± 43	0.61 ± 1.18	Yes	BMD (z-score): DTR: -0.84 ± 1.00 (significant below 0) FN: (% year): -1.50 ± 3.18 (patients) vs. -0.24 ± 1.32 (controls) (p<0.05)
Muller (48)	15	11.2 ± 0.9	200 ± 7	0.09 ± 0.01	Yes	NS
Sijanovic (47)	19 (p<0.05)	9.4 ± 6.4	171 ± 30	0.07 ± 0.062	Yes	DR baseline: 0.670 ± 0.037 4 y: 0.657 ± 0.039
Karner (54)	19	9.4 ± 6.4	171 ± 30	0.07 ± 0.62	No	NS

All values expressed as mean±SD unless indicated otherwise, DTR: Distal Third of the Radius; FN: Femoral Neck, DR= Distal Radius, NS=Not significant

A significant negative correlation was found between thyroxin dose and BMD of the distal radius. Muller *et al.* (48) studied 23 patients: 8 with a non-toxic goitre and 15 DTC, who were followed up for an average of 1.5 years. There were no significant differences in BMD of the lumbar spine, femoral neck, trunk and extremities between patients and age-, gender-, body mass index (BMI)- and years of menopause-matched controls.

Postmenopausal women

The effect of TSH suppressive therapy in post-menopausal women was investigated in 16 studies (Table 3 and 4). Fourteen studies were cross-sectional. Four found a significant difference in BMD between patients and controls. Kung *et al*. (49) studied 34 postmenopausal women. The patients had a significant lower BMD than age-, gender- and menopausal state-matched controls. Jodar *et al.* (45) studied 39 patients. Average TSH levels were 0.50 ± 1.28 mU/l. BMD of the distal radius was significantly lower than the average of controls, although is was still within the normal range. Stepan *et al.* (50) studied 15 patients using both thyroxin and liothyronine. BMD of the lumbar spine was significantly decreased compared to matched controls. Diamond *et al*. (46) studied 10 postmenopausal women. BMD measurements at the lumbar spine, femoral neck and forearm were significantly lower than those of matched controls. There were no differences in BMD observed between patients and controls in the remaining cross-sectional studies analyzed.

There was a significant difference in 2 of the 4 prospective studies analysed. Jodar *et al*. (45) studied 13 postmenopausal women for a period of 2.25 ± 0.6 years. BMD of the femoral neck was significantly lower in DTC patients than in matched controls. Kung *et al*. (51)studied 46 patients who were randomly assigned to treatment with calcitonin and calcium (n=16), calcium (n=15) or placebo (n=15) and followed for 2 years. At the end of the two years, the BMD of patients treated with calcitonin or calcium remained unchanged, whereas BMD was significantly lower in the placebo-group. In the other 2 analyzed studies (48;52) there were no differences in BMD.

Table 3. Postmenopausal women Cross-sectional studies

Author	Patients (N)	Duration (Years)	L-thyroxin Dose (µg/day)	TSH (mU/L)	Controls	Outcome (BMD (z-scores) unless otherwise indicated)
Hawkins (72)	21	6.2 ± 2.5	158.3 ± 43.7	0.03 ± 0.4 80% < 0.3	Yes	NS
Kung (49)	34	12.2 ± 6.6	179 ± 60	< 0.05	Yes	Values versus controls: BMC 1 total body 1652 ± 356 vs. 1994 ± 270 p<0.005) LS 2 0.749 ± 0.147 vs. 0.917 ± 0.161 (P<0.005) FN 3 0.622 ± 0.123vs 0.708 ± 0.127 (p<0.01) T 4 0.552 ± 0.115 vs. 0.635 ± 0.119 (p<0.001) WT 5 0.554 ± 0.139 vs. 0.630 ± 0.144 (p<0.005)
Franklyn (13)	26	8.1 (1-19)	175 (100-200)	0.26 ± 0.54	Yes	NS
Jodar (45)	39	5.8 ± 2.9	160 ± 38	0.50 ± 1.28	Yes	DTR 6 -0.77 ± 0.98 (significant below 0)
Toivonen (64)	10	9-11	215 ± 53	< 0.05	Yes	NS
Stepan (50)	25	7.4 ± 4.5	148.7 ± 49.4	0.05 (0.01-2.26)	Yes	LS -1.08 ± 1.40 vs. -0.05 ± 0.98 (controls, p<0.01)
Goerres (66)	23	10.3 ± 4.4	Cumulative dose: 9195.8 ± 5193 µg/kg	0.019 ± 0.056	Yes	NS
Diamond (46)	10	5.9 ± 1.0	Cumulative dose: 337 ± 72 mg	No quantitative data	Yes	LS 0.876 ± 0.04 vs. 1.069 ± 0.04 (-16% vs. controls, p<0.01) FN 0.702 ± 0.03 vs. 0.916 ± 0.02 (-15%), p<0.001) Forearm 33.5 ± 1.3 vs. 38.8 ± 1.5 (-11%, p<0.05)
Fujiyama (52)	24	11.6 ± 7.36 14.8 ± 9.43	152.1 ± 22.51 95.83 ± 50.94	n=12: < 0.1 n=12: > 0.1	No	NS
Gianinni (67)	13	6.0 ± 1.5	144.2 ± 4.15	< 0.1	Yes	NS
Florkowski (68)	18	9.6 (3-42)	167 (125-300)	< 0.2	No	NS
Chen (69)	25	7.8 ± 3.1	No quantitative data	1.76 ± 0.41 n=8 partly suppressive n=17 suppressive	Yes	NS
Heijckmann (70)	14	median: 5.5 (1-52)	2.2 ± 0.5 µg/kg/day	0.06 (< 0.05-0.35)	No	NS
Reverter (71)	44	12 ± 5	195 ± 43	0.03 ± 0.03	Yes	NS

All values expressed as mean±SD unless indicated otherwise. 1 BMC=Bone Mineral Content, 2 LS=Lumbar Spine, 3 FN=Femoral Neck, 4 T=Trochanter, 5 WT=Ward's triangle, 6 DTR= Distal Third of the Radius, NS=not significant

Table 4. Postmenopausal women Longitudinal studies

Author	Patients (N)	Duration (Years)	L-thyroxin Dose (µg/day)	TSH (mU/L)	Controls	Outcome (BMD (z-scores) unless otherwise indicated)
Jodar (45)	39 13	5.8 ± 2.9	160 ± 38	0.50 ± 1.28	Yes	FN change (%/year): -1.50 ± 3.18 vs. -0.24 ± 1.32 ($p<0.05$)
Muller (48)	10	11.2 ± 0.9	200 ± 7	0.09 ± 0.01	Yes	NS
Kung (51)	46	2	2	< 0.03	No	LS1 -5% vs. baseline FN2 -6.7% T3 -4.7% WT4 -8.8%
Fujiyama (52)	24	11.6 ± 7.36 14.8 ± 9.43	152.1 ± 22.51 95.83 ± 50.94	n=12: < 0.1 n=12: > 0.1	No	NS

All values expressed as mean±SD unless indicated otherwise. 1 LS=Lumbar Spine, 2 FN=Femoral Neck, 3 T=Trochanter, 4 WT=Ward's triangle

Men

Eight studies selected for analysis addressed the effects of TSH suppressive therapy on bone metabolism in men in a cross-sectional study design (table 5). One study was longitudinal (table 6). Only one cross-sectional study found a significant difference between patients and controls. Jodar *et al*. (53) studied 49 men, of whom 17 were treated for DTC and 32 were treated for Graves' disease. DTC patients had a mean TSH concentration of 0.20 ± 0.27 mU/mL. Graves' disease patients had a mean TSH concentration of 1.07 ± 1.85 mU/mL. BMD of patients with Graves' disease and DTC were significantly lower than that of controls. In the longitudinal study (54), 9 men were studied for one year. A significant bone loss at the distal radius, but not the lumbar spine and femoral neck was found.

Discussion

The clinical implications of long-term suppressive thyroxin therapy on bone are critical, largely because of the favourable prognosis of DTC. However, the potential deleterious effects of TSH suppressive therapy on the skeleton remain controversial. Our aim was to review the literature on the effects of TSH-suppressive therapy on bone metabolism focussing on reported changes in BMD measurements. There are many differences in the outcome of studies addressing this issue so that we have systematically categorized studies according to predefined criteria in an attempt to reach a more uniform conclusion.

The majority of studies do not report an effect of TSH suppressive therapy on BMD in men and premenopausal women. A significant effect of TSH-suppressive thyroxin replacement on BMD is reported in a substantial number of studies conducted in postmenopausal women. This suggests that there may indeed be a relevant effect of TSH-suppressive therapy on bone mass in postmenopausal women, whereas these effects are not clear in men and in premenopausal women. This conclusion is in agreement with that of other reviews and meta-analyses addressing this issue (14;15;55-57). Another important aspect of TSH suppressive therapy in young patients is that it may affect bone development and the peak bone mass as investigated together with the contribution of hypoparathyroidism by Schneider *et al*. (58).

Table 5. Men Cross-sectional studies

Author	Patients (N)	Duration (Years)	L-thyroxin Dose (µg/day)	TSH (mU/L)	Controls	Outcome (BMD (z-scores) unless otherwise indicated)
Franklyn (13)	5	7.9 (2-15)	180 (100-200	0.36 ± 0.57 n=2 <0.05 mU/l n=3 normal/smaller than normal	Yes	NS
Jodar (53)	49 (17 DTC)	9.1 ± 4.9	193 ± 50	0.20 ± 0.27	No	LS 1 -0.64 ± 1.22(p=0.046) FN 2 -0.49 ± 0.62 (p=0.007) WT 3 -0.50 ± 0.62 (p=0.004)
Toivonen (64)	4	9-11	215 ± 53	< 0.05	Yes	NS
Marcocci (73)	34 (26 DTC)	10.2 ± 0.8	172 ± 5.9	n=26 undetectable n=6: 0.1 n=2: 0.2	Yes	NS
Stepan (50)	13	4.6 ± 3.0	148.6 ± 55.8	0.06 (0.01-2.49)	Yes	NS
Goerres (66)	17	8.1 ± 5.2	Cumulative dose: 8200.4 ± 5907.0 µg/kg	0.019 ± 0.056	Yes	NS
Florkowski (68)	6	9.6 (3-42)	167 (125-300)	< 0.2	No	NS
Heijckmann (70)	19	median: 6 (1-22)	2.2 ± 0.5 µg/kg/day	0.06 (<0.05-0.35)	No	NS

All values expressed as mean±SD unless indicated otherwise, 1 LS=Lumbar Spine, 2 FN=Femoral Neck, 3 WT=Ward's triangle, NS=not significant

Table 6. Men Longitudinal studies

Author	Patients (N)	Duration (Years)	L-thyroxin Dose (µg/day)	TSH (mU/L)	Controls	Outcome (BMD (z-scores) unless otherwise indicated)
Karner (54)	9	8.1 ± 6.0	200 ± 50	0.06 ± 0.09	No	S BMD DR 0.748 ± 0.086 vs. 0.732 ± 0.083, p<0.05

All values expressed as mean±SD unless indicated otherwise. TSH, thyrotropin; DR, Distal Radius

Estrogen deprivation is the most common cause of osteoporosis. The removal of the physiological block by gonodal steroid hormones allows the release of inflammatory cytokines which in turn enhance the production of M-CSF and RANKL. RANKL is identified as an essential cytokine for the formation and activation of osteoclasts (23;24;59). This effect could be enhanced by the subclinical hyperthyroid state resulting from TSH suppressive therapy. Hofbauer *et al* (59) found that TSH inhibits RANKL mRNA levels by 60 % and upregulates OPG mRNA levels threefold. OPG inhibits osteoclastogenesis by binding to RANKL (27;59-61), thus preventing RANK-RANKL interactions. In the subclinical hyperthyroid state, TSH levels are suppressed resulting in an absence of this block.

The discrepancy in outcome between studies in postmenopausal women might be explained by a difference in duration of thyroxin therapy or additional risk factors. However, no differences in duration of thyroxin therapy or additional risk factors such as smoking, calcium intake, alcohol abuse, and physical activity were observed. A third explanation could be a difference in methodological approaches. However, all authors used BMD measurements to examine the effect of TSH-suppressive therapy on bone mass. Another explanation could be the instability of the TSH concentration in the years of TSH-suppressive therapy as suggested in the study of Pujol *et al*. (62). Other possible factors could be differences in study population with regard to additional determinants of BMD such as dietary factors, physical exercise, endogenous factors and genetic susceptibility, which become relevant only once the powerful contribution of estrogens has disappeared in postmenopausal women (63). For instance, Kung *et al* mentioned that the patients taking part in the study had a low dietary calcium (51)

Regardless of these considerations it is clear that postmenopausal women with DTC treated with TSH-suppressive therapy are most at risk for bone loss. It has also been shown that bone protecting agents such as bisphosphonates are effective in preventing bone loss in patients with DTC on TSH-suppressive therapy (35) The availability of therapeutic interventions that beneficially influence skeletal morbidity in patients with a low bone mass and consequently risk of fractures dictates that patients at high risk should be screened using BMD measurements. We have identified postmenopausal women with DTC receiving TSH suppressive therapy as a high risk group for bone loss. Based on our analysis of available data we strongly advise screening this group of patients at start of TSH suppressive therapy and at regular intervals to allow timely intervention with bone protective agents.

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05

Thyroid Hormone Independent Associations between serum TSH levels and Indicators of Bone Turnover in Cured Patients with Differentiated Thyroid Carcinoma

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Abstract

Objective: It has been proposed that thyrotropin (TSH) has thyroid hormone independent effects on bone mineral density (BMD) and bone metabolism. This concept is still controversial and has not been studied in human subjects in detail. We addressed this question by studying relationships between serum TSH concentration and indicators of bone turnover, after controlling for T3, FT4 and non-thyroid factors relevant to BMD and bone metabolism. We also studied the contribution of the TSHR-Asp727Glu polymorphism to these relationships.
Design: We performed a cross-sectional study with 148 patients, who had been thyroidectomized for differentiated thyroid carcinoma.
Methods: We measured BMD of the Femoral Neck and Lumbar Spine. FT4, T3, TSH, Bone Specific Alkaline Phosphatase, procollagen type 1 aminoterminal propeptide levels, C-crosslinking Terminal Telopeptide of Type I collagen and Urinary N-Telopeptide of Collagen Cross-links were measured. Genotypes of the TSHR-Asp727Glu polymorphism were determined by Taqman assay.
Results: We found a significant, inverse correlation between serum TSH levels and indicators of bone turnover that was independent of serum FT4 and T3 levels as well as other parameters influencing bone metabolism. We found that carriers of the TSHR-Asp727Glu polymorphism had an 8.1% higher femoral neck BMD, which was however no longer significant after adjusting for body mass index.
Conclusion: We conclude that in this group of patients, serum TSH was related to indicators of bone remodeling independently of thyroid hormone levels. This may point to a functional role of the TSHR in bone in humans. Further research into this mechanism needs to be performed.

Introduction

The effects of thyroid hormone on bone metabolism are well established, ranging from decreased skeletal development in childhood hypothyroidism to an increased risk for osteoporosis in hyperthyroidism (1,2,3). The pathophysiology of osteoporosis in hyperthyroidism is multifactorial (4,5), including shortening of the bone remodeling cycle (6) and acceleration of bone turnover (7). Thyroid hormone indirectly promotes osteoclast formation and activation by inducing the expression of cytokines, prostaglandins and the receptor activator of nuclear factor κ-B ligand (RANKL) (8,9,10). Apart from overt hyperthyroidism, subclinical hyperthyroidism also appears to be associated with decreased bone mineral density as reviewed by Heemstra *et al*. (11,12,3).
An important development has been the discovery of the thyrotropin (TSH) receptor (TSHR) in bone (13,14,15). It has been reported that TSHR knockout and haploinsufficient mice with normal thyroid hormone levels have decreased bone mass suggesting that TSH might directly influence bone remodeling (14,5,16). This is intriguing, because effects on bone metabolism that were previously ascribed to high thyroid hormone levels could also be attributed to suppressed TSH levels (17,5,16). Furthermore, in animal studies, low doses of TSH increased bone volume and improved microarchitecture in ovariectomized rats (18), without increasing serum thyroid hormone levels. However, the concept has been challenged by investigations concluding that bone loss in thyrotoxicosis is mediated predominantly by thyroid hormone receptor (TR) α (19). The functional role of a TSHR in bone has been studied in humans to a limited extent. The relationship between low TSH levels and decreased bone mineral density (BMD) has been documented in humans (20,21) but this could still be attributed to elevated thyroid hormone levels. In a study in patients in follow-up for differentiated thyroid carcinoma (DTC), a supraphysiological dose of recombinant human TSH had thyroid hormone independent effects on bone-metabolism parameters (22). We therefore decided to study the independent relation between serum TSH levels and indicators of bone turnover in thyroidectomized patients for differentiated thyroid carcinoma receiving thyroid hormone substitution. The advantage of this group is that these subjects have more uniform FT4 levels. As it was recently reported that the TSHR-Asp727Glu polymorphism was associated with 2.3% higher BMD in elderly carriers (23), we also decided to study this relationship and BMD in these patients. Although the functional consequences of the polymorphism are debated (24), the lower plasma TSH levels in patients carrying the polymorphism could point toward a higher sensitivity of the variant compared to the wild-type TSHR. (25,26). We hypothesized that a group of thyroidectomized patients receiving thyroid hormone substitution would be optimal to study the relationship between the TSHR-Asp727Glu polymorphism and bone as these subjects are not expected to show compensatory lower serum TSH levels if they carry the TSHR-Asp727Glu polymorphism (26,25).

Patients and Methods

Patients were recruited from the outpatient clinic of the Department of Endocrinology of Leiden University Medical Center. Patients were included in the study who had a diagnosis of DTC, for which they had been treated by near-total thyroidectomy, followed by routine postoperative I-131 radioiodine ablation therapy, in all but 4 cases. All patients were cured as defined by the absence of I-131 accumulation at diagnostic scintigraphy, serum thyroglobulin concentrations below 2 μg/L after TSH stimulation, the absence of Tg antibodies, a normal neck ultrasound and no other indication for disease (27). Patients with tumour relapse were only included if they were subsequently cured.

None of the patients used any drug or had a disease known to influence bone metabolism, including estrogen-replacement therapy. Patients taking calcium or vitamine D supplements were also excluded. The Leiden University Medical Center ethics committees approved the study, and written informed consent was obtained from all subjects.

Study design

After an overnight fast, patients had a full clinical examination, including, height (meters [m]) and weight (kilograms [kg]). Blood was collected and measured for TSH, FT4, triiodothyronine (T3), calcium, parathyroid hormone (PTH), 25-hydroxy-vitamin D (25(OH)vitD), bone specific alkaline phosphatase (BAP), C-crosslinking terminal telopeptide of type I collagen (CTx) and procollagen type 1 aminoterminal propeptide (P1NP). Second morning void urine was measured for excretion of N-telopeptide of collagen cross-links (NTx). We choose not to measure Cathepsin K or TRAP levels for various reasons, including age- and gender differences (28,29). Furthermore, CTx seems to reflect bone resorption better than TRAP, especially during hyperthyroidism (29). Plasma, serum and urine samples were handled immediately and stored at –80o C in Sarstedt tubes. BMD (expressed in grams per square centimetre) was measured at the femoral neck and the lumbar spine (vertebrae L2-L4) by dual energy x-ray absorptiometry (NHAMES adjusted, Hologic 4500, Hologic Inc., Bedford, MA, USA). Osteopenia was defined as a T-score between –1 and –2.5 according to the WHO criteria. Osteoporosis was defined as a T-score below –2.5 according to the World Health Organization (WHO) criteria or the presence of fractures at an X-ray of the thoracic and lumbar vertebral column. As fractures of the lumbar vertebrae can affect BMD measurements by increasing X-ray resorption and therefore lead to inappropriate BMD values. We therefore excluded patients with fractures of the lumbar vertebrae from the BMD analyses. The following data were recorded: smoking habits, alcohol use, physical activity, calcium intake, medication (including self-prescription drugs) or vitamin or mineral supplements and daily calcium intake and for females: date of first menstruation (menarche), date of last menstruation, cycle regularity and estrogen substitution.

Serum biochemistry

Serum FT4 and TSH were measured using a chemoluminescence immunoassay with a Modular Analytics E-170 system (intraassay CV of 1.6-2.2 % and 1.3-5.0 % respectively (Roche, Almere, The Netherlands). Serum T3 was measured with a fluorescence polarization immunoassay, CV 2.5-9.0 %, on an ImX system (Abbott, Abbott Park, IL, USA). Thyroglobulin was measured by Dynotest TG-s (Brahms Diagnostica GmbH, Berlin, Germany). Plasma PTH was measured using an immunoradiometric assay (Nichols Diagnostic Institutes, Wijchen, The Netherlands), Calcium by colorimetry and 25(OH)vitD by RIA (Incstar/DiaSorin, Stillwater, MN, USA). Serum BAP was measured by RIA (Hybritech Europe, Liege, Belgium), CTx and P1NP by chemoluminescence immunoassay with the Modular Analytics E-170 system (Roche Diagnostics, Almere, The Netherlands). NTx was measured by ELISA (Ostex International Inc., Seattle, WA, USA). NTx was expressed as the ratio of urine creatinine excretion (NTx/creatinine) to correct for differences in creatinine excretion.

Genotyping

DNA was isolated from peripheral leucocytes by the salting out procedure (30). Genotypes were determined using 5 ng genomic DNA by a 5' fluoregenic Taqman assay and reactions were performed in 384-wells format on ABI9700 2x384well PCR machines with endpoint reading on the ABI 7900HT TaqMan® machine (Applied Biosystems, Nieuwerkerk aan den Ijssel, The Netherlands). Primer and probe sequences were optimized using the single nucleotide polymorphism assay-by-design service of Applied Biosystems.

Statistical Analysis

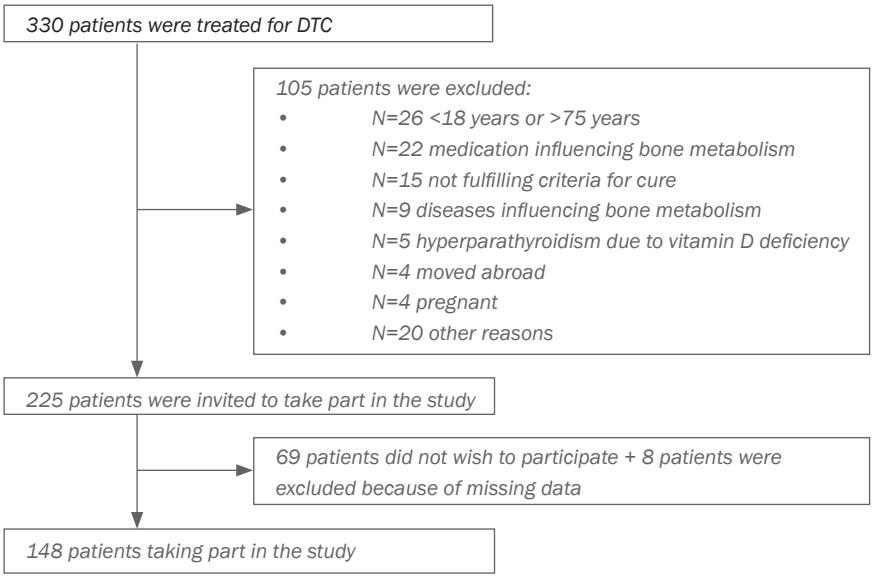
Values are presented as mean ± standard deviation (SD), mean ± SEM, median (range) or as numbers or proportions of patients. Non-normally distributed data (TSH and PTH) were directly log transformed. Factors contributing to BMD and indicators of bone turnover were first identified using univariate regression analysis. During univariate regression analysis, corrected for age, gender and estrogen status (estrogen deplete/replete), the relationship between BMD and the following parameters were analyzed: BMI, smoking, alcohol use, physical activity, duration of follow-up after initial therapy for DTC, total dose of radioiodine received, serum levels of calcium (corrected for an albumin concentration of 42 g/L), 25(OH)vitD, lnPTH, FT4, T3, lnTSH and daily dose of thyroxin. The independent contributions of the TSHR-Asp727Glu polymorphism and serum TSH levels to BMD and indicators of bone turnover were studied by entering FT4, T3, age, gender, estrogen status and all significant covariates at univariate analysis in a multivariate model. Deviation from Hardy-Weinberg Equilibrium was analysed using a X^2-test. All calculations were performed using SPSS 12.0 for windows (SPSS, Inc., Chicago, IL). Differences were considered statistically significant at $P<0.05$

Results

Patient characteristics

Of a potential 330 patients with cured DTC, 105 were excluded for various reasons (Figure 1). Sixty-nine patients did not want or were not able to participate in the study for reasons of time or geographical distance. A total of 156 patients were thus included in the study. Eight patients were left out from analyses because of incomplete data. The basal characteristics of the 148 patients included in the study are shown in Table 1. Thirteen patients had post-surgical hypoparathyroidism for which they were adequately supplemented with active vitamin D metabolites and calcium as required. All patients were receiving L-thyroxin treatment at a mean dose of 182 ± 51 µg/day. The 25th, 50th and 75th percentiles of serum FT4 levels were 19.5, 22.1 and 24.9 pmol/L. A total of 4.3% of patients met the criteria of osteoporosis and 34% of the patients met the criteria for osteopenia (Table 2).

Figure 1 Flowchart of the Study



Serum TSH levels, BMD and indicators of bone turnover

All thyroid related parameters as well as covariates that contributed significantly to BMD and indicators of bone turnover are shown in Table 3. No significant association was observed between serum TSH levels, serum thyroid hormone levels and BMD. However, using univariate analyses, the natural logarithm of serum TSH levels (and the unconverted values) appeared to be significantly inversely correlated to CTx, P1NP, BAP and NTx (Figure 2). The significance was sustained after correction for FT4, T3, gender, age, estrogen status and other significant determinants of bone metabolism using multivariate analysis. The relationship of serum TSH levels with indicators of bone turnover was independent from circulating thyroid hormone levels. Using multivariate analyses no significant or relevant relationship was found between FT4 or T3 and BMD or indicators of bone turnover.

Table 1. Characteristics of Patients

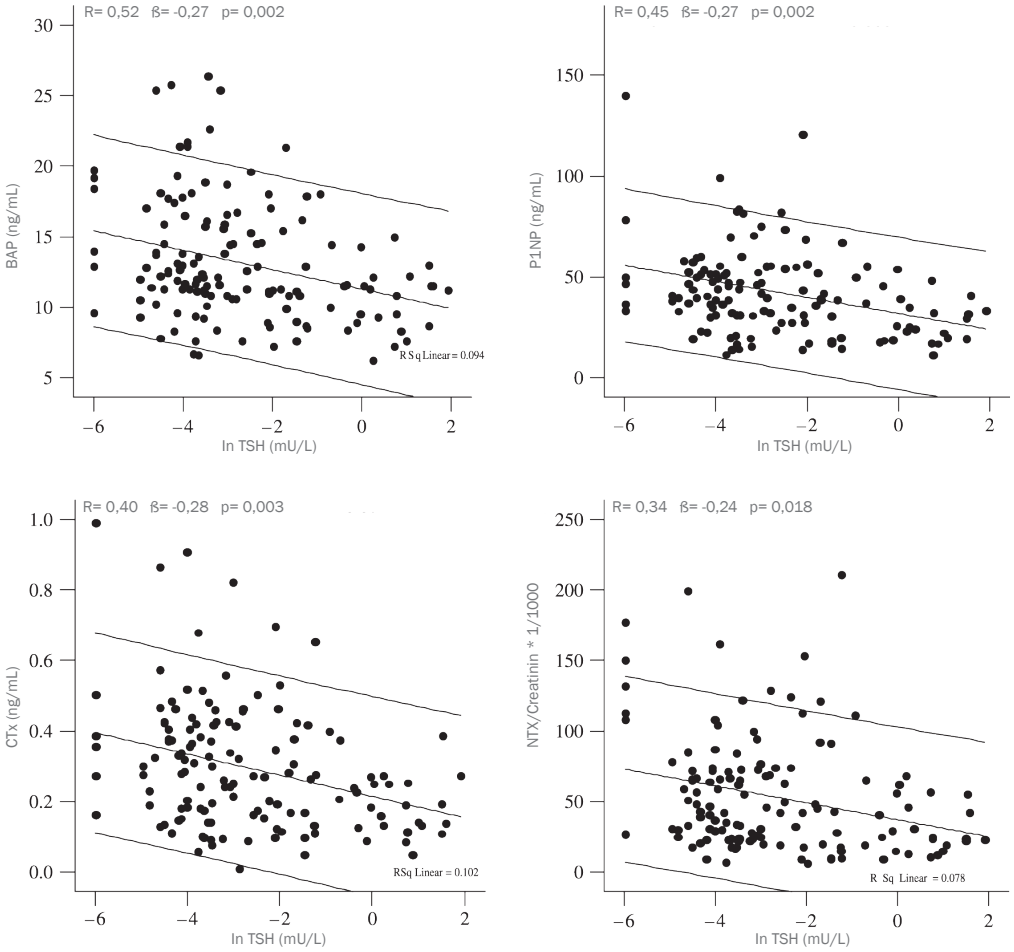
	Total (n=148)
Age (years)	49.4 ± 12.7
Males	27 (18.2%)
Females, premenopausal / postmenopausal	76 (51.4%)/ 45 (30.4%)
Histology	
- Papillary Thyroid Carcinoma	122 (82.4%)
- Follicular Thyroid Carcinoma	26 (17.6%)
pTNM Stage	
- T1-3 N0 M0 / T1-3 N1 M0 / T4 or M1	90 (60.8%)/ 39 (26.4%)/ 19 (12.8%)
Relapse	18 (12.2%)
Follow-up Duration (years)	9.3 (1.2 –43.0)
FT4 (pmol/L)	22.3 ± 4.1
T3 (nmol/L)	1.47 ± 0.34
TSH (mU/L)	0.045 (0.003 – 6.830)
Thyroxin dose / weight (ug/kg)	2.15 ± 0.99
Age menarche women(years)	13.3 ± 1.4
Smoking	18 (12.2 %)
Regular physical activity	77 (52.0%)
BMI (kg/m2)	25.83 ± 4.50

Data are presented as mean ± SD, median (range) or number of patients (percentage).
FT4: Free Thyroxin, T3= Triiodothyronine, TSH= Thyrotropin, BMI: Body Mass Index

The TSHR Asp727Glu polymorphism, BMD and indicators of bone turnover

Genotype frequencies of the TSHR-Asp727Glu polymorphism (Asp/Asp = 131 (88.5%), Asp/Glu = 17 (11.5%) and Glu/Glu = 0 (0.0%)) did not deviate from Hardy Weinberg equilibrium proportions. The Glu727 allele had a frequency of 6.1 %, which is similar to previous studies in Caucasians (26). Patient groups were comparable with respect to age, gender, duration of follow-up, BMI and thyroid hormone parameters. Using univariate analysis, the TSHR-Asp727Glu polymorphism appeared to be a significant determinant of BMD. After correction for age, gender and estrogen status, carriers of the TSHR-Glu^{727} allele had an 8.1% higher femoral neck BMD (p=0.023). When BMI was added as a covariate in the analysis of the relationship of the TSHR Asp727Glu polymorphism with femoral neck BMD, the significance level decreased to p=0.102, the standardized β decreasing from 0.172 to 0.127 (Table 3). There was no significant difference in BMD at the lumbar spine between groups.

Figure 2 Relationship between lnTSH and indicators of bone turnover



No significant differences were observed in indicators of bone turnover between groups (Table 2). The above mentioned relationship between TSH and parameters of bone metabolism was not influenced by the TSHR polymorphism.

Discussion

The purpose of the present study was to identify the relationship between TSH levels, BMD and indicators of bone turnover in 148 thyroidectomized patients. In support for a potential direct effect of TSH on bone, we observed an inverse relationship between serum TSH levels and indicators of bone formation (BAP and P1NP) and bone resorption (CTx and NTx), independent of serum thyroid hormone levels. These results are consistent with a suppressive effect of TSH on bone remodeling and are in keeping with the reported effects of TSH on bone metabolism in animal studies (14). It may well be that the lower range of TSH levels in our patient group, and the more uniform FT4 concentrations (25th and 75th percentiles being 19.5 and 24.9 pmol/L) have allowed to identify this relationship. We noticed no relationship between serum TSH levels and BMD, while we found an inverse relationship between serum TSH levels and indicators of bone turnover.

Table 2. Bone mineral Density and indicators of bone turnover

Bone Mineral Density	Men (n=27)	Women Premenopausal (n=76)	Women Postmenopausal (n=45)	Wild Type TSHR-Asp727glu (n=131)	Heterozygous TSHR-Asp727glu (n=17)
- Femoral neck (g/cm2)	0.90 (0.65-1.25)	0.88 (0.54-1.10)	0.79 (0.56-1.13)	0.86 (0.54-1.25)[1]	0.94 (0.68-1.25)[1]
- z-score	0.58 (-1.64-2.31)	0.51 (-1.95-2.32)	0.98 (-1.67-2.51)	0.55 (-1.95-4.14)[1]	1.13 (-1.18-3.52)[1]
- Lumbar vertebral column* (g/cm^2)	1.14 (0.79-1.37)	1.07 (0.78-1.31)	1.06 (0.67-1.37)	1.06 (0.10-1.74)	1.10 (0.13-1.34)
- z-score	0.84 (-2.31-2.64)	0.68 (-2.07-2.76)	0.96 (-0.77-2.65)	1.25 (-2.31-6.61)	1.38 (-2.07-3.38)
Osteopenia # (%)	33.3	23.0	53.5	33.6	23.5
Osteoporosis# (%)	8.3	1.4	7.0	4.6	0
Calcium (mmol/L)	2.36 ± 0.02	2.40 ± 0.02	2.40 ± 0.02	2.39 ± 0.01 (n=130)	2.35 ± 0.02
25(OH)vitD (nmol/L)	66.04 ± 4.80	63.50 ± 3.57	63.5 ± 3.57	63.92 ± 2.32	65.13 ± 6.68 (n=16)
PTH (pmol/L)	4.36 ± 0.74	5.15 ± 0.46	5.15 ± 0.46	5.38 ± 0.31 (n=129)	3.62 ± 0.52 (n=16)
BAP (ng/mL)	12.96 ± 0.70	14.30 ± 0.78	14.30 ± 0.78	13.33 ± 0.41 (n=116)	12.05 ± 0.64 (n=14)
P1NP (ng/mL)	36.15 ± 3.65	49.88 ± 5.27	49.87 ± 5.27	42.76 ±2.27 (n=116)	40.91 ± 4.46 (n=14)
CTx (mg/mL)	0.25 ± 0.03	0.36 ± 0.04	0.36 ± 0.04	0.29 ± 0.02 (n=116)	0.32 ± 0.04 (n=14)
NTx /creatinine * 1/1000	29.96 ± 7.22	66.44 ± 8.61	66.44 ± 8.61	53.71 ± 3.86 (n=114)	49.86 ± 11.13 (n=14)

Data are presented as median (range), mean ± SEM or percentage.

** Patients with fractures of lumbar vertebrae excluded from BMD measurements of the lumbar spine.*

 # According to WHO criteria.

1 Univariate analysis, $p<0.05$ Corrected for age, gender, estrogen status

PTH: Parathyroid hormone, BAP: Bone Specific Alkaline Phosphatase; P1NP: Procollagen type 1 Aminoterminal Propeptide; CTx: C-crosslinking Terminal Telopeptide of Type I collagen; NTx/Creatinin: Ratio of Urinary N-Telopeptide of Collagen Cross-links and Creatinin Concentration

Table 3. Association between serum TSH levels, BMD and indicators of bone turnover

	Thyroid related Variables			Covariables		
Dependent	Variable	Standardized Beta	p	Co-variable	Standardized Beta	P
BMD-Femoral Neck	FT4	0.063	0.413	BMI #	0.298	<0.001
	T3	-0.039	0.621			
	lnTSH	0.020	0.793			
	$TSHR^{727}Glu$	0.172 (0.127)&	0.023 (0.102)&			
BMD-Lumbar Spine	FT4	0.038	0.635	BMI #	0.160	0.045
	T3	-0.014	0.867			
	lnTSH	-0.026	0.740			
	$TSHR^{727}Glu$	-0.028	0.725			
BAP	FT4	0.148	0.103	BMI	0.177	0.050
	T3	0.251 (0.117)&	0.005 (0.165)&	Ca #	0.351	<0.001
	lnTSH #	-0.303 (-0.273)&	0.001 (0.002)&	lnPTH	0.208	0.021
	$TSHR^{727}Glu$	-0.061	0.509			
P1NP	FT4	0.140	0.112	Ca	0.237	0.006
	T3	0.132	0.131			
	lnTSH #	-0.282 (-0.268)&	0.001 (0.002)&			
	$TSHR^{727}Glu$	0.008	0.926			
CTx	FT4	0.157	0.078			
	T3	0.168 (0.062)&	0.058 (0.481)&			
	lnTSH #	-0.302 (-0.276)&	0.001 (0.003)&			
	$TSHR^{727}Glu$	0.110	0.220			
NTx/Creatinine	FT4	0.234 (0.122)&	0.010 (0.200)&	lnPTH	0.197	0.030
	T3	0.108	0.240			
	lnTSH #	-0.286 (-0.238)&	0.002 (0.018)&			
	$TSHR^{727}Glu$	0.021	0.820			

Univariate regression analysis, all corrected for gender, age and estrogen status. Due to a non-normal distribution, TSH and PTH were transformed by the natural logarithm.

& Values obtained with a multivariate regression model in which covariables that were significant using univariate analysis were included. # Variable with sustained significant association at multivariate analysis.

FT4: Free Thyroxin, T3= Triiodothyronine, TSH= Thyrotropin , BMD: Bone Mineral Density; BAP: Bone Specific Alkaline Phosphatase; P1NP: Procollagen type 1 Aminoterminal Propeptide; CTx: C-crosslinking Terminal Telopeptide of Type I collagen; NTx/Creatinine: Ratio of Urinary N-Telopeptide of Collagen Cross-links and Creatinine Concentration.

This could be due to the fact that the TSH levels Were measured at one occasion. BMD is acquired by a lifelong process, whereas indicators of bone turnover reflect short term biochemical effects. Kim *et al*. and Morris *et al*. found a relationship between not only below normal TSH and BMD, but also between low-normal TSH and BMD (21,31). An explanation for this difference could be that Kim *et al*. and Morris *et al*. excluded patients with thyroid disease whereas we studied DTC patients. It may be objected that TSH measured at one occasion may not reflect the overall suppression of TSH over time. To address this shortcoming, we collected all TSH measurements after initial therapy of the patients who participated in the study, with the exception of stimulated TSH levels. We calculated for each patient the slope of TSH levels, to verify changes over time. An average of 15 TSH measurements per patient were obtained and we calculated a slope of TSH values of -0.0001 (range -0.004-0) mU/L/year, thus indicating stable TSH levels over time.

We also hypothesized that the TSHR-Asp727Glu polymorphism, which is associated with serum TSH, but not FT4, may influence BMD and bone metabolism in humans. Although we found that carriers of the TSHR-Asp727Glu polymorphism had a higher BMD as compared with 131 non-carriers, the relationship in our study was no longer significant after correction for BMI. This may imply that the effect of the polymorphism is explained by BMI, which is also in agreement with the study of van der Deure *et al* (23). An explanation for the difference in outcome of our study and the study of van der Deure *et al*. could be that the number of patients in our study was too small to detect a significant correlation between the polymorphism and BMD. We did not find an association of serum TSH levels with lumbar spine BMD. This might be due to the fact that the BMD measurements of the lumbar spine are influenced by osteoarthritis and therefore cannot be accurately assessed (32).

The mechanisms of TSH effects on bone metabolism have not been fully elucidated. In a provocative study, Abe *et al*. suggested that TSH inhibits osteoclast formation and survival by attenuating JNK/c-jun and NFκB signaling in response to RANK-L and inhibits osteoblast differentiation and type 1 collagen expression as well by downregulating Wnt and VEGF signalling (14). The same group found that TSH directly inhibits Tumour Necrosis Factor-α (TNF-α) production and that TNF-α is the critical cytokine mediating the downstream antiresorptive effects of TSH on the skeleton (33). Other authors suggested that serum TSH activates the type 2 deiodinase in osteoblasts, thereby linking TSH and increased local thyroid hormone availability (15). Our data are in agreement with the notion of a direct effect of TSH on bone, as we found a significantly inverse relationship between serum TSH levels and indicators of bone formation and degradation, which is consistent with an overall suppressive effect on bone turnover. However, 2 recent papers by Bassett *et al*. added to the controversy on the net contribution of TSH to BMD and bone metabolism (19,34). Bassett *et al*. studied mice with complete or haploinsufficiency of TR-α and -β. They found skeletal hypothyroidism and osteosclerosis accompanied by reduced osteoclastic bone resorption in adult mice lacking TR-α, whereas young mice had delayed endochondral ossification, in the presence of normal circulating thyroid hormone and TSH concentrations. Adult mice lacking the TR-β, leading to elevated TSH and thyroid hormone levels, had skeletal hyperthyroidism, with evidence of increased bone resorption. The authors concluded that TR-α regulates both skeletal development and adult bone maintenance, with euthyroid status during development being essential to establish normal adult bone structure and mineralization. In the study of van der Deure *et al*. (23) a stronger effect of FT4 than TSH on BMD was observed, which supports the importance of thyroid hormone effects on bone. In our study, which involved a different patient group in many respects, we did not find a relationship between serum levels of FT4, TSH and BMD, which may well be due to the fact that serum thyroid hormone and TSH levels have changed after the thyroidectomy. Indeed, despite the low serum TSH levels in most patients, overall BMD was within the normal age-corrected range, which is in line with a recent report in DTC patients (35).

It is obvious that the net contribution of TSH in bone development and bone metabolism has not been established yet. We belief that our study may add important human data to the determination of the role of TSH in bone metabolism, since we were able to study the independent relation between serum TSH and bone in thyroidectomized patients.
In summary, we found an independent inverse relationship between serum TSH levels and biochemical indicators of bone turnover, which may point to a functional role of the TSHR in bone in humans. This study documents a direct effect of TSH, independent of thyroid hormone levels on BMD and indicators of bone turnover in humans. Further research into the mechanisms of TSH in bone metabolism needs to be performed.

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06

Thyroid hormone rather than TSH decreases bone turnover during hypothyroidism in athyroid patients with differentiated thyroid carcinoma

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Abstract

Context: Primary hypothyroidism affects bone metabolism. It is not clear whether this has to be attributed to decreased serum thyroid hormone levels per se or to increased TSH levels.
Objective: To document the effects of primary hypothyroidism on bone metabolism and to discriminate between effects mediated by decreased thyroid hormone levels versus those mediated by increased TSH levels.
Design: Prospective study
Setting: University Hospital
Patients: We studied the effects of recombinant human TSH (rhTSH) in 11 athyroid DTC patients on thyroxine substitution. In addition, we included 11 age-, gender- and BMI-matched athyroid patients previously treated for differentiated thyroid carcinoma (DTC), who were studied after 4 weeks of thyroxine withdrawal and during thyroxine replacement therapy.
Main outcome measures: We measured plasma levels of PTH, 25-OH-vitamin D, procollagen type 1 aminoterminal propeptide levels (P1NP), C-cross-linking terminal telopeptide of type I collagen (Ctx), receptor activator for nuclear factor κ B ligand (RANKL) and osteoprotegerin (OPG).
Results: No differences were observed on parameters of bone turnover after rhTSH administration. During thyroxine withdrawal, levels of C-cross-linking terminal telopeptide of type I collagen were significantly lower, whereas levels of osteoprotegerin were significantly higher compared to thyroxine replacement therapy.
Conclusion: Hypothyroidism results in decreased bone turnover. As rhTSH did not impact on bone turnover, it seems that low thyroid hormone levels instead of the increased TSH levels are responsible for the changes in bone turnover during hypothyroidism in DTC patients.

Introduction

The effects of thyroid hormone on bone are established and the conventional view is that hyperthyroidism results in bone loss (1). However, the consequences of hypothyroidism on bone metabolism remain unclear (Table 1). Some studies document low bone turnover as evidenced by decreased markers of bone resorption and formation (2-5), whereas others report normal bone turnover (6-8). Most studies, however, included patients with Hashimoto thyroiditis, in whom the duration of hypothyroidism is not known (3-5;7). Moreover, it is not clear, given the recent suggestion that THS may be a negative regulator of bone remodelling by directly affecting bone independently of thyroid hormone levels (9-11), if the effects of hypothyroidism must be attributed to increased TSH levels or decreased thyroid hormone levels. It has been reported that TSHR knockout and haploinsufficient mice with normal thyroid hormone levels have decreased bone mass, suggesting that TSH might directly influence bone remodeling (10;12;13). However, other studies question the role of TSH in bone metabolism (14;15).

Three studies in humans have investigated the effect of recombinant human TSH (rhTSH) on bone metabolism, but their results were inconclusive by showing either no impact on bone turnover (16), increased markers of bone formation (17;18) or decreased markers of bone resorption (18). In hypothyroidism the relative importance of decreased thyroid hormone levels or increased TSH levels on bone remains thus to be established.

The present study was designed in an attempt at discriminating between potential effects mediated by decreased thyroid hormone levels from those mediated by increased TSH levels in a human model in which the reciprocal relationship between thyroid hormones and TSH was interrupted. To this effect, we studied parameters of bone metabolism after parenteral administration of recombinant human TSH (rhTSH) resulting in exogenously increased TSH levels while preserving normal thyroid hormone levels by uninterrupted thyroid hormone substitution in athyroid differentiated thyroid carcinoma (DTC) patients. We studied the same parameters in age-, gender- and BMI matched athyroid DTC patients during short-term thyroxine withdrawal, resulting in decreased thyroid hormone levels and endogenously increased TSH levels and after reestablishment of thyroid hormone substitution.

Material and methods

Subjects

Patients were recruited from the outpatient clinic of the Department of Endocrinology & Metabolic Diseases of Leiden University Medical Centre, which is a tertiary referral centre for differentiated thyroid carcinoma (DTC). Patients included in the study had a diagnosis of DTC for which they had been treated by near-total thyroidectomy, followed by routine postoperative I-131 radioiodine ablation therapy. Only patients cured of DTC were included, documented by the absence of measurable serum thyroglobulin (Tg) levels during TSH stimulation as well as by negative total-body scintigraphy. Patients with DTC planned for a TSH-stimulated diagnostic protocol were asked to participate in the study. Patients with diabetes mellitus, body mass index (BMI) >35 kg/m^2 or other endocrine diseases were excluded. Patients who used any drugs known to influence bone turnover, such as bisphosphonates, corticosteroids or thiazide diuretics, were also excluded.

The local Ethics Committees of the Leiden University Medical Centre approved the study, and written informed consent was obtained from all subjects.

Table 1. Overview of the literature on the effects of hypothyroidism and rhTSH on parameters of bone turnover

Article	Number of patients	Diagnosis	Control group	Design	Outcome								
					AF	*OC*	*P1NP*	*P1CP*	*OPG*	*Ctx*	*U-DPD*	*U-PD*	*RANKL*
Effects of Hypothyroidism													
Botello-Carretero et al. (2)	19	DTC Thyroxine withdrawal	18 controls	Prospective		↓			↑		↓	↓	
Toivonen et al. (22)	14	DTC Thyroxine withdrawal	38 controls	Prospective		↓	↓	↑		↓			
Sabancu et al.(7)	27 20	Hypothyroidism HT + 3 months T4	5 controls	Cross- sectional	=						=		
Sekeroglu et al. (8)	16	Hypothyroidism (heterogeneous: ineffective surgery/insufficient thyroxine therapy)	15 controls	Cross-sectional	=	=					=		
Nakamura et al. (5)	8	Hypothyroidism (heterogeneous)	-	Prospective							↓	↓	
Guang-Da et al. (3)	20	Hashimoto thyroiditis	20 controls	Prospective					↑				
Nagasaki et al. (4)	53	Hashimoto thyroiditis	53 controls	Prospective					↑				
Effects ofRecombinant human TSH													
Mazziotti et al. (18)	66	DTC + rhTSH	71 controls	Prospective	↑B*				=	↓*			
Giusti et al. (16)	24	DTC + rhTSH	Reference population	Prospective					=		=		=
Martini et al. (17)	30	DTC + rhTSH	80 controls	Prospective			↑*		=	=			↓**

ALP=Alkaline Phosphatase, OC=osteocalcin, P1NP= procollagen type 1 aminoterminal propeptide, P1CP= procollagen type 1 carboxyterminal, OPG=osteoprotegerin (inhibits bone resorption), CTx= C-terminal telopeptide of collagen I, U-PD= Urinary Pyridinium crosslinks, U-DPD= Urinary excretion of Deoxypyridinoline, RANKL= receptor activator nuclear factor κB ligand

*[B] =Bone specific Alkaline Phosphatase, * = in postmenopausal women, **= in men and postmenopausal women*

Two groups matched for age, gender and BMI were studied. The first group consisted of 11 athyroid DTC patients who were receiving uninterrupted thyroxine replacement therapy and were undertaking a TSH stimulation test in the course of monitoring disease state by receiving injections of rhTSH. This resulted in exogenously increased TSH levels with unchanged normal FT4 levels (rhTSH group). The second group also consisted of 11 athyroid DTC patients with short-term thyroxine withdrawal resulting in decreased FT4 levels and endogenously increased TSH levels (thyroxine withdrawal group).

Study Design

Patients in the rhTSH group continued to receive thyroxine substitution and were evaluated prior to receiving recombinant human TSH (Thyrogen, 0.9 mg) which was injected intramuscularly once daily for two consecutive days and patients were also evaluated 1 and 3 days after the last injection of rhTSH.

Patients in the thyroxine withdrawal group were evaluated four weeks after withdrawal of thyroxine substitution and again 8 weeks after restarting thyroxine replacement therapy.

All patients were assessed at 8.00 hr after a 12 hour fast. Height (meters [m]) and weight (kilograms [kg]) were measured and BMI (weight [kg]/lenght^2 [m]) was calculated. Plasma samples were obtained for measurement of FT4, TSH, T3, PTH, 25-OH-vitamin D, procollagen type 1 aminoterminal propeptide levels (P1NP), C-cross-linking terminal telopeptide of type I collagen (Ctx), receptor activator for nuclear factor κ B ligand (RANKL) and osteoprotegerin (OPG). Plasma samples were handled immediately and stored at –20o C in Sarstedt tubes.

Biochemical parameters

All plasma and serum samples were measured in one batch. Serum free thyroxine (FT4) and TSH were measured using an electrochemiluminescent immunoassay with a Modular Analytics E-170 system with an intra-assay CV of 1.6-2.2 % and 1.3-5.0 % respectively (Roche Diagnostics, Almere, The Netherlands). Serum T3 was measured using a fluorescent polarisation immunoassay on an AxSYM system (Abbott, Abbott Park, IL, USA CV 2.5-9.0 %). Plasma Parathyroid Hormone (PTH) was measured by an immunoradiometric assay (Nichols Diagnostic Institutes, Wijchen, The Netherlands), calcium and alkaline phosphatase activity by colorimetry on a fully automated Modular P800 system (Roche, Almere, The Netherlands) and 25(OH) vitamin D by RIA (Incstar/DiaSorin, Stillwater, MN, USA). CTx and P1NP were measured by electrochemiluminescent immunoassays using a Modular Analytics E-170 system (Roche Diagnostics, Almere, The Netherlands). RANKL was measured using the ampli sRANKL human kit (Biomedica, Vienna, Austria), an enzyme linked immunoassay with a detection limit of 0.02 pmol/l (intra-assay CV 8-9%, interassay CV 3-6%). All samples were measured in triplo in single batches for the levels of RANKL and osteoprotegerin. Osteoprotegerin was measured by ELISA (Meso Scale Discovery, Gaithersburg, Maryland, USA) with a detection limit of 5.9 pg/ml. In our hands, the range was 206 to 404 pg/ml; CVs were 0.6-16.2%, with an average of 4.6%.

Statistical Analyses

SPSS 12.0 for windows was used for statistical analyses (SPSS. Inc., Chicago, IL, USA). Values are expressed as mean ± SE. Data within subjects were analysed with the paired samples t-test or the ANOVA for repeated measures. Data between subjects were measured with the Mann-Whitney test. Differences were considered statistically significant at $P<0.05$.

Results

Patient demographic characteristics are shown in Table 2. Patients in the rhTSH-group and thyroxine withdrawal-group were well matched and there were no differences in age, gender, BMI, thyroxine dose or duration of follow-up between groups.

Table 2. Patient characteristics

	Thyroxine withdrawal-study (n=11)	rhTSH stimulation study (n = 11)	P-value
Age (years)	45.5 ± 3.0	47.0 ± 2.8	0.65
Sex (m/f)	4 : 7	4:7	0.67
BMI (kg/m^2)	28.1 ± 1.3	29.7 ± 2.6	0.75
Thyroxine dose (µg/day)	197 ± 13	200 ± 12	0.70
Duration of TSH suppressing treatment (years, (range))	5.0 ± 2.1 (0.6-24.3)	6.7 ± 2.4 (1.2 -25.3)	0.33

Data are expressed as mean ± SE (range) or number of patients

Eleven patients (4 male and 7 female patients) were included in the rhTSH-group. Mean thyroxine dose at time of the evaluation was 200 ± 12 µg/day. TSH levels were significantly increased without any changes in FT4 levels 1 and 3 days after rhTSH was administered (Table 4).

There were no differences in the levels of calcium, PTH, 25-OH-vitamin D, alkaline phosphatase activity, P1NP, Ctx, OPG, RANKL and in the RANKL/OPG ratio between baseline and time points after rhTSH administration.

Eleven patients (4 male and 7 female patients) were included in the thyroxine-withdrawal-group. Mean thyroxine dose prior to withdrawal was 197 ± 13 µg/day. Four weeks after thyroxine withdrawal, TSH levels were significantly increased at 142.4 ± 10.4 mU/L (normal laboratory reference range 0.3-4.8 mU/L) and FT4 levels were significantly decreased at 1.4 ± 0.2 pmol/L (normal laboratory reference range 10-24 pmol/L). Eight weeks after restarting thyroxine replacement therapy, six patients had TSH levels within the normal laboratory reference range and five patients had suppressed TSH levels.

There were no significant differences in levels of calcium, PTH, 25-OH-vitamin D, alkaline phosphatase activity, P1NP, RANKL and the RANKL/OPG ratio between thyroxine withdrawal status and 8 weeks after reintroduction of thyroxine replacement therapy (Table 3). Serum concentrations of Ctx were significantly lower and OPG levels significantly higher during hypothyroidism compared to 8 weeks after reintroduction of thyroxine replacement therapy. There was no significant difference between endogenously and exogenously increased TSH levels respectively obtained 4 weeks after thyroxine withdrawal and 1 day after rhTSH-administration. As expected, FT4 levels were significantly decreased during thyroxine withdrawal compared to the normal levels attained by thyroxine substitution therapy 1 and 3 days after rhTSH administration.

The differences in Ctx levels and OPG levels were significantly different between the thyroxine withdrawal group and rhTSH group. Calcium levels were significantly lower during hypothyroidism compared to rhTSH administration. There were no significant differences observed in any other parameters measured between the groups.

Table 3. Effects of hypothyroidism and rhTSH injections in 11 matched athyroid patients on parameters of bone turnover

	Thyroxine replacement therapy	Hypothyroidism	P-value[$]	Thyroxine replacement therapy	rhTSH day 1	rhTSH day 3	P-value[@]	P-value difference hypothyroidism vs difference rhTSH
TSH (Mu/L)	0.8 ± 0.3[&]	142.4 ± 10.4[#]	0.00	0.06 ± 0.2	143.4 ± 13.6	19.3 ± 2.5	0.00	0.90
FT4 (pmol/L)	*24.8 ± 1.2*	*1.4 ± 0.2# **	*0.00*	*23.4 ± 0.8*	*24.0 ± 0.9*	*24.3 ± 1.0*	*0.13*	*0.00*
T3 (pmol/L)	1.3 ± 0.1	0.3 ± 0.1	0.00	1.8 ± 0.1	1.8 ± 0.1	1.8 ± 0.1	0.25	0.00
Parameters of Bone turnover								
Calcium (mmol/L)	2.20 ± 0.02	2.18 ±0.03[*]	0.68	2.25 ± 0.04	2.28 ± 0.3	2.21 ± 0.06	0.17	0.43
PTH (pmol/L)	3.4 ± 0.5	4.0 ± 0.6	0.13	3.7 ± 0.6	3.5 ± 0.6	3.4 ± 0.6	0.55	0.12
25 (OH)Vit D (nmol/L)	59 ± 6	57 ± 6	0.52	68 ± 8	67 ± 8	65 ± 9	0.27	1.00
P1NP (ng/ml)	28 ± 5	29 ± 6	0.27	38 ± 4	36 ± 4	37 ± 4	0.18	0.28
Ctx (mg/ml)	0.28 ± 0.5	0.24 ± 0.4	0.00	0.33 ± 0.06	0.35 ± 0.06	0.33 ± 0.06	0.23	0.00
OPG (pg/ml)	193 ± 17	246 ± 22	0.00	174.4 ± 11.8	210.4 ± 21.9	198.7 ± 16.5	0.47	0.01
RANKL (pg/ml)	1.1 ± 0.3	1.1 ± 0.3	0.59	1.1 ± 0.3	1.1 ±0.3	1.0 ± 0.3	0.27	1.00
RANKL/OPG ratio	0.006 ± 0.002	0.005 ± 0.002	0.61	0.006 ± 0.002	0.006 ± 0.002	0.006 ± 0.002	0.74	0.44
Alk. Phosphates	66 ± 5	66 ± 5	0.81	76 ± 8	77 ± 8	75 ± 7	0.60	0.70

Data is expresses as mean ± SD. $ Paired samples t-test. @ ANOVA for repeated measurements

[*] Significantly different vs. rhTSH day 1

[#] Significantly different vs. rhTSH day 3

[&] Significantly different vs. rhTSH thyroxine replacement therapy.

Discussion

In this study, we have attempted to dissect the effects of increased TSH levels from those of decreased thyroid hormone levels on bone by studying athyroid DTC patients in which the relationship between thyroid hormone levels and TSH is disrupted. Our findings suggest that acute increases in TSH in the presence of stable thyroid hormone levels obtained by rhTSH administration do not significantly affect skeletal metabolism. The data from our model suggest that hypothyroidism results in decreased bone turnover rather by decreased plasma thyroid hormone concentrations than by increased TSH concentrations, because rhTSH did not impact on bone turnover in DTC patients. To our knowledge, this is the first study comparing rhTSH-injection versus thyroxine withdrawal in age-, gender- and BMI matched DTC patients.

It has been proposed that TSH may modulate bone remodelling independently of thyroid hormones through binding to the TSH receptor on osteoblasts and osteoclasts. (10). However, other studies question these findings. 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, trabecular bone remodeling defects and reduced bone mineralization, indicating that the effects of congenital hypothyroidism on bone are independent of TSH (15). Moreover, Bassett *et al*. showed that osteoblasts and osteoclasts express TSH-receptors, but TSH did not affect a cAMP response or the differentiation or function (15). We used the model of thyroidectomised DTC patients in whom a rhTSH simulation test was performed in an attempt to discriminate the effects of TSH from those of FT4 on bone metabolism. These patients have no endogenous thyroid hormone production and are therefore an excellent model to study the effects of TSH without interfering effects of changes in thyroid hormone concentrations. However, rhTSH did not affect bone turnover. This is in keeping with a study using the same model (16), but ad odds with two others studies (17;18). Mazzioti *et al*. found significantly increased levels of bone specific alkaline phosphatase with decreased levels of cross-linking terminal telopeptide of type I collagen in postmenopausal women after rhTSH administration (18). They found no changes in premenopausal women. Martini *et al*. found significantly increased levels of P1NP and RANKL after rhTSH administration (17). These differences were only significant in postmenopausal women for P1NP levels and in postmenopausal women and men for RANKL levels after stratification for gender and menopausal state. We studied only 2 postmenopausal women. This might explain the differences in outcome. We found no differences in OPG levels, which is consistent with previous studies (16-18) and in agreement with the finding that TSH regulates bone turnover by different mechanisms than OPG (10;19). Osteoprotegerin, a member of the TNF receptor superfamily, inhibits osteoclastogenesis by interrupting the cell-to-cell interaction (20;21).

In the thyroxin withdrawal group, levels of C-cross linking terminal telopeptide of type 1 collagen were lower during hypothyroidism compared to 8 weeks after reinstitution of thyroxine replacement therapy. This is consistent with most reports on hypothyroidism (2;5;22), although Sabancu *et al*. reported no differences in markers of bone turnover during hypothyroidism in a heterogeneous patient population including patients with Hashimoto thyroiditis (7). A disadvantage of the inclusion of patients with Hashimoto thyroiditis may be that the duration and extent of hypothyroidism are not known. OPG levels were significant higher during hypothyroidism compared to thyroxine replacement therapy. This is consistent with previous studies (2-4) and strengthens our finding that thyroxine withdrawal decreases bone turnover.

In summary, bone turnover is decreased during hypothyroidism due to thyroxine withdrawal in DTC patients. As rhTSH did not impact on bone turnover, we conclude that the low thyroid hormone levels instead of the increased TSH levels are responsible for the decreased bone resorption during hypothyroidism in DTC patients.

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07

The Type 2 Deiodinase Thr92Ala Polymorphism is Associated with Increased Bone Turnover and Decreased Femoral Neck Bone Mineral Density

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Abstract

Background: The role of type 2 deiodinase (D2) in the human skeleton remains unclear. The D2 polymorphism Thr92Ala has been associated with lower enzymatic activity, which could result in lower local T3 availability in bone.
Aims: We hypothesized that the D2 Thr92Ala polymorphism may influence bone mineral density (BMD) and bone turnover.
Patients: We studied 154 patients (29 men, 125 women: 79 estrogen replete, 46 estrogen deficient) with cured differentiated thyroid carcinoma.
Methods: BMD and bone turnover markers (bone specific alkaline phosphatase (BAP), C-crosslinking terminal telopeptide of type I collagen (CTx), procollagen type 1 aminoterminal propeptide (P1NP) and N-telopeptide of collagen cross-links (NTx) were measured. Effects of the D2 Thr92Ala polymorphism on BMD and bone turnover markers were assessed by a linear regression model, with age, gender, estrogen state, BMI, serum calcium, 25-hydroxy vitamin D, PTH, TSH and free T4 as covariables.
Results: 60 patients were wildtype (Thr/Thr), 66 heterozygous (Thr/Ala) and 28 homozygous (Ala/Ala) for the D2 polymorphism. There were no significant differences in any covariables between the 3 genotypes. Corrected BMD of the femoral neck was 6% lower in homozygotes than in wild-type subjects ($p=0.028$). Serum P1NP, CTx and urinary NTx/creat were 27%, 32% and 54% higher in homozygotes than in wildtype patients ($p<0.05$).
Conclusion: In patients with cured DTC, the D2 Thr92Ala polymorphism is associated with a decreased femoral neck BMD and higher bone turnover, independently of serum thyroid hormone levels, which points to a potential functional role for D2 in bone.

Introduction

The involvement of thyroid hormone in bone metabolism has been well documented clinically, ranging from decreased skeletal development in childhood hypothyroidism (1-4), accelerated growth in childhood hyperthyroidism (5) to an increased risk for osteoporosis in overt and subclinical hyperthyroidism (6-14).

Although clinical observations suggest a clear involvement of thyroid hormone in bone metabolism, the molecular mechanisms by which thyroid hormone acts on bone is have so far only been partially uncovered. T3 promotes osteoblastic proliferation, differentiation and apoptosis and, by induction of IL-6, prostaglandins and RANKL, probably also promotes osteoclast formation and activation. This suggests that osteoblasts are the primary target cells for T3 in the regulation of bone remodeling (1,2,15-18). A functional role of thyrotropin (TSH) on skeletal development and metabolism has been proposed on the basis of data obtained in animal studies (19-25) and in humans (26-28). This was however disputed by data obtained in thyroid hormone receptor (TR) deficient mice, which indicated that bone remodeling was predominantly mediated by T3 via TRalpha (29,30). It has also recently been reported that in humans there is a significant association between BMD and serum thyroid hormone concentrations than TSH (31).

Most actions of thyroid hormone are mediated by the active form of thyroid hormone, T3. Circulating and local T3 concentrations are mainly regulated by the iodothyronine deiodinases D1, D2 and D3 (32). D2 is essential for the local production of T3 through deiodination of T4. Although earlier studies on the role and functional expression of iodothyronine deiodinase enzymes in the skeleton have been equivocal (18,21,33-36), a recent study reported normal growth in mice with deficiencies in D1 and D2 indicating that D2 may not be critical in skeletal development (37). This notion was supported in a recent study which demonstrated that D2 activity is restricted to mature osteoblasts, suggesting a possible role for D2 in mature osteoblast function (38). Devising a study addressing the potential role of deiodinases, including D2 on skeletal metabolism is difficult in humans, but the study of the effects of functional D2 polymorphisms on BMD and bone turnover in humans may shed light on this role.

Several polymorphisms in D2 have been described (39-41). The single-nucleotide polymorphism D2 Thr92Ala polymorphism has been associated with BMI and insulin resistance in subjects with obesity and type 2 diabetes mellitus (39.40), although this was not confirmed in the Framingham offspring study (42). In the study of Canani *et al* (39), the maximal velocity of D2 was decreased by 3–10-fold in thyroid and skeletal muscle of carriers of the Thr92Ala polymorphism. This effect was observed in the absence of differences in D2 mRNA level or in the biochemical protein properties of the 92Ala allele. It was, therefore, suggested that either a functionally relevant single nucleotide polymorphism occurs in linkage disequilibrium the Thr92Ala polymorphism or the 92Ala allele affects protein translation or stability.

The objective of our study was to try and elucidate a potential role for D2 in skeletal metabolism and BMD by evaluating the relationship between the D2 Thr92Ala polymorphism, BMD and bone turnover markers in cured thyroidectomized differentiated thyroid carcinoma patients receiving thyroid hormone substitution. This human model has the advantage having strictly regulated serum thyroid hormone levels which are kept in a relatively narrow range.

Patients and Methods

Patients included in the study were all under control of the outpatient clinic of the Department of Endocrinology of the Leiden University Medical Center. All patients had a diagnosis of DTC, for which they had been treated by near-total thyroidectomy, followed by standard postoperative I-131 radioiodine ablation therapy. All patients were cured as defined by the absence of I-131 accumulation at diagnostic scintigraphy, serum thyroglobulin (Tg) concentrations below 2 µg/L after TSH stimulation, the absence of Tg antibodies, a normal neck ultrasound and no other indication for disease (43). Patients with tumour relapse were only included if they were subsequently cured. None of the patients used any drug or had a disease known to influence bone metabolism. The Leiden University Medical Center Local Ethics Committees approved the study, and written informed consent was obtained from all subjects.

Study design

On the day of the study, patients had a full clinical examination, including, height (meters [m]) and weight (kilograms [kg]). Blood was collected after an overnight fast, and measured for TSH, FT4, triiodothyronine (T3), calcium, parathyroid hormone (PTH), 25-hydroxy-vitamin D (25(OH)vitD), bone specific alkaline phosphatase (BAP), C-crosslinking terminal telopeptide of type I collagen (CTx) and procollagen type 1 aminoterminal propeptide (P1NP). Second morning void urine was measured for excretion of N-telopeptide of collagen cross-links (NTx). Plasma, serum and urine samples were handled immediately and stored at –80o C in Sarstedt tubes. BMD (expressed in grams per square centimeter) was measured at the femoral neck and the lumbar spine (vertebrae L2-L4) by dual energy x-ray absorptiometry (NHANES III adjusted, Hologic 4500, Hologic Inc., Bedford, MA, USA). Following WHO criteria, osteopenia was defined as a T-score between –1 and –2.5 and osteoporosis as a T-score below –2.5. The following data were additionally recorded: smoking habits, alcohol use, physical activity, calcium intake, medication (including self-prescription drugs) or vitamin or mineral supplements and daily calcium intake and for females: date of first menstruation (menarche), date of last menstruation, cycle regularity and estrogen substitution if applicable.

Biochemical parameters

Serum free T4 (FT4) and TSH were measured using a chemoluminescence immunoassay with a Modular Analytics E-170 system (intraassay CV of 1.6-2.2 % and 1.3-5.0 % respectively (Roche, Almere, The Netherlands). Serum T3 was measured with a fluorescence polarization immunoassay, CV 2.5-9.0 %, on an ImX system (Abbott, Abbott Park, IL, USA). Thyroglobulin was measured by Dynotest TG-s (Brahms Diagnostica GmbH, Germany). Plasma PTH was measured using an immunoradiometric assay (Nichols Diagnostic Institutes, Wijchen, The Netherlands). Calcium was measured by colorimetry and 25(OH)vitD by RIA (Incstar/ DiaSorin, Stillwater, MN, USA). Serum BAP was measured by RIA (Hybritech Europe, Liege, Belgium). Serum CTx and P1NP were measured by chemoluminescence immunoassay using the Modular Analytics E-170 system (Roche Diagnostics, Almere, The Netherlands). NTx was measured by ELISA (Ostex International Inc., Seattle, WA, USA). NTx was expressed as the ratio between NTx and urine creatinine excretion (NTx/creatinine) to correct for differences in creatinine excretion. Insulin sensitivity was estimated by homeostasis model assessment [HOMA _ fasting insulin (milliunits per milliliter) _ fasting glucose (millimoles per liter)/22.5.

Genetic analyses

DNA was isolated from peripheral leucocytes by the salting out procedure. Genotypes were determined using 5 ng genomic DNA by a 5' fluoregenic Taqman assay and reactions were performed in 384-wells format on ABI9700 2x384well PCR machines with endpoint reading on the ABI 7900HT TaqMan® machine (Applied Biosystems, Nieuwerkerk aan den Ijssel, The Netherlands). Primer and probe sequences were optimized using the single nucleotide polymorphism assay-by-design service of Applied Biosystems.

Statistical Analyses

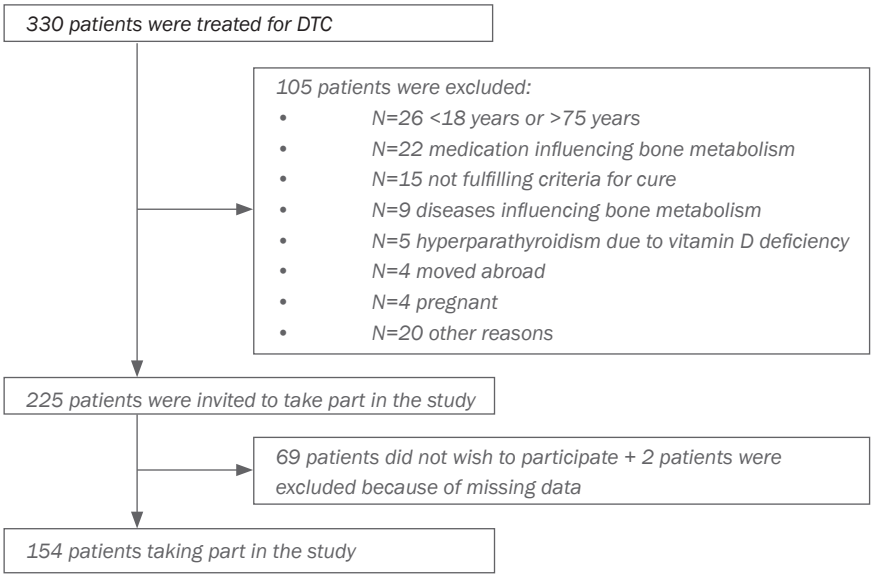
Values are presented as mean ± standard error (SE), median (range) or as numbers or proportions of patients. Non-normally distributed data (TSH and PTH) were log transformed before analyses. Comparisons between groups were analyzed by Anova or Chi-square tests. The relation between the 3 D2 Thr92Ala genotypes (Thr/Thr (wild-type); Thr/Ala (heterozygote) and Ala/Ala (homozygote)), BMD and markers of bone turnover was studied by a stepwise univariate regression analysis. After correction for age, gender and estrogen status (estrogen deplete or replete), the following co-variables were entered: BMI, serum levels of calcium (corrected for an albumin concentration of 42 g/L), 25(OH)vitD, lnPTH, FT4, T3 and lnTSH. Because it has been documented that the D2 Thr92Ala polymorphism is associated with insulin resistance (39), we also compared insulin sensitivity (HOMA) in the 3 genotypes. Deviation from Hardy-Weinberg Equilibrium was analysed using a X2-test. All calculations were performed using SPSS 12.0 for windows (SPSS, Inc., Chicago, IL). Differences were considered statistically significant at $P<0.05$

Results

Patient characteristics

Of a potential of 330 patients with cured DTC, 105 were excluded for various reasons (Figure 1). Sixty-nine patients were not willing or able to participate in the study for different reasons.

Figure 1 Flowchart of the Study



A total of 156 patients were thus included in the study. Two patients were left out from analyses because of incomplete data. Thirteen patients had post-surgical hypoparathyroidism for which they were adequately supplemented with active vitamin D metabolites and calcium as required. Additional analyses were performed leaving out these patients (see below and Tables 2 and 3). In addition, serum PTH levels were included as covariable in the analyses (see below), to correct for potentially confounding effects of hypoparathyroidism. The basal characteristics of the 154 patients included in the study are shown in Table 1. All patients were receiving L-thyroxine treatment at a mean dose of 183 ± 4 µg/day.

Table 1. Characteristics of Patients

	Total (n=154)
Age (years)	49.2 ± 1.0
Males Females: Estrogen Replete / Deplete	29 (18.8 %) 79 (51.3 %) / 46 (29.9 %)
Age at diagnosis	36.6 ± 1.1
Histology	
• Papillary Thyroid Carcinoma (PTC)	107 (69 %)
• Follicular Thyroid Carcinoma	25 (16 %)
• Follicular variant PTC	21 (14 %)
• Hurthle cell Thyroid Carcinoma	1 (1 %)
Total Activity Radioiodine	8067 ± 699 MBq
Lymph Node Surgery	14 (9 %)
pTNM Stage	
• T1-3 N0 M0	90 (63 %)
• T1-3 N1 M0	30 (21%)
• T4 or M1 n=143	23 (16 %)
Relapse DTC	20 (13 %)

(all were cured after relapse)

The D2 Thr92Ala polymorphism, BMD and biochemical parameters of skeletal metabolism
The characteristics of the 3 genotype subgroups are given in Table 2. Genotype frequencies of the D2 Thr92Ala polymorphism (Thr/Thr = 60 (39 %), Thr//Ala = 66 (43 %) and Ala/Ala = 28 (18 %)) did not deviate from Hardy Weinberg equilibrium proportions. The Ala92 allele had a frequency of 45%, which is similar to previous studies in Caucasians (42,44). The characteristics of the 3 genotype subgroups are given in Table 2. The 3 groups were comparable with respect to age, gender, estrogen state (including ages at menarche and menopause) and BMI. Physical activity and smoking habits did not differ either. Biochemical covariables for bone metabolism (serum calcium, 25OHvitD and PTH) were not different as were serum free T4 and T3 levels, serum T3/T4 ratio and TSH levels. Because it has been documented that the D2 Thr92Ala polymorphism is associated with insulin resistance (39), we also compared insulin sensitivity by HOMA in the 3 genotypes, which again did not differ (p=0.361). We also calculated whether HOMA was a significant determinant of BMD and of biochemical parameters of skeletal metabolism (corrected for age, gender, estrogen state and BMI). Univariate analyses revealed that p values for HOMA as an independent variable were respectively 0.912 for femoral neck BMD, 0.583 for lumbar vertebral BMD, 0.826 for

NTX / creatinine, 0.575 for BAP, 0.798 for P1NP and 0.906 for CTx. HOMA was therefore not a determinant of BMD or bone turnover markers.
The relation between the 3 D2 Thr92Ala genotypes, BMD and biochemical parameters of skeletal metabolism were studied by a stepwise univariate regression analysis. After correction for age, gender, estrogen status and BMI, the following co-variables were subsequently entered: serum levels of calcium, 25(OH)vitD, lnPTH, FT4 and lnTSH (Table 3).

Table 2. Characteristics of Patients by the D2-Thr92Ala genotype

	Thr / Thr (60)	Thr / Ala (66)	Ala / Ala (28)	P
Men (n)	13	11	5	0.861 Chi-square
Women (n) Estrogen Replete / Deplete	32 / 15	33 / 22	14 / 9	
Age (years)	47.2 ± 1.6	51.2 ± 1.7	48.3 ± 1.9	0.148 A
Height (m)	1.72 ±0.01	1.70 ± 0.01	1.71 ± 0.02	0.307 A
BMI (kg/m2)	25.6 ± 0.6	26.2 ± 0.4	25.8 ± 1.1	0.773 A
Sports (hrs/week)	3.1 ± 1.1	5.0 ± 1.6	4.5 ± 2.3	0.654 A
Smoking (n)	12 (9%)	7 (5%)	5 (1%)	0.092 Chi-square
Menarche (age)	13.4 ± 0.2	13.1 ± 0.2	13.6 ± 0.3	0.399 A
Menopause (age)	48.2 ± 1.5	47.7 ± 1.1	50.1 ± 1.5	0.484 A
Follow-up duration (years)	13.1 ± 1.2	10.5 ± 1.0	11.3 ± 1.5	0.241 A
Hypoparathyroidism (n)	5 (3%)	6 (4%)	2 (1%)	0.952 Chi-square, #
Vertebral fractures (n)	1 (1%)	2 (1%)	1 (1%)	0.832 Chi-square
HOMA (mmol*22.5/L)	1.75 ± 0.20	2.16 ± 0.21	1.86 ± 0.32	0.361 A
Calcium (mmol/L)	2.39 ± 0.02	2.38 ± 0.01	2.39 ± 0.02	0.943 A
25 OH vitD (nmol/L)	64.5 ± 3.9	60.4 ± 2.9	69.9 ± 4.8	0.277 A
PTH (pmol/L)	4.88 ± 0.36	5.27 ± 0.43	6.19 ± 0.83	0.250 A
TSH (mU/L)	0.051 (0.003-4.620)	0.031 (0.003-4.910)	0.051 (0.003-6.830)	0.753 A
Dose thyroxine (ug/kg)	2.09 ± 1.04	2.23 ± 0.87	2.19 ± 1.03	0.398 A
Free T4 (pmol/L)	22.7 ± 0.1	22.4 ± 0.1	21.6 ± 0.2	0.562 A
T3 (nmol/L)	1.49 ± 0.04	1.47 ± 0.05	1.40 ± 0.07	0.624 A
T3/T4 ratio * 10	6.6 ± 0.2	6.7 ± 0.2	6.6 ± 0.4	0.903 A
BMD femoral neck (g cm^2)	0.90 ± 0.02 #&	0.84 ± 0.01	0.85 ± 0.03	0.028 (0.015) 1
BMD lumbar spine	1.08 ± 0.03	1.04 ± 0.02	1.07 ± 0.04	0.741 (0.094) 1
NTX / Creatinine * 1/1000	44.0 ± 4.1 #	56.5 ± 5.8	67.7 ± 10.6	0.008 (0.002) 1
BAP (ng/mL)	12.5 ± 0.5	13.5 ± 0.6	13.9 ± 0.7	0.063 (0.085) 1
P1NP (ng/mL)	40.0 ± 2.6 #	42.9 ± 3.4	50.9 ± 5.5	0.028 (0.032) 1
CTx (mg/mL)	0.28 ± 0.02 #	0.28 ± 0.02 #	0.37 ± 0.05	0.043 (0.036) 1

Values are presented as mean ± standard error (SE), median (range) or as numbers or proportions of patients. PTH: Parathyroid hormone, BAP: Bone Specific Alkaline Phosphatase; P1NP: Procollagen type 1 Aminoterminal Propeptide; CTx: C-crosslinking Terminal Telopeptide of Type I collagen; NTx/Creatinin: Ratio of Urinary N-Telopeptide of Collagen Cross-links and Creatinin Concentration; A=One-way ANOVA; 1 general linear model, univariate with age, gender, estrogen state, BMI, Ca, lnPTH, 25-OHvitD, lnTSH and Free T4 as covariables; values between brackets: postoperative hypoparathyroidism left out # p<0.05 vs. homozygotes; & p<0.05 vs. heterozygotes

Table 3. Stepwise linear regression for the relationships of the D2-Thr92Ala genotype with bone parameters

Step	Covariables	BMD Femoral neck		BMD Lumbar spine		NTX / Creatinine		BAP		P1NP		CTx	
		B[#]	p	B	p	B	p	B	p	B	p	B	p
1	*D2Thr + Age, Gender, estrogen state, BMI Calcium, 25-OH-vitamin D InPTH*	*-0.168*	*0.021*	*NA*	*0.680*	*0.185*	*0.028*	*NA*	*0.051*	*0.183*	*0.026*	*0.177*	*0.037*
	Without postOK hypoPTH [@]	*-0.195*	*0.010*		*0.073*	*0.269*	*0.002*		*0.064*	*0.190*	*0.026*	*0.196*	*0.029*
2	Step 1 + Free T4 lnTSH/ +lnPTH/	-0.161	0.028	NA	0.741	0.218	0.008	NA	0.063	0.173	0.028	0.165	0.043
	Without postOK hypoPTH	-0.185	0.015		0.094	0.260	0.002		0.085	0.176	0.032	0.177	0.036

[#] *B: regression coefficient (Wild-type as reference (first level), Heterozygote as second level, Homozygote as third level).*

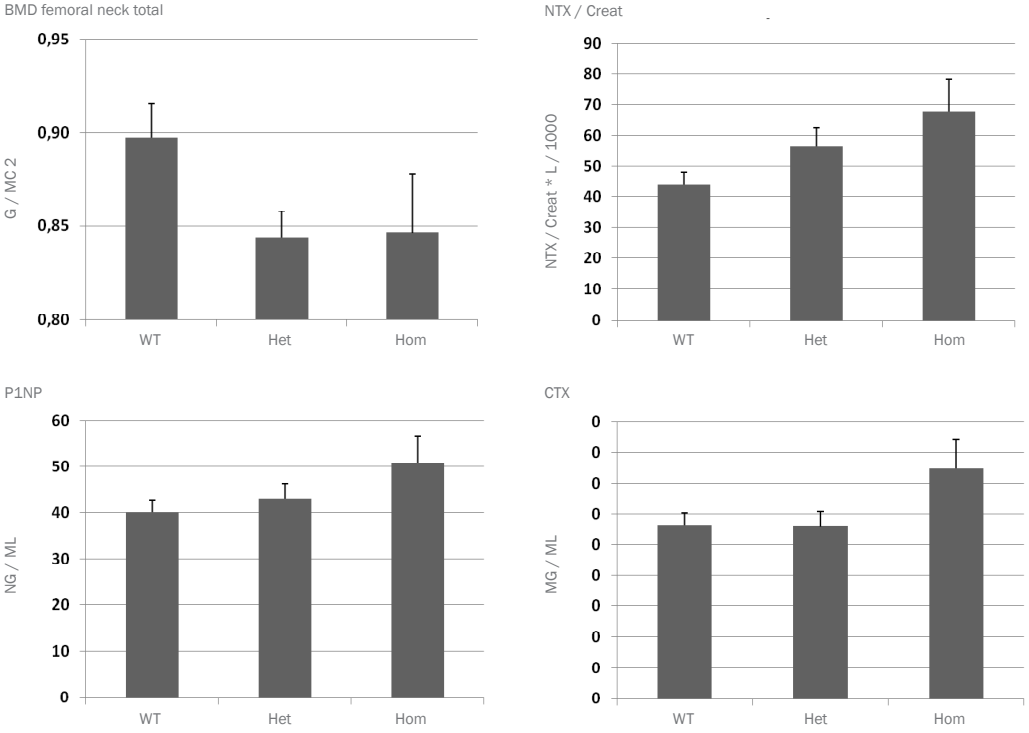
[@] *Post-surgical hypoparathyroidism*

We found a significant independent relationship between the Thr92Ala genotypes and femoral neck BMD (p=0.022) with a 6% lower BMD in homozygotes than in wild-type patients. We also found independent relationships between the D2 Thr92Ala genotypes and biochemical parameters of skeletal metabolism: P1NP (p=0.028), CTx (p=0.043) and NTX / creatinine (p=0.008), which were higher in homozygotes than in wild-type patients. Data for analyses leaving out patients with post-surgical hypoparathyroidism did not influence these results (Tables 2 and 3). The largest difference was observed for NTX / creatinine, being 54% higher in homozygotes than in wild-types.

Discussion

The main objective of the present study was to investigate a potential role for the deiodinase D2 in bone metabolism in humans by studying the relationship between the D2 Thr92Ala polymorphism, BMD and bone turnover. The D2 Thr92Ala polymorphism is associated with a lower D2 Vmax and may therefore lead to decreased local availability of T3 (39), which may in turn affect skeletal metabolism. We studied this relationship in a human model of thyroidectomized patients cured from differentiated thyroid carcinoma receiving thyroid hormone substitution. The advantage of this model is that study subjects have more uniform FT4 levels, which fell between the 25th and 75th percentiles for FT4 (19.5 and 24.9 pmol/L) in our group of patients.

Figure 2. Relationships between D2Thr92ALA Genotypes and Indicators of Bone Turnover. a. Femoral neck BMD, b. Ratio of Urinary N-Telopeptide of Collagen Cross-links and Creatinine Concentration c. Procollagen type 1 aminoterminal propeptide (P1NP) levels, d. C-crosslinking Terminal Telopeptide of Type I collagen. For levels of significance, see text and Table 2.



In support for the involvement of D2 in bone metabolism was the observation of a 6% decrease in femoral neck BMD and increased levels of P1NP (32%), CTx (27%) and NTX/creatinine (54%) in the Ala/Ala subgroup as compared with wildtype. These effects were independent of factors known to influence BMD and bone metabolism such as age, gender, BMI, estrogen state, PTH and vitamin D. These effects were also independent of circulating levels of T3 and TSH and were thus indicative of an independent role of D2 in bone metabolism. We did not find an association of the D2 polymorphism with lumbar spine BMD, possibly due to a differential effect of the polymorphism on predominantly trabecular bone at the lumbar spine versus predominantly cortical bone at the femoral neck. Our data did not confirm earlier observations of an association of the D2 Thr92Ala polymorphism with insulin sensitivity (39.40). This discrepancy may be explained by differences in the populations studied, with a low prevalence of obesity or insulin resistance in our subjects. Our data are however in keeping with the Framingham offspring study, which found no relation between the D2 Thr92Ala polymorphism and insulin resistance (42). We did not observe differences in height, indicating no difference in skeletal development between the 3 genotype subgroups. This is in line with recent observations in the C3H/HeJ D2−/− compound mutant mice with D1 deficiency and deletion of D2, that were shown to maintain normal growth (37).
This notion is supported by a recent study, suggesting that D2 may not play a physiological role in growth plate chondrocytes (38).
The observed effects of the D2 Thr92Ala polymorphism on femoral neck BMD are in line with the importance of local availability of T3 for bone formation. D2 activity has been found on mature osteoblasts (45) which are the primary target cells for T3 regulatory effects on bone formation (1,2,16-18).
The effects of the D2 Thr92Ala polymorphism on bone turnover markers are not easy to explain. It is conventionally accepted that higher rather than lower circulating thyroid hormone levels result in higher bone turnover and decreased bone mass. However, the model we used is unique in the sense that circulating T3 levels were similar between the 3 D2 genotypes, allowing to specifically study the consequences of the polymorphism for local T3 availability in the bone microenvironment. Williams *et al* (38) showed no D2 activity in osteoclasts. The effects of the polymorphism on the markers of bone degradation (NTX/creatinine and CTx) may therefore not be explained by direct effects on osteoclasts but are more likely to result from changes in the interaction between osteoblasts and osteoclasts, possibly by alterations in the RANK/RANKL/OPG signaling pathway which can be possibly modulated by local T3 availability in the bone microenvironment. In the context of conflicting data on a functional role for TSH in skeletal development, our data, which were corrected for serum TSH levels, outline the importance of local T3 for bone metabolism (19-25,27,28). Two recent papers by Bassett *et al*. (29,30) who studied mice with complete or haploinsufficiency of TR alpha and beta, concluded that TR alpha regulates both skeletal development and adult bone maintenance.
Whereas a limitation of our study may be its relatively small size and its cross sectional design, one of its clear strengths is that all subjects were phenotyped for factors other than thyroid status known to modulate bone metabolism. This design enabled us to use regression models, including relevant covariables, the feasible of which is difficult in large cohort studies. A potential further limitation of our study is that thyroid hormone parameters measured at one point of time may not reflect the overall thyroid status over time. To address this issue, we calculated the slope of all TSH measurements routinely obtained after initial therapy in every patient participating in the study to verify the stability over time. An average of 15 TSH measurements were obtained per patient and the slope of TSH values was -

0.0001 (range -0.004-0) mU/L/year, thus indicating stable TSH levels over time.
In summary our data suggest, that a decrease in local availability of T3 potentially due to a D2 polymorphism may result in increased bone turnover and decreased bone mass at the predominantly cortical femoral neck. We believe our study provides additional information on the role of D2 in bone metabolism and the functional consequences of the D2 Thr92Ala polymorphism, supporting a role for D2 in mature bone cells (38).

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08

Glucose Tolerance and Lipid Profile in Long-term Exogenous Subclinical Hyperthyroidism and the Effects of Restoration of Euthyroidism, a Randomised Controlled Trial

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Abstract

Objective: The impact of prolonged subclinical hyperthyroidism on glucose and lipid metabolism is unclear. Therefore, we evaluated glucose and lipid metabolism in patients with differentiated thyroid carcinoma (DTC) on TSH suppressive thyroxin therapy as a model for subclinical hyperthyroidism and investigated whether restoration to euthyroidism affects metabolism.
Design: We performed a prospective, single-blinded, placebo-controlled, randomised trial of 6 months duration with 2 parallel groups.
Patients: Twenty-five subjects with a history of differentiated thyroid carcinoma with >10 years TSH-suppressive therapy with L-thyroxin completed the study. L-thyroxin dose was replaced by study medication containing L-thyroxin or L-thyroxin plus placebo. Medication was titrated to establish continuation of TSH suppression (low-TSH group, 13 patients) and euthyroidism (euthyroidism group, 12 patients).
Measurements: We evaluated glucose metabolism by glucose tolerance test and HOMA (IR) and lipid metabolism by lipid profile. In addition, we measured plasma concentrations of glucoregulatory hormones.
Results: At baseline, glucose tolerance, HOMA (IR), lipid profile and plasma concentrations of glucoregulatory hormones were within the normal range. No significant differences between the low TSH and euthyroidism group were observed. After 6 months, neither glucose- nor lipid metabolism in the low TSH group were different from baseline values.
Conclusion: In summary, glucose- and lipid metabolism in patients with DTC and long-term subclinical hyperthyroidism in general are not affected. Restoration of euthyroidism in general does not affect glucose and lipid metabolism.

Introduction

In *overt hyperthyroidism*, impaired glucose tolerance and increased insulin resistance have long been observed as a frequent complication (1-7)(Table 1), predominantly at the level of the liver (1). The underlying mechanisms have not been completely elucidated, but have been ascribed to a combination of multiple factors, like decreased pancreatic secretion of insulin (8,9), decreased suppression of glucagon by glucose (10) and increased adrenergic activity (11)(Table 1). Regarding lipid metabolism, overt hyperthyroidism is associated with decreased plasma concentrations of total and/or LDL cholesterol (3,12-16), that normalize after correction of hyperthyroidism (17-19) (Table 2).
Subclinical hyperthyroidism is a state, in which the patient has a suppressed thyrotrophin (TSH) level (below 0.4 mU/l), although the free T4 level is within the normal range. This condition affects several organ systems, including bone (20-23) and the cardiovascular system (24-28). Only scarce data are available on the consequences of subclinical hyperthyroidism for glucose- and lipid metabolism. Glucose metabolism in subclinical hyperthyroidism has been studied only by Yavuz *et al.*, who observed a decreased insulin sensitivity index by oral glucose tolerance test in patients with exogenous subclinical hyperthyroidism compared to values after restoration of euthyroidism and compared to controls (29) (Table 1). Most studies report no differences in lipid profile in subclinical hyperthyroidism (29-30), with the exception of 2 studies, that report decreased total and LDL cholesterol levels (32,33). In the study of Franklyn *et al.*, total cholesterol concentrations were decreased only in patients older than 55 years and LDL cholesterol levels were decreased only in patients older than 65 years (32). Most of the above described studies contained patients with endogenous (subclinical) hyperthyroidism. A disadvantage of those studies can be that the duration and degree of (subclinical) hyperthyroidism are not known. Exogenous subclinical hyperthyroidism is a good model to study the effects of subclinical hyperthyroidism on metabolism, because the duration and degree of subclinical hyperthyroidism are known.
In the present study, we therefore conducted a randomised, controlled trial in patients with differentiated thyroid carcinoma to compare the effects of restoration of exogenous subclinical hyperthyroidism to euthyroidism on glucose- and lipid metabolism.

Material and methods

Subjects
Consecutive patients were recruited from the outpatient clinic of the Department of Endocrinology of Leiden University Medical Centre. This department is a tertiary referral centre for differentiated thyroid carcinoma. Patients were included who had been diagnosed with DTC, who had received initial therapy consisting of total-thyroidectomy and radioiodine ablation therapy. Additional therapies were allowed, as long as they resulted in cure of DTC. Cure was documented by the absence of measurable serum thyroglobulin (Tg) during TSH stimulation as well as by a negative total-body scintigraphy with 4 mCi 131-I. The patients had to be on TSH suppressive therapy, defined as TSH levels below the lower reference value for TSH (0.4 mU/l), for at least 10 years. The adequacy of the TSH suppressive therapy had to be documented by yearly TSH measurements.
Patients who had diabetes mellitus according to the WHO criteria (34) or a BMI >30 were excluded. Patients who used any drugs known to influence the metabolic parameters we studied were also excluded. The local ethics committees approved the study, and written informed consent was obtained from all subjects.

Table 1. Overview of the Literature: impact of (subclinical) hyperthyroidism on glucose metabolism

Study	Number of patients	Diagnosis	TSH	Duration subclin. HT	Control group	Design	Outcome
Subclinical hyperthyroidism							
Yavuz (29)	20	Exogenous (T4) MNG	0.2 ±0.3	6 months	20 age- and sex-matched controls	Prospective	ISI (OGTT) ↓
Overt hyperthyroidism							
Tosi (6)	12 healthy subjects	Exogenous (T3)	< 0.1	10 days	-	Prospective	IGT (OGTT)
Jenkins (5)	6	Endogenous	not detectable	?	6 controls	Prospective	4/6 IGT(OGTT)
Karlander (40)	6	Endogenous	<0.1	?	6 controls	Cross-sectional	glucose tolerance = (OGTT)
Ikeda (4)	18	Endogenous	?	?	6 age-matched controls	Prospective	IGT(OGTT)
Gimenez-Palop (2)	24	Endogenous	0.01 ± 0.00	?	45 controls	Prospective	HOMA-IR ↑
Iglesias (3)	20	Endogenous	0.05 ± 0.004	?	20 controls	Prospective	HOMA-IR ↑
Yaturu (7)	69	Endogenous	0.087 ± 0.05	?	-	Prospective	HOMA-IR ↑
Cavallo-Perin (1)	12	Endogenous	?	?	12 sex-, age- and weight-matched controls	Prospective	hepatic insulin resistance (hyperinsulinemic euglycemic clamp)

MNG = Multinodular goitre, HOMA-IR= Homeostatic Model Assessment Insulin Resistance, OGTT= Oral Glucose Tolerance Test, ISI= Insulin Sensitivity Index, IGT= Impaired Glucose Tolerance

Table 2. Overview of the Literature: impact of (subclinical) hyperthyroidism on lipid metabolism

Subclinical hyperthyroidism							
Yavuz (29)	20	Exogenous (T4) MNG	0.2 ±0.3	6 months	20 age- and sex-matched controls	Prospective	TC, TG, LDL, HDL =
Langer (30)	149	Endogenous	011-0.30	?	1750 controls	Cross-sectional	TC, TG =
Lee (31)	35	Endogenous	0.07 ± 0.03	?	100 age- and sex matched controls	Cross-Sectional	TC, LDL, HDL, TG =,
Parle (33)	27	Endogenous	<0.4	?	27 age-, sex- and BMI- matched controls	Prospective	TC, LDL ↓
Franklyn (32)	59	Exogenous (T4)	< 0.5 mU/l	> 1 year (median 7.9 years, range 1-18 years)	Sex, age and menopausal state matched controls	Cross-sectional	TC↓>55 years, LDL ↓> 65 years.
Overt hyperthyroidism							
Riis (46)	9	Endogenous GD	0	?	8 age- and sex- matched controls	Prospective	FFA↑
Muller (45)	6 healthy subjects	Exogenous (T4)	?	10-14 days	-	Prospective	FFA =
Mantzoros (15)	22 healthy subjects	Exogenous (T3)	0.25 ± 0.14	7 days	-	Prospective	TC ↓
Iglesias (3)	20	Endogenous	0.05 ± 0.004	?	20 controls	Prospective	TC↓
Kung (14)	40	Endogenous	< 0.05	?	119 controls	Prospective	TC, LDL, HDL ↓
Cachefo (12)	5	Endogenous	?	?	20 controls	Cross-sectional	TC, HDL ↓ TG ↑
Sundoram (16)	7	Endogenous	< 0.04	?	-	Prospective	TC, LDL ↓
Costantini (13)	16	Endogenous	0.02 ± 0.035	?	16 age- and sex- matched controls	Cross-sectional	TC, LDL, HDL ↓
Lam (42)	16	Endogenous	< 1.3 µi.u/ml	?	24 controls	Cross-sectional	TC, HDL, TG =
Abrams (44)	13	Endogenous	2.6 ± 0.2 units	?	Controls	Prospective	TG ↑ VLDL-TG ↓
Raiszadeh (43)	24	Endogenous	0.07 ± 0.08	?	23 age- and sex- matched controls	Prospective	TG ↓ after treatment: TC,TG ↑
Azizi (17)	50	Endogenous	< 0.3	?	50 age- and sex-matched controls	Cross-sectional	TG ↓
Diekman (18)	47	Endogenous	< 0.01	?	-	Prospective	after treatment: HDL, LDL, TG, TC↑
Oge (19)	16	Endogenous	0.01 ± 0.2	?	-	Prospective	after treatment: C, LDL ↑

TC = Total Cholesterol, TG = Triglycerides, LDL = LDL cholesterol, HDL = HDL cholesterol, FFA= Free Fatty Acids

Study design

The study was a prospective, single-blinded randomised controlled trial with 2 parallel groups with a duration of 6 months. After inclusion, patients were randomised in a single-blinded way to continue TSH suppressive therapy (low TSH group, target TSH level < 0.4 mU/L) or to restore euthyroidism by decreasing the L-thyroxin dose (euthyroidism group, target TSH levels within the normal reference range (0.40-4.8 mU/L)).

After randomisation, standard thyroxin therapy of all patients was replaced in part by study medication according to an algorithm. Study drugs consisted of either 25 µg thyroxin or placebo tablets with similar appearance. Serum TSH levels were checked every 6 weeks in every patient, and study medication was adjusted if necessary to obtain the target TSH levels. Before and after 6 months, a physical examination was performed and fasting blood samples were drawn for hormonal and metabolic parameters.

Experimental protocol

Subjects were admitted to the clinical research unit, where they handed in the urine collected over the previous 48 hours. Patients were asked to follow a diet free of potential catecholamine stimulating food or medication (excluding coffee, alcohol, bananas, nuts and acetaminophen) from two days before urine collection. All subjects fasted from the preceding evening (18.00 hr) until the end of the study. On the study day, at 08.00 hr, height (meters [m]) and weight (kilograms [kg]) were measured. Body composition was measured by DEXA (Hologic 4500, Hologic Inc., Bedford, MA, USA). Patients were subsequently requested to lie down on a bed in a semi recumbent position. A catheter was inserted in a dorsal hand vein to collect plasma samples for measurement of glucose, insulin, cortisol, growth hormone, leptin, free fatty acids (FFA), total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides. To investigate insulin sensitivity an Oral Glucose Tolerance Test (OGTT) was performed. After an overnight fast patients were given 75 gram of glucose. At 0, 30, 60, 90 and 120 minutes serum glucose and insulin was measured. Plasma and serum samples were handled immediately and stored at –20o C in Sarstedt tubes.

Blood Chemistry

All plasma and serum samples were measured in one batch. Serum free thyroxin (FT4) and TSH were measured with an electrochemoluminescentic immunoassay with a Modular Analytics E-170 system with an intraassay CV of 1.6-2.2 % and 1.3-5.0 % respectively (Roche, Almere, The Netherlands). Serum free triiodothyronine (FT3) was measured with a fluorescentic polarisatic immunoassay, CV 2.5-9.0 %, on an ImX system (Abbott, Abbott Park, IL, USA). Serum insulin was measured by IRMA (Medgenix, Fleurus, Belgium) with a CV of 4.8-8.5 %, leptin by RIA (Linco Research, St. Charles, MO, USA) with an intraasaay CV of 3.0-5.1 %, growth hormone by IFMA on a Delfia system (Wallac OY, Turku, Finland), cortisol by TDX (Abbott) with an intraassay CV of 3.5-6.5 %, ACTH by IRMA (Nichols Institute, san Juan Capistrano, CA, USA) and FFA by spectrofotometry by a validated kit (Boehringer, Mannheim, Germany). Glucose, total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides were measured with a fully automated Modular P800 system (Roche).

Twenty-four hour excretion of urinary catecholamines (epinephrine, norepinephrine) was measured by HPLC with electrochemical detection.

Calculations

Insulin resistance was assessed by HOMA score (HOMA-IR) with the formulas as described by Matthews *et al.* (35). Insulin Sensitivity Index (ISI) was calculated as described by Matsudo and DeFronzo (36).

Statistical Analysis

Values are presented as mean ± standard deviation (SD). Data between groups were analysed using an unpaired T-test. Data within groups were analysed using a paired T-test. All calculations were performed using SPSS 12.0 for windows (SPSS. Inc., Chicago, IL, USA). Differences were considered statistically significant at $P<0.05$.

Results

Patient characteristics

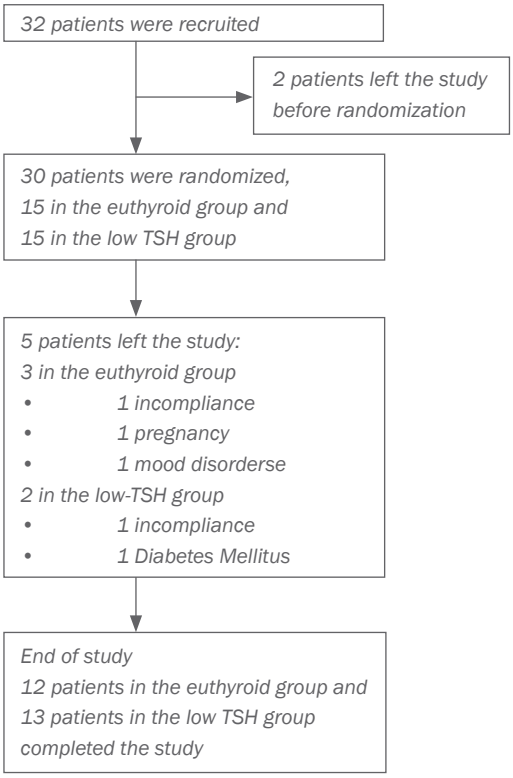
Thirty-two patients were recruited initially (Figure 1). Before randomisation 2 patients left the study; one patient because of comorbidity and the other patient without a clear reason. The other 30 patients were randomised in two groups of 15 patients. Two patients in the euthyroidism group left during the study, one because of pregnancy, the other because of mood disorders. In the subclinical hyperthyroidism group one patient left the study, because diabetes mellitus was diagnosed on the basis of a fasting glucose > 7 mmol/l. At the end of the study 2 patients, one in each group, were excluded because of incompliance. Consequently, 25 patients were included in the calculations, 8 men and 17 women (Table 3). Thirteen patients (9 women and 4 men, mean age 48.18 ± 8.60 years) were treated with thyroid hormone to suppress TSH level below 0.4 mU/l. Mean dose of thyroid hormone before randomisation was 179 ± 31 µg per day. Twelve patients (8 women and 4 men, mean age 50.81 ± 9.99 years) were treated to restore euthyroidism. Mean dose of thyroid hormone treatment before randomisation was 185 ± 39 µg.

Table 3. Patient Characteristics at baseline

	Low TSH (n=13)	Euthyroidism (n=12)	p-value
Age (yr)	48.18 ± 8.60	50.81 ± 9.99	0.487
Sex (m/f)	4 : 9	4 : 8	0.891
Weight (kg)	68.07 ± 19.84	74.8± 9.9	0.302
Length (m)	1.70 ± 0.08	1.73 ± 0.07	0.458
BMI (kg/m^2)	23.46 ± 6.74	24.98 ± 2.20	0.463
DEXA LBM (kg)	49.8 ± 8.5	48.8 ± 13.0	0.809
DEXA total fat (kg)	21.9 ± 6.4	21.1 ± 3.0	0.693
Tumor Stage			0.328
T2 N0 M0	9	5	
T2 N1 M0	3	3	
T3 N0 M0	1	2	
T3 N1 M0	0	2	
Histology Tumor			0.564
Papillary	10	10	
Papillary-follicular variant	2	1	
Follicular	0	1	
Follicular Hurthle	1	0	
Dose I-131	3174 ± 1817#	2418 ± 602	0.183
Duration of TSH suppressing treatment (years, (range))	13 ± 2	13 ± 2	0.724

 # 1 patient received 6900 MBq I-131 for persisting thyroid remnants

Figure 1 Study Flow Chart



Thyroid hormone levels

Thyroid hormone levels are summarized in Table 4. All patients had suppressed TSH levels at baseline. TSH, free T4 (FT4) and free T3 (FT3) concentrations were not different between the 2 groups at baseline. At 6 months no differences were observed in TSH, FT4 and FT3 concentrations and thyroxin dose in the subclinical hyperthyroid group compared to baseline. In the euthyroidism group, TSH, FT4 and FT3 concentrations were significant different at 6 months compared to baseline. Serum TSH concentrations were significantly lower (0.07 ± 0.10 vs. 4.35 ± 3.63 µmol/ml, p=0.002) and serum free T4 (22.97 ± 4.23 vs. 18.29 ± 4.76 nmol/l, p=0.012) and FT3 (3.59 ± 0.65 vs. 2.63 ± 0.60 pmol/l, p=0.001) concentrations were significantly higher in the low-TSH group compared with the euthyroidism group.

Anthropometric data

At baseline, BMI, DEXA total fat and DEXA Lean Body Mass were not different between the subclinical hyperthyroidism group and the euthyroidism group (Table 3). In addition, these parameters were not different within each groups at 6 months compared to baseline, or between groups at 6 months.

Glucose metabolism

At baseline, 2 of 13 patients in the subclinical hyperthyroidism group had impaired glucose tolerance on the basis of the OGTT according to the WHO criteria (34). In the euthyroidism group, 2 of 12 patients had impaired glucose tolerance. There were no differences between both groups. At 6 months, 1 of 13 patients in the subclinical hyperthyroidism group had impaired glucose tolerance. In the euthyroidism group, 1 of 12 patients had impaired glucose tolerance and 1 patient had type 2 diabetes mellitus based on the OGTT. There were no differences between groups or compared to baseline in both groups.

Table 4. Thyroid hormone parameters

	Baseline					6-months		
	Low TSH (n=13)	Euthyroidism (n=12)	p-value vs. Low TSH	Low TSH (n=13)	p-value vs. baseline	Euthyroidism (n=12)	p-value vs. baseline	p-value vs. Low TSH
Thyroxin dose (µg/day)	170 ± 30	185 ± 39	0.308	180 ± 32	0.077	129 ± 37	0.000	0.001
TSH (mU/l)	0.20 ± 0.25[a]	0.19 ± 0.29[a]	0.943	0.07 ± 0.10	0.063	4.35 ± 3.63	0.002	0.002
FT4 (pmol/l)	22.08 ± 5.71	22.60 ± 4.09[a]	0.777	22.97 ± 4.23	0.484	18.29 ± 4.76	0.004	0.018
FT3 (pmol/l)	3.30 ± 0.79	3.55 ± 0.39	0.336	3.59 ± 0.65	0.200	2.63 ± 0.60	0.000	0.001

[a] *p <0.05 in independent t-test compared to placebo group*

Table 5. Glucose and lipid metabolism

	Subclinical hyperthyroidism (n= 13)			Restoration to euthyroidism (n=12)		
	0 months	6 months	p-value	0 months	6 months	p-value
Glucose Metabolism						
Glucose (mmol/l)	5.1 ± 0.8 (4.0-6.8)	4.7 ± 0.7 (4.0-6.3)	0.510	5.2 ± 0.5(4.3-5.9)	5.1 ± 0.5(4.4-6.1)	0.696
Insulin (pmol/l)	69.5 ± 41.7 (20.8-173.6)	55.6 ± 13.9 (27.8-83.3)	0.226	76.4 ± 13.9 (27.8-83.3)	69.5 ± 17.8 (27.8-111.1)	0.302
HOMA-IR (median 2.8, interquartal range 1.55)(24)	2.39 ± 1.90 (0.54-7.40)	1.70 ± 0.58 (0.77-2.69)	0.193	2.73 ± 1.57 (0.85-6.21)	2.23 ± 1.07 (0.80-3.88)	0.268
Insulin Sensitivity Index (2.28±1.2)(14)	4.62 ± 1.75(1.77-7.38)	5.35 ± 2.17(2.34-9.44)	0.104	3.80 ± 1.14	4.60 ± 2.19	0.140
Lipid metabolism						
Cholesterol (mmol/l)	5.26 ± 0.71(4.38-6.93)	5.03 ± 0.96(3.94-7.37)	0.118	5.30 ± 1.35(3.46-7.80)	5.44 ± 0.99(3.96-7.54)	0.581
HDL-cholesterol (mmol/l)	1.51 ± 0.31(0.99-2.03)	1.49 ± 0.17(0.85-2.10)	0.588	1.75 ± 0.77(1.06-3.96)	1.68 ± 0.51(1.04-2.87)	0.518
LDL-cholesterol (mmol/l)	3.53 ± 0.72(2.34-5.09)	3.27 ± 0.71(2.45-4.28)	0.559	3.50 ± 1.11(2.09-5.85)	3.69 ± 1.07(2.58-5.97)	0.159
Triglycerides (mmol/l)	1.05 ± 0.48(0.51-2.24)	1.04 ± 0.64(0.39-2.76)	0.902	0.91 ± 0.43(0.47-2.09)	0.95 ± 0.49(0.43-2.03)	0.643
FFA (mmol/l)	0.55 ± 0.17(0.31-0.90)	0.52 ± 0.20(0.28-1.07)	0.602	0.45± 0.21(0.11-0.85)	0.43 ± 0.09(0.28-0.58)	0.781

Baseline plasma insulin concentrations, HOMA-IR and ISI were not different between the subclinical hyperthyroidism and euthyroidism group (Table 5). At 6 months, plasma insulin concentrations, HOMA-IR and ISI were not different compared to baseline values in both experimental groups and not different between groups. In our study, basal glucose concentrations and HOMA-IR were within the normal range of a Dutch study including 277 healthy controls with normal glucose tolerance with a median BMI of 25.6 kg/m^2 (37).

Lipid metabolism

At baseline, concentrations of total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides and FFA were not different between the subclinical hyperthyroidism group and the euthyroidism group (Table 5). After 6 months, no differences were seen in these parameters in both groups compared to baseline values. In addition, there were no differences between the two groups in any of these parameters at 6 months.

Hormone concentrations

At baseline and after 6 months plasma concentrations of cortisol, ACTH, growth hormone, leptin and 24 hour urinary excretion of catecholamines were not different between both groups. At 6 months those values were not different within each groups.

Discussion

The present study was performed to investigate the effects of restoration of euthyroidism after long-term subclinical hyperthyroidism on glucose- and lipid metabolism. The study was a prospective, placebo-controlled randomised study and as such the first using this design. The findings indicate, that restoration to euthyroidism in patients with long-term subclinical hyperthyroidism had no appreciable influence on several parameters of glucose- and lipid metabolism.

In our study, according to reference values using WHO criteria, 4 of 26 patients (15.4%) had impaired glucose tolerance at baseline. These percentages are in accordance with previous studies in The Netherlands and USA. Mooy *et al.* (38) found a prevalence of impaired glucose tolerance of 10.3% in the Dutch population, Harris *et al.* (39) a prevalence of 15.6% in the USA population. Two patients had a familiarity for type 2 diabetes mellitus. However, these patients did not have impaired glucose tolerance at baseline or at 6 months. The findings of our study on glucose metabolism are in conflict with the study of Yavuz *et al.* (29), which reports a decreased insulin sensitivity index after 6 months of exogenous subclinical hyperthyroidism compared to matched controls. Basal glucose values are not reported in that study. Most studies, performed in overt hyperthyroidism (1-7) found insulin resistance, whereas one study found no difference in glucose tolerance (40). An explanation for these differences in outcome of subclinical hyperthyroidism on glucose metabolism could be 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 (29). Alternatively, it might imply that the “dose” (the extent of subclinical hyperthyroidism) in our study was not relevant to result in a “response” (glucose intolerance), However, this is unlikely, because TSH values in our study were comparable to values in the study of Yavuz *et al.* (29).

Table 6. Glucoregulatory hormones.

	Subclinical hyperthyroidism (n= 13)			Restoration to euthyroidism (n=12)		
	0 months	6 months	p-value	0 months	6 months	p-value
Cortisol (nmol/l)	428.5± 173.2(160.0-720.0)	419.9 ± 174.0(160.0-870.0)	0.839	445.8 ± 126.6(300.0-660.0)	476.7 ± 169.4(220.0-730.0)	0.493
ACTH (pmol/l)	3.74 ± 2.64 (1.10-11.44)	2.86 ± 1.32 (1.54-6.38)	0.219	4.84 ± 2.42 (1.98-9.90)	5.50 ± 4.84 (1.54-18.48)	0.694
GH (mU/l)	4.90 ± 6.03(0.50-18.63)	7.16 ± 9.01(0.07-29.72)	0.215	5.46 ± 6.92(0.07-18.09)	5.29 ± 5.55(0.39-18.54)	0.911
Leptin (µmol/l)	12.4 ± 7.4(2.7-24.0)	12.9 ± 7.9(3.5-28.7)	0.626	16.0 ± 7.4(5.6-32.2)	16.3 ± 6.6(4.4-27.4)	0.714
Adrenaline-U (µmol/24 hour)	0.02 ± 0.01(0.00-0.06)	0.02 ± 0.02(0.00-0.07)	0.439	0.02 ± 0.02(0.00-0.08)	0.02 ± 0.02(0.00-0.05)	0.391
Noradrenalin-U (µmol/24 hour)	0.24 ± 0.12(0.09-0.53)	0.24 ± 0.08(0.14-0.43)	0.948	0.30 ± 0.10(0.18-0.44)	0.25 ± 0.06(0.15-0.35)	0.188

Alternatively, it might indicate the absence of a direct relationship between plasma levels of hormones and tissue specific hormone effect parameters (41). It might be argued that the number of patients included in our study was too low, resulting in underpowering of the study. A posthoc power analysis, however, for some important items of the different questionnaires, showed a sufficient power, e.g. range 70-97 %. Therefore, it seems unlikely that underpowering of our study plays a major role in the negative findings.

Restoration of subclinical hyperthyroidism to euthyroidism did not affect lipid profile. This is in accordance with previous studies (29-31). Only one study in 27 patients with endogenous subclinical hyperthyroidism (33) and one study in 59 patients with exogenous subclinical hyperthyroidism (32) found a decrease in total- and/or LDL cholesterol. In the latter study, also a decrease in LDL-cholesterol was observed.

In overt hyperthyroidism, total cholesterol was mostly decreased (3,12-16). Only one study (42) found a total cholesterol level within the normal range. Triglycerides are either decreased (17,18,43), within the normal range (42), or increased (44,12). Free fatty acids were within the normal range (45) or increased (46).

In our study, basal glucoregulatory hormones were all within the normal range and were not different after restoration of subclinical hyperthyroidism. In contrast to our findings, Hsieh *et al*. (47) noticed a significant increase in serum leptin in patients with exogenous subclinical hyperthyroidism. This change was more profound in females.

Successful restoration of subclinical hyperthyroidism to euthyroidism, as indicated by normalization of TSH concentration and a decrease in FT3/FT4 concentration by approximately 40% and 30%, respectively, did not result in any changes in parameters of glucose metabolism or lipid profile, which is comparable with baseline observations that glucose- and lipid metabolism are not affected to a considerable extent in subclinical hyperthyroidism. In addition, minimally if any changes were observed in any other of the studied metabolic parameters. We cannot exclude that a period of 6 months of restoration of euthyroidism is too short to detect differences in metabolic parameters.

In summary, we investigated the effects of subclinical hyperthyroidism in patients treated for differentiated thyroid carcinoma on several metabolic parameters. A prospective, single-blinded, randomised controlled trial was performed to investigate whether restoration of euthyroidism has effects on these metabolic parameters. We observed no relevant differences in glucose- and lipid metabolism during long-term subclinical hyperthyroidism and after restoration of subclinical hyperthyroidism to euthyroidism.

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09

Autonomic nervous system function in chronic exogenous subclinical thyrotoxicosis and the effect of restoring euthyroidism

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Abstract

Context: Knowledge on the relationship between the autonomic nervous system and subclinical hyperthyroidism is mainly based upon cross-sectional studies in heterogeneous patient populations and the effect of restoration to euthyroidism in subclinical hyperthyroidism has not been studied.
Objective: We investigated the long-term effects of exogenous subclinical hyperthyroidism on the autonomic nerves system and the potential effects of restoration of euthyroidism.
Design: Prospective single blinded, placebo-controlled, randomized trial.
Setting: University Hospital.
Patients: 25 patients who were on >10 years of TSH suppressive therapy after thyroidectomy.
Intervention: Patients were studied at baseline and subsequently randomized to a 6-months thyroid hormone substitution regimen to obtain either euthyroidism or maintenance of the subclinical hyperthyroid state.
Main outcome measures: Urinary excretion of catecholamines and heart rate variability were measured. Baseline data of the subclinical hyperthyroidism patients were compared to data obtained in patients with hyperthyroidism and controls.
Results: Urinary excretion of NE and VMA was higher in the subclinical hyperthyroidism patients compared to controls and lower compared to patients with overt hyperthyroidism. Heart rate variability was lower in patients with hyperthyroidism, intermediate in subclinical hyperthyroidism patients and highest in the healthy controls. No differences were observed after restoration of euthyroidism.
Conclusions: Long term exogenous subclinical hyperthyroidism has effects on the autonomic nerves system measured by heart rate variability and urinary catecholamine excretion. No differences were observed after restoration to euthyroidism. This may indicate occurrence of irreversible changes or adaptation during long-term exposure to excess thyroid hormone that are not remedied by 6 months of euthyroidism.

Introduction

Overt hyperthyroidism has profound effects on the heart, including tachycardia and/or arrhythmias, increased systolic pressure, increased systolic function, left ventricular hypertrophy and diastolic dysfunction (1,2,3). These effects are thought to be the result of direct effects of thyroid hormone on the cardiovascular system and the interaction of thyroid hormones with the sympathetic nervous system (2,4). This interaction has been shown to result from a sympathovagal imbalance, characterized by increased sympathetic activity in the presence of diminished vagal tone, which coincides with increased urinary excretion of catecholamines (5,6,7). Hence, the current consensus is that manifestations of altered autonomic nervous system function play a role in the pathophysiology and clinical presentation of thyrotoxicosis.

For subclinical hyperthyroidism, defined as low serum thyroid stimulating hormone (TSH) concentrations despite normal free thyroxin (FT4) and tri-iodothyronine (T3) concentrations, cardiovascular effects may also occur, but these are less well known and seemingly less severe. The most consistent findings include increased heart rate, supraventricular arrhythmias and abnormalities of LV morphology and function (8,2,9,10). Altered autonomic nervous system function in subclinical hyperthyroidism is also less well defined. Petretta et. al. (9), Goichot et. al. (11) and Portella et. al. (12), using measures of heart rate variability , found evidence that in patients with endogenous subclinical hyperthyroidism a reduction of cardiac parasympathetic control is present and this is supported by findings on heart rate turbulence by Osman *et al* (13). However, in the study of Goichot (11) there were no differences in the heart rate variability measure (the ratio of low frequency power over high frequency power: LF/HF) that is commonly used to characterize the balance between vagal and sympathetic influences in these patients. In addition, it seems that the most prominent differences between patients with (subclinical) hyperthyroidism and controls were present during a challenge of the autonomic nervous system. Apart from this, the interpretation of these findings is difficult as studies on the role of the possibly altered autonomic nervous system abnormalities and the cardiovascular consequences of subclinical hyperthyroidism are complicated by several factors. First, subclinical hyperthyroidism is a heterogeneous clinical syndrome with many possible etiologies with as sole common denominator the (biochemical) definition of low TSH and normal T3/T4 concentrations. Second, the duration and course of the underlying disease is often not known and therefore it cannot be excluded that the underlying disease itself, treatment with thyreostatic medication and use of β-blockers may have influenced cardiovascular parameters independent of serum thyroxin levels.

These considerations suggest that the most appropriate population to study the consequences of subclinical hyperthyroidism are patients treated for differentiated thyroid carcinoma (DTC) in whom, after thyroidectomy, continuous suppression of TSH occurs with individualized doses of levothyroxine (L-thyroxin). In these patients, subclinical hyperthyroidism is solely the result of exogenous L-thyroxin. We therefore performed a prospective, randomized, placebo-controlled study to assess autonomic nervous function in patients with DTC with longer than 10 years exogenous subclinical hyperthyroidism and to investigate whether restoration to euthyroidism affects autonomic nervous function. Autonomic nervous function was assessed using urinary catecholamine excretion, heart rate variability measurements during rest and by measuring the response in heart rate to a standardized mental stress test.

Subjects and methods

The ethics committee of Leiden University Medical Center (LUMC) approved the study protocol, and written informed consent was obtained from all subjects. The study was performed in compliance with the principles of the Declaration of Helsinki.

Subjects

Patients treated for DTC were recruited from the outpatient clinic of the Department of Endocrinology of the LUMC, a tertiary referral centre for DTC. Patients were included who had been diagnosed with DTC, and had received initial therapy consisting of total-thyroidectomy and radioiodine ablative therapy. Cure was documented by the absence of measurable serum thyroglobulin (Tg) during TSH stimulation as well as by a negative total-body scintigraphy with 4 mCi I-131. Patients had been on TSH suppressive therapy, defined as TSH levels below the lower reference values for normal serum levels of TSH (0.4 mU/L), for at least 10 years. The adequacy of this therapy was documented by yearly TSH measurements. Patients were excluded when they used medication affecting the sympathetic nervous system or when they were currently treated for or had experienced major cardiovascular events as uncontrolled hypertension or a myocardial infarction.

The study was a prospective, single-blinded randomized study of 6 months duration with 2 parallel groups. After inclusion, patients were randomized in a single-blinded fashion (patients were blinded) to a maintenance group or an intervention group. Only the treating physician prescribing the study medication was aware of the randomization. The other research staff involved in study-related activities was also blinded to treatment. In the maintenance group the existing TSH-suppressive therapy was continued (target TSH level <0.4 mU/L). In the intervention group it was attempted to reach a restoration of euthyroidism by decreasing the L-thyroxin dose target TSH levels within the normal reference range (0.4-4.8 mU/L)). This was achieved by replacing in all patients the standard L-thyroxin therapy in part by study medication according to an algorithm. Study medication consisted of either L-thyroxin 25 µg or identically looking placebo tablets. Serum TSH levels were checked every 6 weeks in every patient, and study medication was adjusted if necessary to obtain the target TSH levels.

Patients were compared to data obtained in patients with overt hyperthyroidism and healthy controls using similar methodology (5).

Before and after 6 months, identical assessments were performed. After an overnight fast, subjects were admitted to the clinical research unit, where the urine collected over the previous 48 hrs was handed in. After a medical history and physical examination, blood samples to assess thyroid hormone status were taken. At least 30 minutes after blood sampling, continuous ECG and blood pressure measurements were made while the subject was in supine position for at least 15 minutes. During this period the patients were acquainted with the test procedures that were about to follow. The measurements consisted of a 1-lead electrocardiogram (ECG) registration (recording 600 subsequent beats). The subjects were instructed to relax, to breathe regularly, not to speak and to stay awake. ECG signals were sampled at a rate of 500 Hz and the arterial pulse wave at a rate of 300 Hz. The signals were digitized using a customized laboratory interface (model 1401, Cambridge Electronic Design, Cambridge, UK), and analyzed with software supplied with the interface. Each registration was screened for artefacts and subsequently analyzed for heart rate variability parameters in the time domain: mean RR-interval (RR-int), the coefficient of variation (CV) of the successive RR-intervals (reflecting total variability), and the standard deviation of differences between adjacent R-R intervals (SDSD) reflecting “beat-to-beat” and, therefore, vagally mediated variability) as previously described (5) and according to the applicable guidelines (14).

The registrations were also analyzed for heart rate variability parameters in the frequency domain according to the same guidelines (14). Upon completion of the recording at rest, another 5-min recording was started during which the subjects were subjected to a mental stress test (15). During this test the subjects had to perform a standardized arithmetic test about which they had been instructed before. The registration made during this test was used to determine the percentage increase in heart rate from baseline.

Assays

Thyroid hormones, thyroid-stimulating hormone (TSH), and urinary creatinine and catecholamines concentrations were determined using standardized routine methodology at the clinical chemistry laboratories of the LUMC.

FT_4 was measured on an IMx (Abbott, Abbott Park, IL; intra-assay variability: 2.5-7.6%, interassay variability: 5.6-12.4%) at different levels). Total T_4 was determined on the TDx (Abbott; interassay CVs: 2.4-5.9%). Free tri-iodothyronine (FT3) was measured by RIABEAD (Abbott; interassay CVs of 2.0-4.4%). Serum TSH was determined with a Modular Analytics E-170 system (Roche Diagnostic Systems, Basle, Switzerland), interassay variability:0.88-10.66%). Reference values for FT4, FT3 and TSH are respectively 10-24 pmol/L, 2.5-5.4 pmol/L and 0.4-4.8 mU/L. Urinary norepinephrine (NE), dopamine (DOPA) and vanillylmandelic acid (VMA) were determined by routine HPLC methodology.

Statistical analysis

Data are expressed as mean ± standard deviation (SD). For assessment of the treatment effect between groups, the variables were log-transformed to meet the requirements for analysis of variance. Subsequently the transformed data were analyzed using analysis of covariance (ANCOVA, SAS Proc MIXED) with the baseline value as co-variate. Treatment least square means were back-transformed resulting in geometric mean treatment estimates corrected for differences in the baseline values. Contrasts and 95% confidence intervals (95% CI) between treatments were back-transformed resulting in geometric mean ratios, which were subsequently translated into percentage increase of the therapy treatment relative to the maintenance treatment.

The data obtained at baseline in the subclinical hyperthyroidism patient cohort were compared with data obtained in patients with overt hyperthyroidism and healthy controls using ANOVA and unpaired Student's t-test assuming unequal variances. The latter data were obtained using similar methodology and were reported earlier by our group (5). These data were also used to perform a post-hoc power analysis (using power=80% and alpha=5%) to calculate the required sample size per group for detecting relevant changes in the study parameters. Relevant changes were defined as the change required for normalizing the values obtained in the subclinical hyperthyroidism patients to the values observed in healthy controls.

All analyses were performed using SAS software (V9.1.2, SAS Institute, Inc., Cary, NC, USA).

Results

Patient characteristics

Thirty-three patients who fulfilled all inclusion criteria were included in the study. Four patients left the study before randomization: 1 patient because of abdominal surgery, 2 patients withdrew consent because of the perceived burden of the study and 1 patient did not present at the randomization visit.

During the study 3 patients from the intervention group were withdrawn: 1 patient (at 12 weeks) because of fatigue, headache and diarrhea, a second patient left (at 6 weeks) because of pregnancy and the third patient was excluded because of apparent incompliance: despite lowering the thyroxin dose, serum FT4 levels increased throughout the study. Two patients in the maintenance group (persistent low TSH) were also excluded for apparent incompliance; TSH levels rose despite being in the TSH suppression group. Thus 25 subclinical hyperthyroidism patients completed the study, 12 patients in the intervention group and 13 patients in the maintenance group. A summary of the subject characteristics is given in table 1.

Table 1. Characteristics of the study population

	Maintenance group (persistent TSH suppression) (n=13)	Intervention group (restoration euthyroidism) (n=12)	Control group (n=15)	Hyperthyroidism group (n=15)
Gender (F : M)	9 : 4	8 : 4	14:1	14:1
Age (yr)	49 ± 7.2 (36-64)	51 ± 10.5 (36-67)	40 ± 10.3 (21 - 56)	39 ± 9.7 (21-56)
Weight (kg)	77 ± 11.5 (60-103)	74 ± 9.8 (58-91)	68 ± 13.9 (47-97)	65 ± 11.9 (44-83)

Data are presented as mean ± SD (range).

L-thyroxin dose and thyroid hormones

The mean ± SD L-thyroxin dose in the maintenance group was 164 ± 34 µg/day before randomisation and remained virtually unchanged at 173 ± 28 µg/day at the second assessment. In the intervention group, the L-thyroxin dose was reduced from 185 ± 39 µg/day to 129 ± 37 µg/day in order to restore euthyroidism. The thyroid hormone levels are summarized in table 2. Thyroid hormone concentrations were not different between the groups at baseline. Particularly, the range of TSH concentrations was 0.003 - 0.339 mU/L in the maintenance group and 0.003 - 0.302 mU/L in the intervention group. At the end of the study, TSH concentrations were higher in the intervention group (range: 0.218 - 6.09 mU/L), while these remained virtually unchanged in the maintenance group (0.005- 0.210 mU/L). Eight patients in the intervention group became euthyroid 2 months after thyroxin dosage reduction, one patient 3 months after thyroxin dosage reduction and 2 patients 4 months after thyroxin dosage reduction. In these latter patients, FT4 levels decreased significantly every month, whereas TSH levels stayed behind. FT3 concentrations decreased by 42% (95% CI: 19-69%) and FT4 concentrations by 29% (95% CI: 12-48%) in the intervention group compared to the maintenance group. One patient in the intervention group had a persistently low TSH (<0.4 mU/L) for the duration of the study. After 6 months, this patient had a TSH of 0.218 mU/L, however, her baseline TSH was 0.0025 mU/L. Since her FT4 decreased from 21 tot 14 pmol/L, we considered the intervention in this patient successful, although her TSH at 6 months was still below 0.4 mU/L. Two patients in the intervention group had a TSH>4.8 mU/L (5.80 and 6.09 mU/L) at the end of the study. FT4 levels were 18.8 and 26.7 mmol/L.

Urinary catecholamine excretion

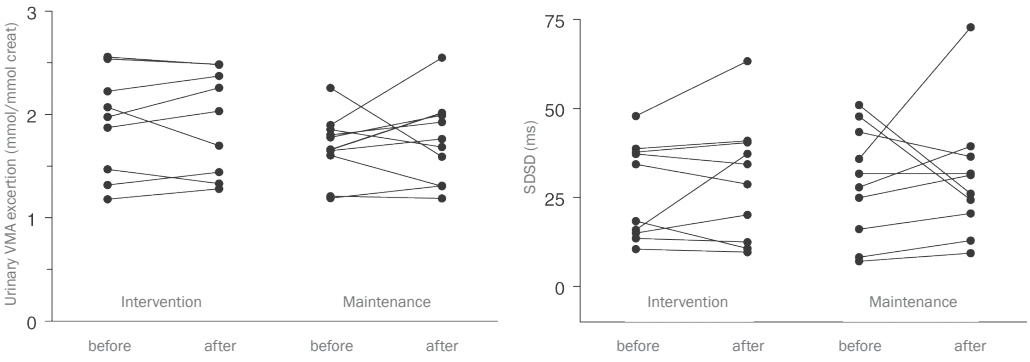
The urinary excretion of NE, DOPA and VMA (normalized for creatinine) is summarized in table 2. In order to illustrate the effects of longitudinal follow-up and the intervention, the urinary excretion of VMA for both groups is depicted in figure 1. This shows that in general the urinary excretion is remarkably stable during a 6-month follow-up period without treatment. Restoration to euthyroidism did not result in significant reductions in catecholamine excretion.

Table 2. Summary of thyroid hormone status and urinary catecholamine excretion normalized for creatinine excretion

	Maintenance group (persistent TSH suppression)		Intervention group (restoration euthyroidism)		Between group comparison
	before	after	before	after	p-value
TSH (mU/L)	0.08 ± 0.10	0.05 ± 0.02	0.10 ± 0.11	2.97 ± 2.30	p <0.001
FT3 (pmol/L)	3.4 ± 0.6	3.6 ± 0.6	3.6 ± 0.4	2.6 ± 0.6	p <0.001
FT4 (pmol/L)	22.8 ± 4.3	23.6 ± 1.0	22.6 ± 3.9	18.5 ± 4.1	p <0.001
Norepinephrine*	21.0 ± 6.5	23.6 ± 7.6	26.1 ± 7.4	24.7± 6.6	p =0.40
Dopamine*	0.17 ± 0.07	0.13 ± 0.04	0.15 ± 0.07	0.10 ± 0.04	p =0.40
VMA*	1.76 ± 0.35	1.72 ± 0.42	1.97 ± 0.48	2.03 ± 0.48	p =0.83

Data are presented as Mean ± SD. In the last column the p-value for the difference between the groups is given (using ANCOVA with baseline as co-variate).
TSH: thyroid stimulating hormone; FT3: free tri-iodothyronine; FT4: free thyroxin (FT4); VMA: vanillylmandelic acid.
** expressed as mmol/mmol creatinine*

Figure 1 Scatter plots showing the urinary vanillylmandelic acid excretion (VMA; left) and the SDSD measurements (right) illustrating that, except for the occasional outlier, both parameters were remarkable stable for the patients whether treated or not.



Autonomic nervous system function tests

The RR-interval in the intervention group increased from a mean value of 899 ± 135 milliseconds (ms) to 956 ± 135 ms (p =0.04). In the patients in whom thyroid suppression was continued, the RR-interval before the study was 849 ± 29 ms and this was not changed at the end of the study (869 ± 25 ms). Between the groups the change in RR-interval was not significantly different (6.7%; 95%CI: -0.5, 14.4%; p= 0.07). Both the time domain parameter reflecting the overall variability (CV) and the parameter reflecting the vagal influence on heart rate (SDSD) remained unchanged both within and between the treatment groups. The difference between the groups in CV and SDSD at the end of the treatment period were 3.6 (95% CI: -18.9, 32.3%) and 9.8% (95%CI: -25.0, 60.8%) respectively. The measurements of the SDSD for both groups are also depicted in figure 1. The data in the frequency domain were also not different between the groups (data not shown). All data are summarized in table 3. The difference in the increase in heart rate observed during the mental stress test was 9.0% (95%CI: -37.4; 32.3%) between the groups.

Table 3. Heart rate variability parameters in the time domain.

	Maintenance group (persistent TSH suppression)		Intervention group (restoration euthyroidism)		Between group comparison
	before	after	before	after	p-value
RR-int (ms)	849 ± 29	869 ± 25	899 ± 135	956 ± 135*	0.068
CV RR-int (%)	4.7 ± 1.6	4.7 ± 1.7	4.6 ± 2.4	4.4 ± 1.4	0.765
SDSD (ms)	35.8 ± 3.5	42.5 ± 5.6	26.3± 12.5	35.5 ± 24.2	0.614
HR response (%)	23 ± 3	23 ± 3	28 ± 17	24 ± 16	0.606

Data is expressed as mean ± SD The last column shows the p-value for the difference between the groups (using ANCOVA with baseline as co-variate).

RR-int: RR-interval; CV RR-int: coefficient of variation in RR-interval; SDSD: standard deviation of differences between adjacent R-R intervals; HR response: increase in heart rate during a mental stress test.

**p=0.04 for difference between before and after treatment*

Comparison between patient groups and power calculation

First, it is of note that the groups were comparable (ranges in age and weight show great overlap) albeit there were minor differences, particularly in weight which is obviously not surprising as patient with overt hyperthyroidism tend to lose weight (table 1). There was a difference between the groups regarding gender distribution with a female predominance in the comparison groups. However, there are no indications that gender is an important determinant of autonomic nervous system function (16).

There was a slight difference in age between the groups, and it has been shown that increasing age is related to a decline in heart rate variability-related parameters (17). However, the difference in age between the groups was small and even overlapping, making it unlikely that this may have caused important differences in the heart rate variability presented here.

Table 4 summarizes the results obtained at baseline in the current study and the data obtained in patients with overt hyperthyroidism and healthy controls.

The ANOVA analysis showed that the urinary excretion of NE (p=0.03) and of VMA (p=0.003) differed between the groups. The analysis shows that urinary excretion of the catecholamines was lower in the healthy controls compared to patients with subclinical hyperthyroidism; the mean (95% CI) difference was 3.80 mmol/mmol creatinine (-0.46/+8.061; p=0.053) for NE excretion and 0.434 mmol/mmol creatinine (+0.194/+0.675; p<0.001) for VMA excretion. Comparing the patients with subclinical hyperthyroidism with patients with overt hyperthyroidism showed a difference for the NE excretion of 4.00 mmol/mmol creatinine (-1.83/+9.83; p=0.217) and a difference in VMA excretion of -0.139 mmol/mmol creatinine (-0.4/+0.1223; p=0.275).

Analysis of variance showed significant differences between the groups for the measures of heart rate variability; the p-value was <0.001 for the RR-interval, a p-value of 0.0018 was observed for the coefficient of variation and for the differences in SDSD the p-value was <0.001. Patients with hyperthyroidism had on average a lower RR-interval of 280 ms (+201/+359; p<0.0001) than the patients with subclinical hyperthyroidism. This was accompanied with lower measures of heart rate variability; the coefficient of variation was 1.50% (+0.30/+2.70; p= 0.0088) lower, and the SDSD was 22.14 ms lower (+8.95/+35.33; p=0.0002). Comparing the patients with subclinical hyperthyroidism to the healthy controls showed that the RR-interval was 33 ms lower (-48/+114; p= 0.402). The coefficient of variation in heart rate was 1.88% (-0.04/+3.72; p= 0.08) lower and the SDSD was 12.92

Table 4. Urinary catecholamine excretion (normalized for creatinine) and heart rate variability parameters.

	Urinary catecholamine excretion		Heart rate variability		
	NE (mmol/mmol creatinine)*	VMA# (mmol/mmol creatinine)	RR-interval& (ms)	CV RR-int‡ (%)	SDSD† (ms)
Subclinical hyperthyroidism patients (n=25)	23.4 ± 7.3	1.85 ± 0.42	903 ± 135	4.46 ± 1.67	30 ± 13
Hyperthyroid patients (n=15)	27.5 ± 11.1	1.70 ± 0.37	621 ± 102	2.86 ± 0.80	9 ± 3
Healthy controls (n=15)	19.0 ± 4.3	1.40 ± 0.26	936 ± 114	6.66 ± 3.76	45 ± 28
Effect size for normalization of parameter	- 19.3%	- 24.3%	16.7%	43.0%	47.4%
Population required (n per group)	17	7	6	11	22

Data is expressed as mean ± SD. The bottom part of the table indicates the effect size needed for each for complete normalization for patients with subclinical hyperthyroidism and the size of the population that would be required to detect this normalization.

VMA: vanillylmandelic acid; CV RR-int: coefficient of variation of the RR-intervals (measure of overall heart rate variability); SDSD: standard deviation of the differences in subsequent RR-intervals (measure of beat-to-beat heart rate variability).

** ANOVA p=0.03, # ANOVA p=0.003, & ANOVA p<0.001, ‡ ANOVA p=0.0018, † ANOVA p<0.001*

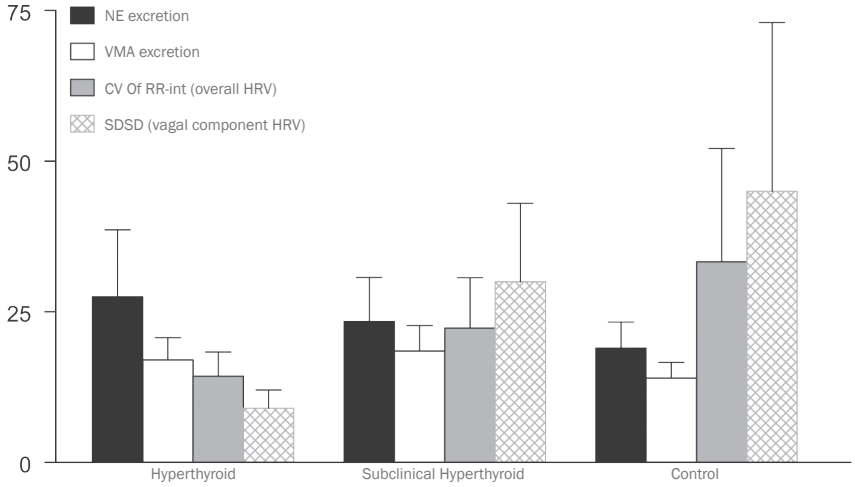
ms (-4.13/+29.98; p=0.142) lower. The findings are summarized in figure 2 which shows that the patients with subclinical hyperthyroidism have intermediate urinary excretion of NE excretion compared to patients with thyrotoxicosis and controls. Also, the measures for overall heart rate variability and the parameter reflecting the vagally mediated component of heart rate variability (SDSD) are between the values of the patients with frank hyperthyroidism and control subjects.

Table 4 also shows the effect size and the size of the population that would have been required if restoration to euthyroidism in the subclinical hyperthyroidism patients would have resulted in complete normalization of the effects parameters.

Discussion

This study was performed to investigate the long-term effects of exogenous subclinical hyperthyroidism on the autonomic nervous system and the potential effects of restoration of euthyroidism. The autonomic nervous system was characterized by assessment of the urinary catecholamine excretion and by heart rate variability parameters. Our study is the first prospective, placebo-controlled randomized study in which the effects of restoration of euthyroidism on the autonomic nervous system in patients with long-term exogenous subclinical hyperthyroidism were studied. The main finding of the study was that restoration to euthyroidism in patients with long-term subclinical hyperthyroidism due to TSH suppression had no appreciable influence on the autonomic nervous system. Urinary catecholamine excretion, the heart rate variability parameters in the time domain and the response to a mental stress test remained virtually unchanged between the patients who remained on TSH suppression and those in whom biochemical euthyroidism was restored.

Figure 2. Graph (mean ± SD) for urinary norepinephrine excretion (in mmol per mmol creatinine), urinary VMA excretion (in mmol per mmol creatinine), overall heart rate variability (CV of RR-intervals in %) and the vagal component of heart rate variability (SDSD in ms) for patients with overt hyperthyroidism, subclinical hyperthyroidism and control subjects. The values for urinary VMA excretion were multiplied 10 times for legibility reasons. The values for CV were multiplied 5 times for legibility reasons.



If the current data are compared with data obtained using similar methodology reported earlier by our group (5), values for activation of the autonomic nervous system in the current patient group with subclinical hyperthyroidism seem to be in between the group of patients with thyrotoxicosis and healthy controls. There were some differences between the groups particularly with regard to age and gender distribution. However, these are not confounding factors. It has been shown that the autonomic nervous system and its activity are not substantially influenced by gender (16). Admittedly, there are reports indicating that increasing age is associated with a decrease in heart rate variability (17,18) due to an age-related decline in parasympathetic regulation (19). These reports however show that this decline occurs over the age range of 20 to 80 years and that the change in heart rate variability occurring in the age range of the population that we studies is very small (17).

We could show that urinary NE excretion in the patients with subclinical hyperthyroidism was indeed lower compared to patients with overt hyperthyroidism and higher compared to the healthy controls. This seems at odds with data reported by Mercuro *et al*. (20) who showed that plasma NE concentrations were significantly lower in patients with exogenous subclinical hyperthyroidism than in controls. However, these data are based on plasma NE concentrations in a single sample of venous forearm blood, while it is known that catecholamine levels are more appropriately determined in arterial(ized) blood, inasmuch as extraction from venous circulation occurs across various organs (21,22), while the urinary excretion of catecholamines and their metabolites is considered to better reflect their average plasma concentrations and whole body turnover in plasma (22,23). We also showed that the heart rate, its total variability (coefficient of variation) and the vagally mediated influence on heart rate variability (SDSD) of the patients were between the values found for patients with thyrotoxicosis and healthy controls.

Interestingly, however, restoration to the euthyroid state in subclinical hyperthyroidism patients did not result in relevant changes in most autonomic nervous system parameters. Apparently, restoring a biochemical euthyroid state in patients who have been subclinical hyperthyroid for >10 years is not reflected in a state of the autonomic nervous system state that is identical to the situation in healthy euthyroid subjects.

It is important to note that in the present study, the intervention of restoring euthyroidism in the patients was successful. TSH concentrations normalized and FT3/FT4 concentrations decreased by approximately 40% and 30% respectively and became in the normal ranges utilized by the laboratories of our hospital. Obviously, it is of crucial importance that the study was sufficiently powered to detect relevant differences. As it was impossible to perform *a priori* power analysis because of lack of data, a population size was chosen which at least would allow exploratory analyses. Subsequently the data that were obtained were used to perform a post-hoc power analysis. This analysis showed that the present study was sufficiently powered to detect differences that would have changed most of the parameters in the Subclinical hyperthyroidism patients to the normal values for these parameters. In order to demonstrate normalization of the urinary NE and VMA excretion two groups of 17 or 7 patients respectively would have been needed. Also for the heart rate variability parameters, it seems that the study was sufficiently powered as for normalization of the RR-interval two groups of 7 patients were required and for the normalization of the overall heart variability (CV) two groups of 6 patients would have sufficed. Admittedly, more patients, (namely 22 patients per group) would have been necessary to also demonstrate normalization of the parameter that is commonly used to characterize the beat-to-beat variability. Nevertheless, we would like to argue that the current study was sufficiently powered for most of the parameters. We feel that our approach in which the restoration to euthyroidism in a homogenous group of patients with exogenous subclinical hyperthyroidism was studied, in a seemingly sufficiently powered randomized experiment is the most appropriate approach to study the effects of subclinical hyperthyroidism on the autonomic nervous system.

Notwithstanding this, the interpretation of these findings is not straightforward. It could be that irreversible changes or adaptation occurs during long term exposure to excess thyroid hormone. If the latter would be true, this would imply that restoration of the autonomic nervous system set-point takes a longer time than half a year. This may explain the difference with studies with a shorter duration in overt hyperthyroidism (5,6) or subclinical hyperthyroidism (13). In addition, another probably crucial difference between our and other studies is that our study the population of patients with subclinical hyperthyroidism was homogenous regarding etiology and duration of the syndrome whereas in other studies more heterogeneous patient populations or populations with endogenous subclinical hyperthyroidism are studied.

In conclusion, long-term exogenous subclinical hyperthyroidism affects the autonomic nerve system as measured by heart rate variability and urinary catecholamine excretion. No differences were observed 6 months after restoration of euthyroidism. This may indicate irreversible changes or adaptation during long-term exposure to excess thyroid hormone that are not remedied by 6 months of euthyroidism. To explore this further additional research is needed.

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10

Short-term overt hypothyroidism induces sympathovagal imbalance in thyroidectomized differentiated thyroid carcinoma patients

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Clinical Endocrinology in press

Abstract

Context: Thyroid hormone impacts on the cardiovascular system. (Subclinical) Hyperthyroidism results in sympathovagal imbalance due to a decreased vagal tone. However, conflicting data have been reported on the effects of hypothyroidism on the activity of the autonomic nervous system (ANS). In hypothyroidism; both increased and decreased sympathetic activity and an increased vagal tone have been found.
Objective: To study the effects of acute short term overt hypothyroidism and thyroxin replacement therapy on the ANS by measuring urinary excretion of catecholamines and heart rate variability (HRV).
Design: Prospective study
Setting: University hospital
Patients: We studied 11 patients, previously treated by thyroidectomy for differentiated thyroid carcinoma during hypothyroidism caused by cessation of thyroxin substitution for 4 weeks and during thyroxin replacement therapy and 21 matched healthy controls.
Main outcome measures: The activity of the ANS was assessed by measuring urinary excretion of catecholamines and HRV in rest and during a challenge of the ANS by a mental stress test.
Results: Urinary dopamine excretion was significantly lower during hypothyroidism. Although in the patients total variability was unchanged, HRV analysis showed a significantly lower LF/HF ratio, indicating sympathovagal imbalance with sympathetic withdrawal. The mental stress test in the patients resulted in a significant increase in heart rate of 16-18%. This response was not different between the hypothyroid state and during thyroxin replacement therapy suggesting that cardiovascular reflexes in these patients remain intact.
Conclusion: Acute short-term overt hypothyroidism results in sympathovagal imbalance with sympathetic withdrawal with preservation of the cardiovascular reflexes to (mental) stress.

Introduction

Changes in thyroid hormone concentrations influence the cardiovascular system. Both overt and subclinical hyperthyroidism have been associated with cardiac arrhythmias, left ventricular hypertrophy, diastolic dysfunction and increased systolic function (1-5). In addition, (subclinical) hyperthyroidism results in sympathovagal imbalance with increased sympathetic activity and a decreased vagal tone (6-12). Osman et al. found decreased vagal modulation during overt hyperthyroidism which persisted when patients became in the subclinical hyperthyroid state (13). In contrast, in hypothyroidism bradycardia, decreased cardiac output, diastolic cardiac dysfunction, mild diastolic hypertension, and increased peripheral cardiovascular resistance are observed (2;14-16). Acute short-term hypothyroidism shows similar cardiovascular abnormalities as in chronic hypothyroidism including bradycardia, diastolic dysfunction, impaired systolic function during effort and increased systemic vascular resistance (17). Although hypothyroidism has also been associated with sympathovagal imbalance (18-20), current literature shows conflicting results with either increased sympathetic activity (18), decreased sympathetic modulation (19) or an increased vagal tone in hypothyroidism (20). These differences can be due to the heterogeneity of the study populations regarding the cause and duration of hypothyroidism. Patients with differentiated thyroid carcinoma (DTC) being withdrawn from thyroxin for diagnostic purposes represent an excellent group to study the effects of acute short-term hypothyroidism on the autonomic nervous system. Only one study has documented the consequences of hypothyroidism on the autonomic nervous system in this situation. (21) In that study, however, no healthy controls were studied. In addition, the response to a challenge of the autonomic nervous system, which seems to reveal the most prominent differences in the autonomic nervous system during (subclinical) hyperthyroidism (9;11;22), was not studied in these hypothyroid patients. We therefore conducted a study on the autonomic nervous system in DTC patients during acute short-term hypothyroidism and subsequently during treatment with thyroxin, and compared these to healthy age-, gender- and BMI-matched controls. We studied urinary catecholamine excretion and heart rate variability measurements at rest and during a mental stress test (23).

Materials and methods

Subjects

Patients were recruited from the outpatient clinic of the Department of Endocrinology of Leiden University Medical Centre, which is a tertiary referral centre for differentiated thyroid carcinoma. Patients were included who had been diagnosed with DTC and had received initial therapy consisting of total-thyroidectomy and radio iodine ablation therapy. Additional therapies were allowed, as long as they resulted in cure. Cure was documented by the absence of measurable serum thyroglobulin (Tg) during TSH stimulation as well as by a negative total-body scintigraphy with 4 mCi 131-I.

Patients who had cardiovascular diseases, diabetes mellitus, other endocrine diseases or had a BMI >35 kg/mm2 were excluded. Patients who used any drug known to influence the heart rate variability were also excluded. The local ethics committees approved the study and written informed consent was obtained from all subjects.

Study design

Eleven consecutive patients with DTC undergoing TSH stimulated I-131 scan were asked to participate in the study. Four weeks after thyroxin withdrawal and 8 weeks after restarting thyroxin replacement, patients were admitted to the clinical research unit. For each patient, two healthy age-, gender- and BMI-matched controls were included. Subjects handed in the urine collected over the previous 48 hours. Subjects were asked to follow a diet free of potential catecholamine stimulating food or medication (excluding coffee, alcohol, bananas, nuts and paracetamol) from two days before and during urine collection. All subjects fasted from the preceding evening (18.00 hr) until the end of the study. On the study day, at 08.00 hr, height (meters [m]), weight (kilograms [kg]) and BMI (weight [kg]/height2 [m]) were measured. Plasma samples were collected for measurement of FT4, TSH and FT3. Plasma samples were handled immediately and stored at –20o C in Sarstedt tubes.

Heart rate variability

At least 30 minutes after blood sampling, a continuous ECG registration was made while the subject was in supine position for at least 15 min. The measurements consisted of a 1-lead electrocardiogram (ECG) registration (recording 600 subsequent beats). The subjects were instructed to relax, to breathe regularly, not to speak and to stay awake. The ECG signal was sampled at a rate of 500 Hz, digitized using a customized laboratory interface (model 1401, Cambridge Electronic Design, Cambridge, UK), and analyzed with software supplied with the interface. Each registration was screened for artefacts and subsequently analyzed for HRV parameters in the time domain: mean RR-interval (RR-int), the coefficient of variation (CV) of the successive RR-intervals (reflecting total variability), and the standard deviation of differences between adjacent R-R intervals (SDSD) reflecting “beat-to-beat” variability) as previously described (6) and according to the applicable guidelines (24). The registrations were also analyzed with HRV Analysis Software 1.1 for windows developed by The Biomedical Signal Analysis Group, Department of Applied Physics, University of Kuopio, Finland. The software is distributed free of charge upon request (http://venda.uku.fi/research/biosignal), and conforms with the same guidelines (27). The power spectra were analyzed for the Low Frequency (LF) and high frequency (HF) components and are expressed as normalized units. Upon completion of the recording at rest, another 5-min recording was started during which the patients were subjected to a mental stress test (23). During this test the subjects had to perform a standardized arithmetic test with which they had been familiarized before. The registration made during this test was used to determine the percentage increase in heart rate from baseline. This test was not performed in the healthy controls as the increase in heart rate reported in the literature in healthy subjects is always between 15 and 25% (25-28) and this is also the case in our experience (data on file at CHDR).

Assays

All plasma and serum samples were measured in one batch. Serum free thyroxin (FT4) and TSH were measured with an electrochemoluminescentic immunoassay with a Modular Analytics E-170 system with an intraassay CV of 1.6-2.2 % and 1.3-5.0 % respectively (Roche, Almere, The Netherlands). Serum free thyronine (FT3) was measured with a fluorescentic polarisatic immunoassay, CV 2.5-9.0 %, on an ImX system (Abbott, Abbott Park, IL, USA). Reference values for FT4, FT3 and TSH are respectively 10-24 pmol/L, 3.9-6.7 pmol/L and 0.4-4.8 mU/L. Urinary excretion of norepineprhine, epinephrine and dopamine were determined by routine HPLC methodology.

Statistical Analyses

Values are expressed as mean ± SD. Data between subjects were analyzed with the paired samples t-test. Data between DTC patients and control group was analyzed with the independent t-test. Differences were considered statistically significant at P<0.05. SPSS 12.0 for windows was used for statistical analyses (SPSS. Inc., Chicago, IL, USA).

Results

Patient characteristics

Eleven patients were included (4 male, 7 female). Mean age was 45.5 ± 10.0 years and BMI was 28.1 ± 4.2 kg/m2. All patients had been treated by total thyroidectomy followed by radioiodine ablation. Median (range) duration after initial therapy was 1.3 (0.6-24.3) years. Before thyroxin withdrawal the majority of patients was receiving TSH suppressive therapy (n=8). Three patients were receiving thyroxin replacement therapy, because their duration after initial therapy was more then 15 years. None of the patients had recurrent disease. Thyroid hormone levels are presented in table 1. All patients were overtly hypothyroid during the first study day, 4 weeks after withdrawal of thyroxin replacement therapy. Eight weeks after restarting thyroxin replacement therapy, six patients had thyroid parameters within the reference range, whereas 5 patients had a TSH below the reference range with normal plasma T3 and T4 levels. Mean thyroxin dose was 197 ± 42 mg/day. Diastolic blood pressures were significantly higher during the hypothyroidism state (84 ± 0 vs. 79 ± 6 mm Hg, p=0.0049), whereas systolic blood pressures were not significantly different.

Table 1. Thyroid hormone levels and urinary catcholamine excretion in 24 h urine collections

	Overt hypothyroidism	Thyroxin replacement	P-value
TSH (Mu/L)	142.4 ± 34.4	0.8 ± 1.0	<0.001
FT4 (pmol/L)	1.4 ± 0.7	24.8 ± 4.06	<0.001
FT3 (pmol/L)	0.1 ± 0.2	4.8 ± 0.8	<0.001
BMI (kg/m^2)	28.08 ± 4.19	27.41 ± 4.42	0.004
BP systolic (mm Hg)	121 ± 6	123 ± 11	0.460
BP diastolic (mmHg)	84 ± 9	79 ± 6	0.049
Norepineprhine (µmol/mmol creatinine)	25.1 ± 10.0	23.6 ± 11.4	0.690
Epinephrine (µmol/mmol creatinine)	1.6 ± 1.2	2.8 ± 2.2	0.064
Dopamine(µmol/mmol creatinine)	97 ± 33	132 ± 48	0.016

Paired samples t-test. Data are expressed as mean ± SD

BMI= body mass index, BP=blood pressure

Urinary catecholamine excretion

Urinary catecholamine excretion (normalized for creatinine excretion) is summarized in Table 1. Urinary excretion of dopamine was significantly lower during hypothyroidism compared to thyroxin replacement (97 ± 33 vs. 132 ± 048 µmol/mmol creatinine, p=0.016). Urinary epinephrine and norepinephrine excretion rates were not significantly different.

Table 2. Parameters of heart rate variability during hypothyroidism and thyroxin replacement therapy

	Overt hypothyroidism (n=11)	Thyroxin replacement (n=11)	P-value
RR-interval (ms)	1074 ± 123	948 ± 156	0.003
CV (%)	4.3 ± 2.3	4.4 ± 1.8	0.767
SDSD (ms)	40.1 ± 33.7	32.3 ± 19.7	0.159
LF (nu%)	54.7 ± 22.7	67.4 ± 14.5	0.050#
HF (nu%)	45.6 ± 22.7	32.6 ± 14.5	0.081#
LF/HF	2.20 ± 1.80	2.56 ± 1.33	0.041#

Data are expressed as mean ± SD

CV: coefficient of variation; SDSD: standard deviation of the successive differences in RR-intervals; LF: power in low frequency band; HF power in high frequency band; LF/HF: ratio of power in low frequency band over power in high frequency band

 # p-value for log-transformed variables

Heart rate variability

Parameters of heart rate variability are presented in Table 2. The RR-interval was significantly prolonged during hypothyroidism compared to during thyroxin replacement (1074 ± 123 vs. 948 ± 156 ms, p=0.03). Total variability (represented by the CV) and SDSD were not significantly different between hypothyroidism and thyroxin replacement. Frequency domain analysis showed that LF tended to be lower during hypothyroidism (54.7 ± 22.7 vs. 67.4 ± 14.5 nu, p=0.050) during hypothyroidism compared to thyroxin replacement. The LF/HF power ratio was significantly lower during hypothyroidism compared to thyroxin replacement therapy (p=0.042).

Mental stress test

The mental stress test produced a significant increase in heart rate of 16 ± 11% in the hypothyroid state and 18 ± 14.0% during thyroxin treatment. The response did not differ between the two conditions (p= 0.377) and has the same order of magnitude as previously reported for healthy subjects (25-28).

Table 3. Parameters of heart rate variability between patients and controls

	Thyroxin replacement (n=11)	Control group (n=21)	P-value
RR-interval (ms)	948 ± 156	863 ± 124	0.118
CV (%)	4.4 ± 1.8	3.5 ± 1.5	0.121
SDSD (ms)	32.3 ± 19.7	27.4 ± 18.6	0.517
LF (nu%)	67.4 ± 14.5	54.0 ± 22.0	0.037#
HF (nu%)	32.6 ± 14.5	46.0 ± 22.0	0.141#
LF/HF	2.56 ± 1.33	1.83 ± 1.69	0.112#

Data are expressed as mean ± SD

CV: coefficient of variation; SDSD: standard deviation of the successive differences in RR-intervals; LF: power in low frequency band; HF power in high frequency band; LF/HF: ratio of power in low frequency band over power in high frequency band

 # p-value for log-transformed variables

Comparison between controls and patients

Comparisons were made between patients on thyroxin substitution and healthy controls. The control group consisted of 8 men and 14 women. One male subject was excluded because the ECG recording had too many artefacts to be analyzed properly. Mean age was 45.5 ± 8.7 years and mean BMI was 28.3 ± 4.3 kg/m2, which was not different compared to controls. There were no differences in the RR-interval or CV between patients and controls (Table 3). The LF component was significantly higher in patients compared to controls, whereas there were no differences in LF component or the LF/HF ratio between patients and controls.

Discussion

We investigated the impact of acute short-term hypothyroidism and restoration to subclinical hyperthyroidism on the autonomic nervous system in thyreoidectomized patients with DTC by measuring urinary catecholamine excretion and heart rate variability at rest and in response to a mental stress test.

Heart rate was significantly lower during hypothyroidism compared to thyroxin replacement therapy and controls. As expected, the RR-interval was significantly prolonged during hypothyroidism in the patients. The LF/HF power ratio, representing sympathovagal balance with lower levels suggesting sympathetic withdrawal (24), 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 (24), other studies report that the LF component, especially when expressed in normalized units, reflects sympathetic activity (8;24;29). Our findings are consistent with some studies (19-21;30), but at odds with two other studies which reported a decreased LF/HF power ratio with an increased LF component and a decreased HF component in hypothyroid patients (18;19). We hypothesize that the discrepancies between the different studies may be explained by differences in duration, cause and severity of hypothyroidism as in these studies patients with hypothyroidism caused by Hashimoto thyreoiditis were investigated, whereas we studied thyreoidectomized patient with DTC with a controlled duration and degree of hypothyroidism. Our patients did not have any endogenous thyroid hormone production and therefore represent a unique model population to study controlled hypothyroidism.

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 (6-13),13. 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 (31).

It has been suggested that the VLF component reflects influences on heart rate variability mediated by thermoregulatory and angiotension-mediated mechanisms (32). We noticed a substantial difference between patients and controls. In controls, the absolute VLF power (92 ± 115 ms2) was much lower than in the patients (208 ± 152 ms2 when hypothyroid and 321 ± 369 ms2 when on thyroxin replacement therapy). It may well be that this difference in the contribution of the VLF frequency band influenced our findings on the LF and HF component. We suggest that investigations in the VLF component should receive more attention in patients with thyroid disorders.

To our knowledge, this is the first report showing that the cardiovascular adaptation mediated by the autonomic nervous system during mental stress is preserved in thyreoidectomized patients irrespective of a hypothyroid state or when on thyroxin replacement. Apparently, short term thyroid hormone withdrawal is not crucial in the (short-term) reflex-mediated cardiovascular regulation as indicated by the mental stress test.

Urinary excretion of dopamine was significantly lower during hypothyroidism. This is consistent with the results reported by Guasti et al (21). There were no differences in urinary excretion rates of norepinephrine and epinephrine.

In conclusion, acute short-term hypothyroidism in thyreoidectomized DTC patients results in a sympathovagal imbalance with sympathetic withdrawal. This, however, does not compromise the ability of the cardiovascular system to react normally to a (short-term) challenge of the autonomic nervous system.

10 Autonomic Nervous System during hypothyroidism

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11

Quality of Life in Cured patients with Differentiated Thyroid Carcinoma

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Abstract

Objective: This study was performed to evaluate the impact of cured differentiated thyroid carcinoma (DTC) on quality of life. Previous studies on quality of life in patients with DTC were hampered by small patient numbers, limited of quality of life parameters or were uncontrolled.
Design: Cross-sectional case-control study.
Method: We assessed quality of life in 153 cured DTC patients with a median duration of cure of 6.34 years (range 0.3-41.8) and studied the contribution of disease specific, biochemical and social variables, focusing on the degree of TSH suppression. Four validated health-related questionnaires were used (Short Form-36, Multidimensional Fatigue Index-20, Hospital Anxiety and Depression Scale and Somatoform Disorder Questionnaire), including multiple aspects of physical, psychological and social functioning. Patients were compared with 113 controls selected by patients themselves (control group-I), and with 336 pooled age- and gender-matched controls from other Leiden quality of life studies (control group-II).
Results: Patients had significantly decreased quality of life in 11 of 16 subscales when compared with control group-I. Comparison with control group-II, decreased scores in 13 of 16 items were observed. An important independent predictor for quality of life was duration of cure. Quality of life parameters were not influenced by serum TSH levels, both measured at the time of quality of life assessment and measured over time since initial therapy.
Conclusions: Patients cured for DTC have impaired quality of life, independently of TSH level. Quality of life parameters were inversely affected by duration of cure and consequently may be restored after prolonged follow-up.

Introduction

Well-differentiated thyroid carcinoma (DTC) is associated with an excellent medical prognosis, with 10-year survival rates reaching 90-95% (1). Following initial therapy, usually consisting of total thyroidectomy and radioiodine thyroid remnant ablation therapy, most patients used to be treated with high doses of L-thyroxin in order to suppress TSH levels (1). On one hand, the excellent prognosis and the moderate invasiveness of the initial therapy may implicate that quality of life in cured DTC patients may be relatively normal. On the other hand, TSH suppressive thyroxin replacement therapy may lead to a decreased quality of life (2,3,4).
Only a few studies have evaluated quality of life in cured DTC patients (5,6,7,8,9). These studies are limited by small patient numbers (6,7), limited number of quality of life questionnaires (5,9) or the absence of a healthy control group (5,6,8).
Studies that focused on the relation between the level of TSH suppression and quality of life in DTC patients are inconclusive because of small patient numbers, selection of patients with symptoms of hyperthyroidism or selection of patients with a long duration of cure (2,10). Therefore, the aim of the present study was to assess quality of life in a large cohort of cured DTC patients and to investigate the determinants of quality of life, including serum TSH levels. We used four validated, health-related questionnaires and included controls matched for age, gender and socioeconomic status.

Patients and methods

Cured DTC patients, 18-70 years old, were recruited from the outpatient clinic of the Department of Endocrinology of the Leiden University Medical Center. Other medical conditions or drugs that could influence quality of life were not permitted.
Initial therapy consisted of near-total thyroidectomy, followed by postoperative radioiodide ablation therapy with I-131. Cure after initial therapy was defined as the absence of I-131 accumulation at diagnostic 185 MBq scintigraphy, serum Tg concentrations below 2 µg/L after TSH stimulation in absence of Tg antibodies and no other evidence of disease (11). Patients with tumor relapse were only included if they were subsequently cured.
Initially, 157 DTC patients who met these criteria were asked to participate. Four validated questionnaires were sent to their homes together with a list of general questions about level of education, country of origin and marital state. Four patients specifically wished not to participate. Each patient was also asked to provide a control person of comparable sex, age and socio-economic status (friend, neighbour, relative) (control group-I). We received 153 completed questionnaires from patients and 113 questionnaires from controls. To exclude bias in the selection of control group-I, we also compared the patients with a larger cohort of age, gender and socioeconomic status matched healthy controls (n=336) obtained from other quality of life studies performed in our centre (12,13,14,15) (indicated as control group-II).
The study protocol was approved by the medical ethics committee of the Leiden University Medical Centre and written consent was obtained from all patients.

Study parameters
Primary study parameters were the outcomes of the four health related questionnaires and the contribution of patient characteristics (age, gender, educational level, marital status), disease specific characteristics (initial TNM stage, recurrent disease, duration of cure), treatment (extent of surgery, radioiodine therapy and additional treatments) and biochemical parameters (serum free T4, T3 and TSH levels) to quality of life.

The influence of TSH on quality of life was investigated both by evaluation of serum TSH levels at time of the survey (expressed as continuous variable or stratified as 'profoundly suppressed' (<0.1 mU/l), 'moderately suppressed' (<0.4 mU/l) and 'unsuppressed' (>0.4 mU/l) and by summary TSH parameters over time since initial therapy for each patient. Summary TSH parameters over time were the mean, 25th, 50th and 75th percentiles and the percentage of profoundly suppressed, suppressed and unsuppressed TSH values from all available unstimulated TSH measurements since initial therapy.

Quality of life questionnaires

Short-form-36 (SF-36)

The SF-36 questionnaire comprises 36 items and records general well-being during the previous 30 days (16), subdivided in 8 health concepts. Scores are expressed on a 0–100 scale, and higher scores are associated with a better quality of life.

Multidimensional Fatigue Index-20 (MFI-20)

The MFI-20 comprises 20 statements (5 dimensions) to assess fatigue, which are measured on a five-point scale (17). Scores vary from 0-20; higher scores indicate greater fatigue.

Hospital Anxiety and Depression Scale (HADS)

The HADS consists of 14 items pertaining to anxiety and depression. Scores for the anxiety and depression subscale range from 0 to 21, and values for the total score range from 0-42. Higher scores indicate more anxiety or depression (18).

Somatoform disorders questionnaire (SDQ)

All somatoform disorders mentioned in classification DSM-III were comprised in this questionnaire (19). The total score varies from 0-51 for women and 0–55 for men. The total score expresses the extent of physical complaints that were present in the previous week.

Assays

Serum free thyroxin (FT4, normal range 10-24 pmol/L) and TSH levels (normal range 0.4-4.5 mU/L) were measured by electrochemoluminescentic immunoassay using a Modular Analytics E-170 system (Roche, Almere, The Netherlands).

Statistical analysis

SPSS for Windows, Version 12.0 (SPSS Inc., Chicago, IL. USA) was used to perform all analyses. Data are expressed as mean ± SD unless indicated otherwise. As dependent variables, we calculated delta scores between each patient and age and gender matched Leiden-controls by subtracting age and gender specific means of the controls from patient scores for all questionnaire subscales. Stepwise univariate linear regression analysis was used to identify independent variables for quality of life. Differences were considered statistically significant at $p<0.05$.

Results

One hundred and fifty three patients (28 males, 125 females, age 49±13 years, 127 papillary- and 27 follicular carcinomas) were analyzed. Tumor stages were T1-3 M0 in 131, T4 in 18 and M1 in 4 patients. Median duration of cure was 6.3 years (range 0.3-41.8). At the time of the survey, median TSH was 0.1 mU/L (range 0.005-6.8) and FT4 was 22.4±4 pmol/L. An average of 15 unstimulated TSH measurements per patient was obtained since initial therapy. Summary parameters of TSH over time per patient were: mean 0.4 mU/L (range 0.1-3.4), median 0.05 mU/L (range <0.005 - 2.18); proportions of profoundly suppressed values: 58% (range 0 –100) and moderately suppressed values: 80% (range 0-100%). The slope of TSH values was -0.0001 mU/year (range -0.004-0.000 mU/year), indicating that the TSH levels were reasonably stable. Mean dose of L-thyroxin was 183 ± 51 µg/day.

Quality of life in DTC patients and controls

Quality of life scores in patients were significantly reduced in 11 of the 16 items assessed when compaired to control group-I. According to the SF-36 questionnaire, patients had significantly worse scores on social functioning and general health perception. All MFI-20 subscales and HADS subscales were affected in DTC patients. The SDQ total score was also significantly worse than in control group-I. Comparison of the patients with control group-II (12,13,14,15) showed similar results: 13 of 16 quality of life parameters differed significantly between patients and controls (Table 1).

Determinants of quality of life

Marital status, country of birth, initial TNM-stage, total activity of I-131, tumor recurrence, L-thyroxin dose, post-surgical hypoparathyroidism, and serum FT4-level did not affect any of the questionnaire items.

TSH levels measured at the time of the assay (both continuous and stratified) and summary TSH values over time appeared not to be a significant independent predictor for quality of life. Post-hoc power calculation revealed sufficient power (all items > 0.9) to draw this conclusion.

A longer duration of cure was correlated with better scores on SF-36 social functioning (standardized β=0.21, p=0.030), role limitations due to physical problems (β=0.17, p=0.049), general health perception (β=0.32, p=0.001), MFI-20 general fatigue (β= -0.17, p=0.035), physical fatigue (β= -0.24, p=0.003) and mental fatigue scores (β=-0.17, p=0.038). We calculated the duration of cure needed for the quality of life scores to reach the normal range of all healthy subjects (Figure 1). The 95% CI intervals of quality of life scores only included 0 (no difference between quality of life of patients and controls) after a relatively long duration of cure (approximately 12 to 28 years for SF-36 respectively MFI-20).

Discussion

The purpose of this study was to evaluate quality of life in a large cohort of cured DTC patients using multiple quality of life parameters and a matched healthy control group. We found that quality of life scores assessed by the majority of subscales are reduced in patients previously treated for DTC, compared to controls. Although our observations are in line with other studies on quality of life in DTC patients (5,9,6,7,8), our study includes a higher numbers of patients, uses more quality of life questionnaires and uses matched control groups.

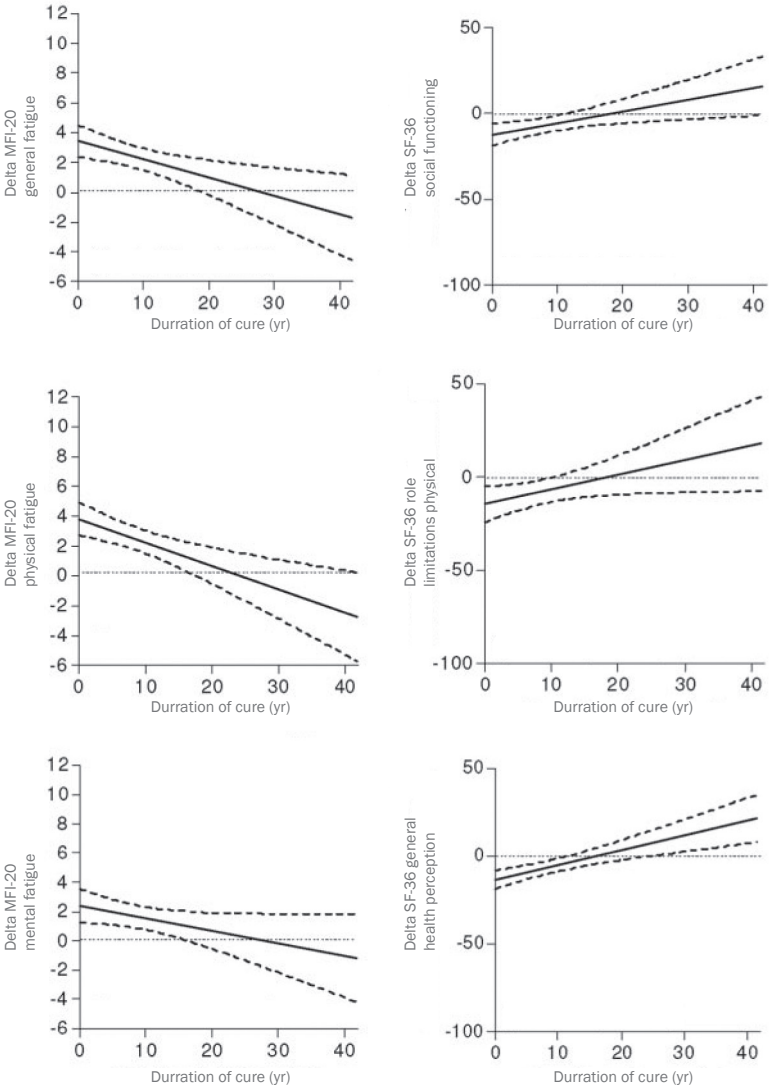
Table 1: Quality of life in patients treated for DTC compared with controls selected by patients themselves (Control Group I) and age and gender matched controls from other Leiden quality of life studies (Control Group II) (12,13,14,15). Data shown are mean ± SD.

Questionnaire	Patients (n=153)	Control group I (n=113)	P (patients vs. control group-I)	Control group II (n=336)	P (patients vs. control group-II)
Age	49.10	48.08	0.522	49.99	0.496
M/F	28/125	19/94	0.754	67/269	0.672
SF-36					
Physical functioning	83.70±21.02	88.27±16.78	0.052	87.77±17.14	0.040
Social functioning	81.09±24.90	87.39±20.01	0.037	88.06±19.28	0.007
Role limitations due to physical problems	75.35±40.04	81.42±34.36	0.194	83.38±32.43	0.035
Role limitations due to emotional problems	83.22±35.43	84.66±31.82	0.734	85.93±30.21	0.422
Bodily pain	82.74±21.70	84.78±18.93	0.426	85.17±19.24	0.216
General health perception	65.59±20.48	71.45±18.43	0.027	71.34±18.79	0.007
Change in health	52.15±18.37	55.18±18.19	0.185	54.77±18.64	0.105
MFI-20					
General fatigue	11.03±4.72	8.11±3.35	<0.001	8.60±4.01	<0.001
Physical fatigue	9.95±4.93	6.65±2.64	<0.001	7.60±3.69	<0.001
Reduced activity	8.79±4.15	6.85±3.30	<0.001	7.18±3.57	<0.001
Reduced motivation	8.64±3.76	6.67±2.79	<0.001	7.26±3.53	<0.001
Mental fatigue	9.53±4.50	7.93±3.60	0.002	7.92±3.31	<0.001
HADS					
Anxiety	5.69±3.95	4.14±3.15	<0.001	4.21±3.21	<0.001
Depression	3.61±3.08	2.37±2.52	<0.001	2.86±2.99	0.011
Total	9.30±6.30	6.51±4.92	<0.001	7.07±5.39	<0.001
SDQ					
SDQ total	5.92±6.20	1.66±2.51	<0.001	1.65±2.50	<0.001

Longer duration of cure was associated with better scores on different quality of life items. This finding is in line with studies by Dagan *et al* (7) and Crevenna *et al* (5), but this is the first study to quantify the predicted duration of affected quality of life in relation to duration of cure. After a long duration of cure, approximately 15 to 20 years (MFI-20 respectively SF-36) the 95% confidence intervals of 6 of the 16 quality of life subscales included a normal score (Figure 1).

In our study, quality of life was not influenced by TSH levels at the time of the survey and by TSH levels over time since initial therapy, although it can be objected that generic questionnaires were used. Other studies on the effects of subclinical hyperthyroidism on well-being have yielded inconclusive results. Most of these studies have been performed in patients with endogenous subclinical hyperthyroidism (3) who cannot easily be compared with DTC patients or contained selected patients with DTC (2).

Figure 1 Differences between age- and gender-matched controls and patients for the quality-of-life parameters plotted against duration of cure; linear regression line and 95% confidence interval are shown (standardized B and significance of linear regression analysis). The horizontal line represents the value for quality-of-life parameters where there is no difference between patients and the means of age- and gender-matched controls



Comparison of DTC survivors to survivors of other cancer types is complicated because of the many differences between the several cancer types. A large study (20) revealed that DTC survivors had similar quality of life as patients with breast cancer, worse than survivors of melanoma or colorectal cancer but better than hematological malignancies. Despite cure, excellent prognosis and moderate aggressive treatment, DTC patients have an evident decrease in quality of life that may only be restored after years of follow-up. The findings of our study have therefore implications for the approach of the cured DTC patients: attention for the psychological well-being of the patient and availability of professional support may be important aspects in follow-up.

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11 Quality of Life in Differentiated Thyroid Carcinoma

12

The Thr92Ala polymorphism in the type 2 deiodinase is not associated with thyroxin dose in athyroid patients or patients with Hashimoto thyroiditis

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Clinical Endocrinology in press

Abstract

Objective: The type 2 deiodinase (D2)-Thr92Ala polymorphism has been associated with decreased D2 activity in some in vitro experiments but not in others. So far no association between the D2-Thr92Ala polymorphism and serum thyroid hormone levels has been observed in humans, but in a recent study in athyroid patients, it was suggested that patients homozygous for the Ala92 allele needed higher thyroxine doses to achieve TSH suppression.

We studied the association between the D2-Thr92Ala polymorphism with thyroid hormone levels and thyroxine dosage, in patients treated for differentiated thyroid carcinoma (DTC) and in a group of patients treated for Hashimoto thyroiditis.

Design: Cross sectional study

Patients: We studied 154 patients with DTC treated with TSH suppressive thyroid hormone replacement therapy for longer than 3 years and 141 patients with Hashimoto thyroiditis treated for at least 6 months with thyroxine.

Measurements: In all patients, serum levels of TSH, free thyroxine, triiodothyronine and reverse T3 were measured and genotypes of the D2-Thr92Ala polymorphism were determined by Taqman assay. Univariate regression analysis was performed to determine the relation between thyroxine dosages and the D2-Thr92Ala polymorphism corrected for age, gender, BMI and serum TSH levels.

Results: Both in DTC patients and Hashimoto patients, no association was observed between serum thyroid hormone levels or thyroxine dosages in presence of the D2-Thr92Ala polymorphism. Categorization of DTC patients according to degree of TSH suppression did not change these results.

Conclusion: The D2-Thr92Ala polymorphism is not associated with thyroid hormone levels or thyroxine dose in patients treated for DTC or Hashimoto thyroiditis.

Background

Most actions of thyroid hormone are mediated by the active form of thyroid hormone, triiodothyronine (T3). Serum and local T3 concentrations are mainly regulated by the iodothyronine deiodinases D1, D2 and D3(1). D2 is essential for the local production of T3 through deiodination of T4. D2 is thus essential for the negative feedback regulation of thyroid hormone on thyrotropin (TSH) production in the pituitary. Several polymorphisms in D2 have been described (2-5). Controversy exists about the functional implications of the D2-Thr92Ala polymorphism, which has been associated with a decreased D2 activity in some in vitro experiments (2) but not in others (5). So far no associations were found between the D2-Thr92Ala polymorphism and serum thyroid hormone levels in studies in healthy subjects(4;6;7).

Torlontano *et al*. reported in thyroidectomized differentiated thyroid carcinoma (DTC) patients that homozygous carriers of the D2-Ala92 allele needed higher dosages of thyroxine(8). This difference was most prominently observed in the group with near-suppressed TSH (TSH values between 0.1 and 0.5 mU/L). Limitations of this study were that actual values of serum TSH levels for wild-type and homozygous groups within the near-suppressed TSH group were not given. It is therefore unclear whether TSH levels in both groups were indeed identical, which would be a key finding to ascribe the slight differences in thyroxine dose indeed to the polymorphism. The fact that serum T4 and T3 levels did not differ between the wild-type group and D2-Thr92Ala homozygotes is also remarkable. Moreover, as TSH is a continuous variable, we believe that the optimal analytic strategy would be by regression analysis, rather than a categorized approach. We therefore performed this study to reconfirm the findings of Torlontano *et al*.

For this reason, we studied the association between the D2-Thr92Ala polymorphism and thyroid hormone levels and thyroxine dosage in 154 patients treated for DTC and 141 patients substituted with thyroxine for Hashimoto thyroiditis, using a linear regression model. In addition, we performed a categorized analysis to allow maximal comparability with the Torlontano study.

Patients and Methods

Patients

Patients treated for DTC were recruited from the outpatient clinic of the Department of Endocrinology of the Leiden University Medical Center. All patients had been treated by near-total thyroidectomy followed by radioiodine ablation. After initial treatment, thyroxine therapy was started in a dose intended to suppress TSH levels below 0.4 mU/l for 15 years. All patients were cured as defined by the absence of I-131 accumulation at diagnostic scintigraphy, serum thyroglobulin (Tg) concentrations below 2 µg/l after TSH stimulation in the absence of Tg antibodies, a normal neck ultrasound and no other indication for disease. Patients with tumor relapse were only included if they had been subsequently cured. The Local Ethics Committee of the Leiden University Medical Centre approved the study, and written informed consent was obtained from all subjects.

We also included 141 patients treated for at least 6 months with L-thyroxine for Hashimoto thyroiditis. Serum TSH levels were between 0.11 and 4.0 mU/L. These patients were described earlier by Appelhof *et al* (9).

Study design

After an overnight fast, patients had a physical examination, including, height (meters [m]) and weight (kilograms [kg]). Blood was collected for determination of TSH, free thyroxine (FT4), T3 and reverse T3 (rT3). Serum samples were handled immediately and stored at –80 oC in Sarstedt tubes. DNA was collected for genotyping of the D2-Thr92Ala polymorphism. To be able to compare our study with the study of Torlontano *et al*. (8), patients were categorized in groups with a suppressed TSH (< 0.1 mU/L), near-suppressed TSH (0.1-0.5 mU/L) or non-suppressed TSH (>0.5 mU/L).

Serum biochemistry

In the patients treated for DTC, serum FT4 and TSH were measured using a chemoluminescence immunoassay with a Modular Analytics E-170 system (intraassay CV of 1.6-2.2 % and 1.3-5.0 % respectively (Roche, Almere, The Netherlands). Serum T3 was measured with a fluorescence polarization immunoassay, CV 2.5-9.0 %, on an ImX system (Abbott, Abbott Park, IL, USA). Reverse T3 was measured using a RIA as described previously (10).

In the patients treated for Hashimoto thyroiditis, serum TSH and FT4 were measured by time-resolved fluoroimmunoassay and serum T4 and T3 by in-house RIA methods (6).

Genotyping

DNA was isolated from peripheral leucocytes by the salting out procedure (11). Genotypes of the D2-Thr92Ala polymorphism (rs 225014) were determined using 5 ng genomic DNA in a 5' fluoregenic Taqman assay and reactions were performed in 384-wells format on ABI9700 2x384well PCR machines with endpoint reading on the ABI 7900HT TaqMan® machine (Applied Biosystems, Nieuwerkerk aan den IJssel, The Netherlands). Primer and probe sequences were optimized using the single nucleotide polymorphism assay-by-design service of Applied Biosystems.

Statistical Analysis

Values are presented as mean ± standard deviation (SD), median (range) or as numbers or proportions of patients. Deviation from Hardy-Weinberg Equilibrium was analysed using a X2-test. Dominant (Thr/Thr vs. Ala/X) and recessive (Thr/X vs. Ala/Ala) effects of the polymorphism were analyzed. The association between D2-Thr92Ala genotypes and thyroxine dosages or thyroid hormone levels were analyzed using multivariate regression analyses. This was corrected for age, gender, BMI and the natural logarithm of TSH levels. In addition, differences between the different D2 genotype groups were analyzed using unpaired t-test or ANCOVA. All calculations were performed using SPSS 12.0 for windows (SPSS, Inc., Chicago, IL). Differences were considered statistically significant at $P<0.05$.

Results

Patient characteristics

We studied 154 DTC patients. Mean duration of TSH suppressive therapy was 9.2 years (range 0.5-42.6 years). Median duration of cure was 8.9 years (range 1.0-41.8 years). The mean dose of thyroxine was 183 ± 51 µg/day. Mean thyroxine dose was 2.2 ± 1.0 µg/kg body weight. We also studied 141 patients with Hashimoto thyroiditis on thyroxine replacement therapy. Genotyping of the D2-Thr92Ala polymorphism failed in two subjects. The remaining 139 patients were treated with thyroxine for a mean duration of 7.3 ± 5.8 years. Mean thyroxine dose was 125 ± 46 µg/day.

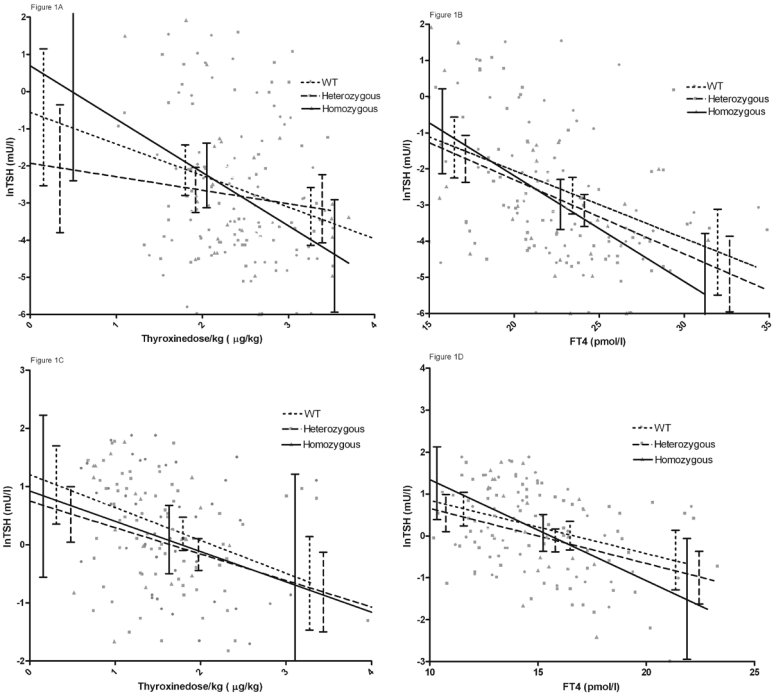
Thyroid hormone parameters and D2-Thr92Ala

Allele frequencies of the D2-Thr92Ala polymorphism in the DTC patients and Hashimoto thyroiditis patients were 39.6% and 40.3%, respectively. The genotype distributions did not deviate from Hardy-Weinberg equilibrium. Thyroid hormone levels and thyroxine dose for patients with DTC and Hashimoto thyroiditis are presented in Table 1. No differences were observed in thyroid hormone levels and thyroxine dose, corrected for BMI and TSH levels between wild-type, heterozygous and homozygous carriers of the D2-Thr92Ala polymorphism. Analyses were comparable when thyroxine dose was corrected for BMI. No differences were observed in the correlation between lnTSH and thyroxine dose/kg or FT4 level for the different carriers of the D2-thr92ala polymorphism (figure 1 A-D).

No differences were observed either in thyroid hormone levels or thyroxine dose when patients were categorized according to the degree of TSH suppression in the cohort of DTC patients.

Linear regression analysis showed no association between the D2-Thr92Ala polymorphism and thyroid hormone levels or thyroxine dose corrected for TSH levels and BMI (DTC: p=0.960, r=0.564, Hashimoto thyroiditis: p=0.274, r=0.302)

Figure 1.



a. Correlation between the natural logarithm of TSH and thyroxine dosage/kg for the different alleles of D2-Thr92Ala polymorphism in 154 patients with differentiated thyroid carcinoma. Lines: regression lines; bars: 95% confidence intervals of regression lines.

b. Correlation between the natural logarithm of TSH and FT4 for the different alleles of D2-Thr92Ala polymorphism in 154 patients with differentiated thyroid carcinoma. Lines: regression lines; bars: 95% confidence intervals of regression lines.

c.c. Correlation between the natural logarithm of TSH and thyroxine dosage/kg for the different alleles of D2-Thr92Ala polymorphism in 139 patients treated for Hashimoto thyroiditis.

d.d. Correlation between the natural logarithm of TSH and FT4 for the different alleles of D2-Thr92Ala poly morphism in 139 patients treated for Hashimoto thyroiditis.

Table 1. Deiodinase type 2 genotypes and thyroid hormone parameters.

	Genotype	Patients (n)	Age (yr)	Gender (m/f)	Weight (kg)	BMI (kg/m²)	TSH (mU/l)	FT4 (pmol/l)	T3 (nmol/l)	rT3 (nmol/l)	T4 dose (µg/day)	T4 dose (µg/kg)	Dose / kg x lnTSH
DTC	WT Thr/Thr	60	47.2±12.4	13/47	76.1±15.3	25.6±4.7	0.05 (0.003-4.6)	22.72±3.89	1.49±0.28	0.60±0.23	186.3±58.2	2.09±1.04	-6.74±5.28
	HeZ Ala/Thr	66	51.5±13.5	11/55	75.7±12.2	26.2±3.5	0.03 (0.003-4.9)	22.42±4.48	1.46±0.38	0.51±0.21	178.2±41.5	2.22±0.87	-6.81±4.96
	HoZ Ala/Ala	28	48.3±10.2	5/23	74.7±14.8	25.8±5.9	0.05 (0.003-6.8)	21.66±4.27	1.40±0.33	0.56±0.19	185.9±58.4	2.19±1.07	-7.82±5.67
	P-value	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS		
HT	WT Thr/Thr	47	46.6±8.6	5/42	78.5±17.8	27.8±5.4	2.02±1.76	14.46±2.87	1.73±0.36	ND	124.2±41.4	1.64±0.62	0.24±1.81
	HeZ Ala/Thr	72	47.3±10.9	11/61	80.4±18.3	28.2±5.6	1.59±1.41	15.16±3.05	1.73±0.30	ND	127.6±50.6	1.64±0.71	-0.22±2.01
	HoZ Ala/Ala	20	52.1±8.2	4/16	91.0±31.3	32.2±9.1	1.96±1.68	14.42±2.48	1.71±0.36	ND	116.3±38.1	1.36±0.51	0.17±1.45
	P-value	NS	NS	0.046	0.026	NS	NS	NS	NS	NS	NS	NS	NS

Table 1. Deiodinase type 2 genotypes and thyroid hormone parameters

DTC= Differentiated thyroid carcinoma, HT= Hashimoto thyroiditis, WT= wild-type, HeZ= Heterozygous, HoZ= Homozygous

Data are expressed as mean ± SD or number of patients, except for TSH which is median (range)

Analyses for TSH, FT4, T3 and T4 dose in HT patients are corrected for age, gender and BMI.

Discussion

We studied the association between the D2-Thr92Ala polymorphism and thyroid hormone levels and thyroxine dose in 2 separate groups of patients, treated for DTC or Hashimoto thyroiditis. Frequencies of the alleles of D2-Thr92Ala are in agreement with previous studies varying between 30-38.8 % in patient with normal thyroid function or not taking thyroid replacement or thyreostatic medication (3;4;6;7). The D2-Thr92Ala polymorphism was not associated with thyroid hormone parameters or thyroxine dosages in the 2 separate group of patients included in our analyses. This is in accordance with previous studies (4-7). Torlontano *et al*. found that homozygous DTC carriers of the D2-Ala92 allele need higher thyroxine dosages (8). This association was observed in the near-suppressed TSH group, but not in the suppressed group. The study of Torlontano *et al*. has however several limitations. TSH levels in the near-suppressed group of the different alleles were not given, which would have been useful to investigate whether the differences in thyroxine dose are not caused by alterations in TSH levels. In our study, no differences were observed in TSH levels or thyroxine dose for the different alleles with and without categorization according to the degree of TSH suppression in the DTC patients or Hashimoto patients. In addition, we believe that the analysis strategy should be primarily based on regression analysis rather than TSH categories, because for alterations in TSH levels should be corrected.

Remarkably, they did not find any differences in thyroid hormone levels suggesting that patients with D2-Ala92 alleles need a higher thyroxine dose to reach the same serum FT4 level. By inference, the Ala allele would not affect T4 feedback but rather T4 resorption. Torlontano *et al*. explain the discrepancies of their findings with previous studies by two arguments. First, they state that in previous studies thyroid hormone levels were within the wide reference range, which makes is difficult to detect subtle differences in thyroid hormone levels for different carriers of the D2-Thr92Ala polymorphism. However, they found this difference only in the near-suppressed group, which is an ill-defined group with a wide plasma TSH range including patients with normal TSH levels. Second, Torlontano *et al*. argue that the difference between their finding and earlier studies may be explained by the absence of a thyroid gland in their patients. However, in our analysis with athyroid DTC and Hashimoto patients, we could not confirm this. A posthoc power analysis for thyroxine dose and thyroxine/kg showed a sufficient power of 100 %. Therefore, it seems unlikely that underpowering of our study plays a major role in the negative findings.

In summary, we found no association between the D2-Thr92Ala polymorphism and thyroid hormone levels and thyroxine dose in 2 separate groups of patients treated for DTC or Hashimoto thyroiditis.

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12 Thr92Ala polymorphism in the type 2 deiodinase is not associated with thyroxinedose

13

Summary and General Discussion

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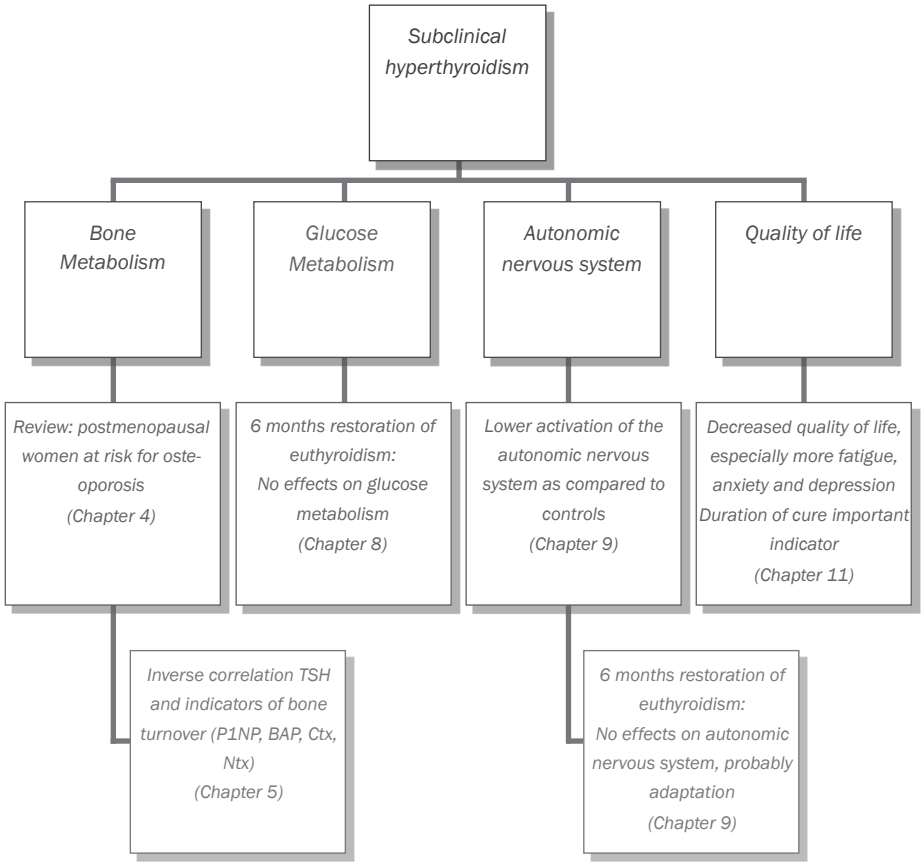
VIII. D2-Thr-92-Ala and thyroxin dose

I. Introduction

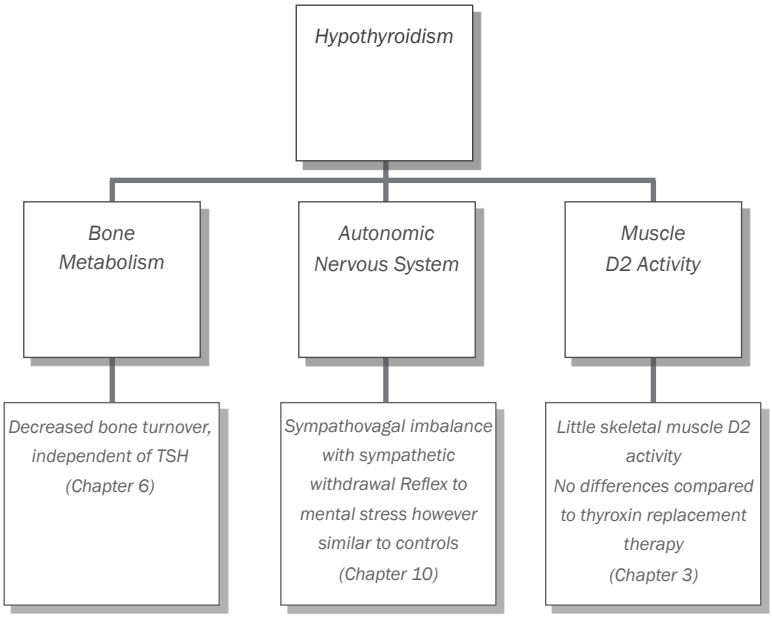
Patients with differentiated thyroid carcinoma (DTC) have an excellent prognosis due to the biological behaviour of the tumor as well as the efficacy of initial therapy. Although the recent publications of Dutch, European and American guidelines and consensus papers have improved the implementation of 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.

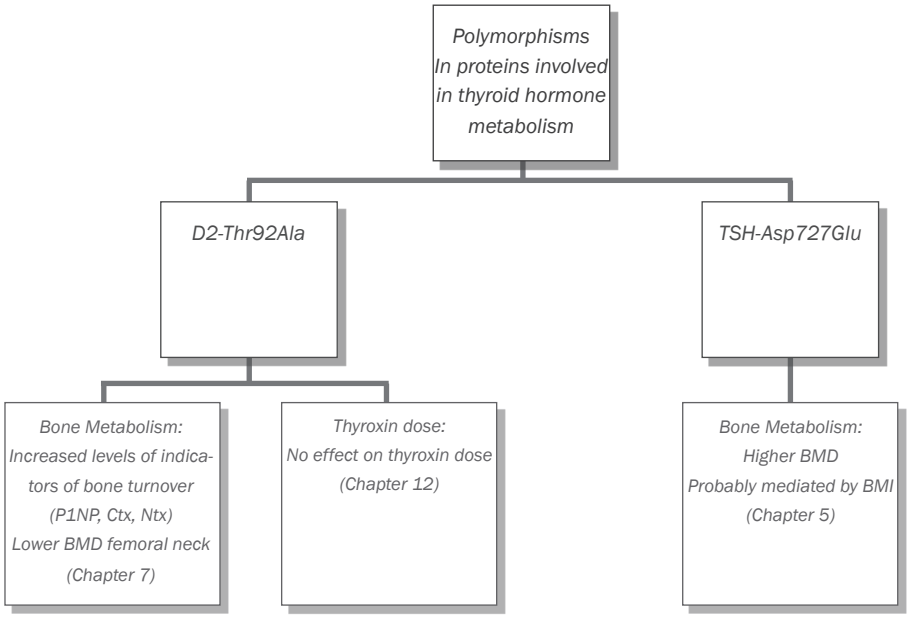
Clinical implications

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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Nederlandse samenvatting

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I. Inleiding

Patiënten met gedifferentieerd schildkliercarcinoom hebben een goede prognose. Dit is het gevolg van een combinatie van het biologische gedrag van de tumor en de effectiviteit van de initiële behandeling. Hoewel de implementatie van uniforme protocollen voor diagnose, behandeling en follow-up van gedifferentieerd schildkliercarcinoom is verbeterd door recente publicaties van Nederlandse, Europese en Amerikaanse richtlijnen, zijn er nog veel beperkingen aanwezig. Sommige beperkingen zijn het gevolg van het feit dat de prevalentie van gedifferentieerd schildkliercarcinoom laag is, wat het moeilijk maakt om gerandomiseerde trials uit te voeren. Andere beperkingen zijn het gevolg van het feit dat gedifferentieerd schildkliercarcinoom een bijzondere maligne ziekte is met fascinerende biologische fenomenen, zoals de pathofysiologie van het jodide-transport en (gecontroleerde) veranderingen in de schildklierhormoonwaarden. Deze zorgen er voor dat de meest algemene principes van de oncologie niet geëxtrapoleerd kunnen worden naar gedifferentieerd schildkliercarcinoom. Een ander probleem is de gedecentraliseerde aanpak van deze ziekte. Ondanks de lage incidentie behandelen vele centra patiënten met gedifferentieerd schildkliercarcinoom.
Er bestaan veel onzekerheden over de optimale follow-up strategie van gedifferentieerd schildkliercarcinoom. Het meeste onderzoek wordt gedaan naar de detectie en behandeling van gedifferentieerd schildkliercarcinoom. In dit proefschrift hebben we de diagnostische en prognostische waarde van thyreoglobuline onderzocht. Echter, het grootste gedeelte van het proefschrift richt zich op de consequenties van een thyreoïdectomie en schildklierhormoonsubstitutie op metabole eindpunten en op de kwaliteit-van-leven in patiënten met gedifferentieerd schildkliercarcinoom. Patiënten met gedifferentieerd schildkliercarcinoom worden behandeld met een hoge dosis schildklierhormoon om het TSH te onderdrukken, omdat TSH de groei van eventueel achtergebleven tumorcellen kan stimuleren. Dit weerspiegelt in feite een status van subklinische hyperthyreoïdie en kan gevolgen hebben voor het botmetabolisme, glucosemetabolisme, het autonome zenuwstelsel en de kwaliteit-van-leven.
Tijdens de follow-up van gedifferentieerd schildkliercarcinoom wordt schildklierhormoon soms onthouden aan patiënten of wordt de patiënt behandeld met recombinant humaan TSH om een eventuele tumorrest of recidief te ontdekken. Tijdens het onthouden van schildklierhormoon worden patiënten hypothyreoot, wat het botmetabolisme, glucosemetabolisme en autonome zenuwstelsel ook kan beïnvloeden.
Patiënten met gedifferentieerd schildkliercarcinoom vormen een uniek substitutiemodel om het effect van exogeen schildklierhormoon, overmaat en deficiëntie, op de hierboven genoemde systemen te onderzoeken. In dit proefschrift worden de effecten van exogene subklinische hyperthyreoïdie en het onthouden van schildklierhormoon op het botmetabolisme, glucosemetabolisme, autonome zenuwstelsel en kwaliteit-van-leven besproken. Tevens zal de deiodinase 2 activiteit in skeletspierweefsel tijdens hypothyreoïdie worden besproken. Verder zal het effect van het D2-Thr92Ala polymorfisme op het botmetabolisme en de gevolgen voor de toe te dienen schildklierhormoondosis worden bediscussieerd.

II. Diagnostische & prognostische waarde van thyreoglobuline

Serum thyreoglobuline (Tg) metingen zijn de belangrijkste marker in de follow-up van gedifferentieerd schildkliercarcinoom. Een Europese consensus heeft geadviseerd om institutionele Tg afkapwaarden te definiëren. Daarnaast is de prognostische waarde van Tg nauwelijks onderzocht.

Daarom hebben we de prognostische en diagnostische waarde van Tg metingen voor recidief en overlijden onderzocht in een groot cohort van patiënten met gedifferentieerd schildkliercarcinoom met behulp van ROC curven (hoofdstuk 2). Onze resultaten tonen een hoge sensitiviteit (100 %) van Tg-waarden onder TSH stimulatie 6 maanden na initiële behandeling met een hogere Tg-afkapwaarde (10.0 µg/l) dan normaal wordt gebruikt. De specificiteit en de positief voorspellende waarde daalden aanzienlijk wanneer de meer gangbare Tg-afkapwaarde van 2.0 µg/l werd gebruikt. Voordelen van onze studie zijn de homogeniteit van de studiepopulatie met betrekking tot de initiële behandeling, het feit dat meerdere Tg metingen werden geanalyseerd op specifieke tijdstippen tijdens de follow-up en het gebruik van ROC-curven tijdens de analyse. De hogere Tg-afkapwaarde kan verklaard worden door het lagere initiële I-131 ablatiepercentage in ons instituut vergeleken met andere instituten. Een Tg-waarde vóór de I-131 ablatie kleiner dan 27.5 µg/l voorspelt curatie onafhankelijk van andere prognostische factoren zoals stadium T4, folliculaire histologie, metastasen en hogere leeftijd. TSH gestimuleerde Tg metingen 6 maanden na initiële behandeling en 2 en 5 jaar na initiële behandeling waren onafhankelijke voorspellers van overlijden gerelateerd aan gedifferentieerd schildkliercarcinoom . We vonden een specificiteit van minder dan 100 %, welke verklaard kan worden door de beperkingen van de huidige beeldvormende technologieën om tumor recidief te ontdekken. Wij zijn het daarom eens met het beleid om patiënten met verhoogde Tg-waarden te behandelen met een hoge dosis I-131. Verder, geloven wij dat Tg zou moeten worden gezien als een risico-indicator en daarom zou moeten worden geïncludeerd in het panel van risicofactoren. Een beperking van onze studie is dat de analyses gebaseerd zijn op retrospectieve data. De prognostische waarde van Tg afkapwaarden moet daarom met voorzichtigheid worden geïnterpreteerd en hiernaar zal prospectief onderzoek moeten worden verricht.

Klinische implicaties
Onze studie toont aan dat het definiëren van institutionele Tg-afkapwaarden belangrijk is in de follow-up van gedifferentieerd schildkliercarcinoom. Een Tg-meting onder TSH stimulatie 6 maanden na initiële behandeling heeft een uitstekende diagnostische waarde. Tevens zijn Tg-waarden onafhankelijke prognostische indicators van ziektevrije regressie en overlijden en door deze strategie te gebruiken kunnen groepen patiënten met een hoog risico worden geïdentificeerd als aanvulling op de conventioneel gebruikte prognostische indicatoren.

Perspectief
De methodologische tekortkomingen en de inter-institutionele verschillen van de Tg-metingen creëren de behoefte aan nieuwe methodes om tumorrecidief te ontdekken. Naar onze mening moet harmonisatie van Tg-metingen tussen instituten, op nationaal en internationaal niveau, worden nagestreefd. RT-PCR die cellen die schildklier specifieke eiwitten, zoals TPO, produceren detecteert, zou een nieuwe marker kunnen zijn om tumorrecidieven te detecteren. Echter, meer onderzoek naar de rol van deze nieuwe methoden in de follow-up van gedifferentieerd schildkliercarcinoom is nodig.

III. Deiodinase 2 activiteit in skeletspier tijdens hypothyreoïdie

Recentelijk is er beschreven dat D2 een grote rol speelt in de T3 productie tijdens euthyreoïdie en hierdoor kan D2 ook een belangrijke rol spelen tijdens hypothyreoïdie. De perifere conversie van T4 naar T3 is verhoogd tijdens hypothyreoïdie, wat het resultaat kan zijn van verhoogde D2-activiteit.

In ratten werd er een verhoogde conversie van T4 naar T3 door D2 aangetoond tijdens hypothyreoïdie. Dierenstudies tonen een significant verhoogde D2-activiteit in de cerebrale cortex en hypofyse aan tijdens hypothyreoïdie. Omdat D2-activiteit is gevonden in skeletspier van de mens, hebben we D2-activiteit en expressie van D2 en D3 mRNA in skeletspier tijdens hypothyreoïdie en na schildklierhormoonsubstitutie onderzocht in patiënten met gedifferentieerd schildkliercarcinoom (hoofdstuk 3). We vonden weinig D2-activiteit in skeletspier en geen verschillen tussen hypothyreoïdie en na schildklierhormoonsubstitutie. Om een interfererend effect van lidocaïne op D2-activiteit uit te sluiten, werd de D2-activiteit in D2-COS1-cellen bij stijgende lidocaïne concentraties gemeten. Er werden geen verschillen in D2-activiteit waargenomen. Desondanks kunnen we een locaal direct effect van lidocaïne, dat tot een verlaging van de D2-activiteit zou kunnen leiden, niet uitsluiten. Er werden geen verschillen gevonden tussen de expressie van D2 of D3 mRNA in skeletspier tijdens hypothyreoïdie en schildklierhormoonsubstitutie. Daarom is een verhoogde D2-activiteit in andere weefsels waarschijnlijk verantwoordelijk voor de verhoogde omzetting van T4 naar T3 in hypothyreote mensen.
Samenvattend, D2-activiteit en -expressie worden niet beïnvloed door de schildklierhormoonstatus. De waargenomen lage D2-activiteit maakt de relevantie van D2-activiteit in skeletspier van de mens op de T3 productie buiten de schildklier twijfelachtig.

IV. Botmetabolisme

Het effect van subklinische hyperthyreoïdie op het botmetabolisme is een relevant vraagstuk, want patiënten met gedifferentieerd schildkliercarcinoom hebben een goede prognose en worden daarom langdurig behandeld met TSH-onderdrukkend schildklierhormoonsubstitutie. Bestaande studies zijn inconsistent door gebreken in studieontwerp, de geïncludeerde patiëntengroepen, de gebruikte methodologie, de duur van de follow-up en de keuze van uitkomstparameters. Hoewel er meerdere studies over dit onderwerp zijn verschenen, zijn er geen studies geweest die deze studies categoriseren naar de hierboven genoemde parameters. Daarom bespreken wij systematisch alle studies die het effect van subklinische hyperthyreoïdie op het botmetabolisme onderzochten met het zwaartepunt op veranderingen in botdichtheid (hoofdstuk 4).
De meeste studies in premenopausale vrouwen en mannen lieten geen veranderingen in de botdichtheid zien tijdens TSH onderdrukkende behandeling, terwijl enkele studies in postmenopausale vrouwen wel een verandering in botdichtheid aangeven. Dit kan erop wijzen dat er een klinisch effect is van TSH onderdrukkende behandeling op de botdichtheid in postmenopausale vrouwen. Andere studies laten geen verandering in de botdichtheid in postmenopausale vrouwen zien. Dit kan het gevolg zijn van de instabiliteit van TSH-waarden van jaar tot jaar of van verschillen in additionele determinanten van de botdichtheid zoals voedselinname, lichaamsbeweging, endogene factoren of genetische aanleg. Deze determinanten worden relevant als de bijdrage van de oestrogenen is verdwenen in postmenopausale vrouwen. Het gebrek aan oestrogenen resulteert in een verhoogde vrijlating van cytokines en daardoor een verhoogde productie van M-CSF en RANKL, welke essentiële cytokines zijn voor de botvorming en de activatie van osteoclasten. Subklinische hyperthyreoïdie kan dit effect vergroten. TSH kan de RANKL mRNA-waarden verlagen en de OPG mRNA-waarden verhogen, wat leidt tot een verlaagde osteoclastvorming en -overleving. TSH wordt onderdrukt tijdens subklinische hyperthyreoïdie, wat kan leiden tot een verminderde remming van de botresorptie.

We onderzochten ook de relatie tussen TSH en indicators van het botmetabolisme in 148 patiënten met gedifferentieerd schildkliercarcinoom (hoofdstuk 5). Het voordeel van een studie in patiënten met gedifferentieerd schildkliercarcinoom is dat deze patiënten meer uniforme schildklierhormoonwaarden hebben. We vonden een significante omgekeerde relatie tussen serum TSH-waarden en parameters van zowel botvorming (bot-specifiek alkalisch fosfatase (BAP) en procollageen type 1 aminoterminal propeptide (P1NP)) als parameters van botresorptie (C-cross-linkende terminal telopeptide van type 1 collageen (Ctx) en N telopeptide van collageen cross-links (Ntx)), onafhankelijk van serum schildklierhormoonwaarden. Er werd geen relatie gevonden tussen TSH-waarden en botdichtheid. Dit kan verklaard worden door het feit dat botdichtheid bepaald wordt door levenslange processen, terwijl de parameters van het botmetabolisme de korte termijn effecten reflecteren. Om instabiliteit van de TSH-waarden van jaar tot jaar te ontdekken werd de gradiënt van de TSH-waarden per patiënt berekend. De gemiddelde gradiënt was -0.0001 (bereik -0.0004 tot 0) per jaar, wat aantoont dat de TSH-waarden stabiel waren. Het TSH-Asp727Glu polymorfisme wordt geassocieerd met lagere TSH-waarden zonder verschillen in schildklierhormoonwaarden. Omdat patiënten met gedifferentieerd schildkliercarcinoom meer uniforme TSH- en schildklierhormoonwaarden hebben, onderzochten we de relatie tussen het TSH-ASP727Glu polymorfisme en botmetabolisme. Een significant hogere botdichtheid werd gevonden in dragers van het polymorfisme. Echter, na correctie voor body mass index was deze relatie niet meer significant, wat suggereert dat het effect van het polymorfisme wordt gemedieerd via de body mass index. Dit is in overeenstemming met de studie van Van der Deure *et al*.

Er bestaat echter onduidelijkheid over de bijdrage van TSH aan de botdichtheid en aan het botmetabolisme. Bassett *et al*. vonden dat muizen die geen schildklierhormoonreceptor-α hebben (TRα knock-out) osteosclerose met een verminderde botresorptie door de osteoclasten hebben in de aanwezigheid van normale schildklierhormoon- en TSH-waarden. Muizen die geen schildklierhormoonreceptor-β hadden (TRβ knock-out), waren osteoporotisch in de aanwezigheid van verhoogde schildklierhormoon- en TSH-waarden. T3-gen-expressie toonde hypothyreoïdie in het skelet van de TRα knock-out muizen, terwijl de TRβ knock-out muizen hyperthyreoïdie van het skelet toonden. Bassett *et al*. beschreven ook dat Pax -/- muizen en hyt/hyt muizen -twee muizenmodellen voor congenitale hypothyreoïdie waarbij de feedback tussen TSH en schildklierhormoon intact of beschadigd is- beiden verlate verkalking, verminderd corticaal bot, een gebrek in de trabeculaire bothervorming en verminderde botmineralisatie vertoonden, wat er op wijst dat de effecten van congenitale hypothyreoïdie op het botmetabolisme onafhankelijk van TSH zijn. Verder toonden zij aan dat osteoblasten en osteoclasten een TSH-receptor hebben, maar TSH had geen effect op de cAMP response, differentiatie of functie.

Om de effecten van gecontroleerde hypothyreoïdie op het botmetabolisme te onderzoeken en om onderscheid te maken tussen de effecten gemedieerd door de verlaagde schildklierhormoonwaarden versus de effecten gemedieerd door de hoge TSH-waarden, onderzochten we 11 patiënten met gedifferentieerd schildkliercarcinoom tijdens het korte termijn onthouden van schildklierhormoon en 8 weken na het herstarten van schildklierhormoonsubstitutie versus 11 leeftijd-, geslacht- en BMI- gematchte patiënten met gedifferentieerd schildkliercarcinoom na injectie met recombinant menselijk TSH (rhTSH), wat leidt tot hoge TSH-waarden met ongewijzigde schildklierhormoonwaarden (hoofdstuk 6). Voor zo ver wij weten, is dit de eerste studie die beide situaties vergelijkt in leeftijd-, geslacht- en BMI-gematchte patiënten. Tijdens hypothyreoïdie werden er significant lagere Ctx-waarden gevonden met significant hogere OPG-waarden, wat wijst op een verminderde botresorptie. Dit stemt overeen met andere studies. Na injectie met rhTSH werden er geen verschillen in parameters van het botmetabolisme gevonden. Een recente studie vond een zwakke expressie van de TSH-receptor in menselijke osteoblasten. Dit kan onze bevindingen verklaren.

D2-activiteit is ook gevonden in osteoblasten, waardoor er een mogelijke rol voor D2 in het botmetabolisme kan zijn. Omdat het D2-Thr92Ala polymorfisme wordt geassocieerd met een verminderde enzymsnelheid, onderzochten wij de relatie tussen het D2-Thr92Ala polymorfisme en botmetabolisme in patiënten met gedifferentieerd schildkliercarcinoom (hoofdstuk 7). We vonden een 6% lagere botdichtheid van de heup in de homozygote dragers van het D2-Thr92Ala polymorfisme. Tevens werden er verhoogde parameters van botvorming (P1NP) en botresorptie (Ctx en Ntx) gevonden in de homozygote dragers van het polymorfisme onafhankelijk van andere determinanten van het botmetabolisme zoals leeftijd, geslacht, BMI, oestrogeen status, calcium- en vitamine D-intake en onafhankelijk van TSH. Deze relatie was ook onafhankelijk van de schildklierhormoonwaarden wat wijst op een reëel effect van het D2-Thr92Ala polymorfisme.
Er werd geen relatie gevonden tussen het D2-Thr92Ala polymorfisme en de botdichtheid van de lumbale wervelkolom. Dit kan verklaard worden door het feit dat botdichtheidmetingen van de lumbale wervelkolom kunnen worden beïnvloed door artrose en daarom niet nauwkeurig kunnen worden bepaald. Een recente studie vond een relatie tussen het D2-Thr92ala polymorfisme en artrose, wat de rol van D2 in het botmetabolisme versterkt. We hadden verwacht een lagere bot-turnover te vinden in homozygote dragers van het D2-Thr92Alal polymorfisme, omdat het D2-Thr92Alal polymorfisme wordt geassocieerd met een lagere enzymsnelheid in vitro in de schildklier en skeletspier, omdat T3 de osteoblast proliferatie stimuleert en omdat T3 zich primair richt op de osteoblasten. We vonden echter een verhoogde bot-turnover in de homozygote dragers van het D2-thr92Ala polymorfisme. D2-activiteit is gevonden in volgroeide osteoblasten, echter niet in osteoclasten. De verhoogde botresorptie kan daarom niet verklaard worden door een direct effect op osteoclasten, maar waarschijnlijk wel, indirect, door veranderingen in de interactie tussen osteoblasten en osteoclasten. Deze studie laat zien dat de relatie tussen de lokale T3-beschikbaarheid door D2-activiteit en botmetabolisme complex is en niet kan worden verklaard door de traditioneel waargenomen directe effecten van T3 op bot. Vele bekende en onbekende componenten van het micromilieu van het bot kunnen hierbij betrokken zijn. Wij geloven dat onze bevindingen in andere studies moeten worden herbevestigd. Echter, aangezien we multipele regressie analyses gebruiken om deze associatie te onderzoeken en aangezien we gecorrigeerd hebben voor alle covariaten geloven wij dat deze studie belangrijke data kan toevoegen met betrekking tot de rol van D2 en het D2-Thr92Ala polymorfisme op het botmetabolisme.
We kunnen concluderen dat er geen effect werd gevonden van TSH suppressieve therapie op de botdichtheid in mannen en premenopausale vrouwen. Echter, postmenopausale vrouwen lopen een groter risico op osteoporose. Hoewel we een relatie tussen TSH en parameters van het botmetabolisme vonden in patiënten met gedifferentieerd schildkliercarcinoom, werd er geen effect gevonden van TSH op het botmetabolisme tijdens stimulatie met TSH. Dit is consistent met de studie van Bassett *et al*. in muizen. Een mogelijke verklaring voor dit verschil zou kunnen zijn dat in de cross-sectionele studie waarin we de relatie tussen schildklierhormoonwaarden en indicatoren van botmetabolisme onderzochten postmenopausale vrouwen werden geïncludeerd, terwijl in de hypothyreoïdie-studie maar 2 postmenopausale vrouwen werden bestudeerd. Een andere verklaring zou het verschil in studieontwerp kunnen zijn, aangezien de effecten van hypothyreoïdie op korte-termijn (4 weken) werden onderzocht, terwijl in de cross-sectionele studie patiënten met langdurige (mediaan (range) 9.3 (1.2-43) jaar) TSH suppressieve therapie werden behandeld. Wij geloven dat de veranderingen in schildklierhormoonwaarden een belangrijker effect hebben op het botmetabolisme dan veranderingen in TSH-waarden. We vonden dat homozygote dragers van het D2-Thr92Ala polymorfisme een lagere botdichtheid van de femur hadden met verhoogde parameters van de bot turnover. Deze resultaten tonen aan dat D2 een functionele rol heeft in mensen voor de locale beschikbaarheid van T3 in bot. Echter, deze associatie is complex en wordt waarschijnlijk indirect verklaard door een interactie tussen osteoclasten en osteoblasten.

Klinische implementatie
Onze bevindingen wijzen op een groter risico op osteoporose in postmenopausale vrouwen die behandeld worden met TSH suppressieve therapie. In deze patiënten is screening op baseline en tijdens regelmatige intervallen tijdens de TSH suppressieve therapie geadviseerd om vroegtijdig te kunnen behandelen met botbeschermers. Aangezien we een relatie tussen TSH en parameters van de bot-turnover hebben aangetoond, geloven wij dat herstel van euthyreoïdie moet worden gepropageerd in patiënten met een laag risico op recidief of na langdurige follow-up.

V. Glucose metabolisme

Er is maar weinig data beschikbaar over de gevolgen van subklinische hyperthyreoïdie op het glucosemetabolisme. Daarom hebben we een prospectieve, gerandomniseerde placebo-gecontrolleerde studie uitgevoerd om de effecten van herstel van euthyreoïdie na langdurige subklinische hyperthyreoïdie op het glucosemetabolisme te onderzoeken (Hoofdstuk 8). We vonden geen effect van herstel van euthyreoïdie op parameters van het glucose- en vetmetabolisme. Het percentage van patiënten met een gestoorde glucosetolerantie, gemeten met de orale glucose tolerantie test, was 15.4 % in onze studie, en is vergelijkbaar met vorige studies in Nederland en Amerika (10.3 % en 15.6%). Yavuz *et al*. vonden, in tegenstelling tot onze studie, een verminderde insuline gevoeligheid index na 6 maanden van exogene subklinische hyperthyreoïdie vergeleken met gematchte gezonde vrijwilligers. De verschillen in uitkomst zouden kunnen worden verklaard door verschillen in de duur van de hyperthyreoïdie. We onderzochten een populatie die meer dan 10 jaar behandeld was met subklinische hyperthyreoïdie, terwijl Yavuz *et al*. 20 patiënten onderzocht die 6 maanden behandeld werden. Een andere verklaring zou kunnen zijn dat de “dosis”(de mate van subklinische hyperthyreoïdie) in onze studie niet hoog genoeg was om te resulteren in een “response” (glucose intolerantie). Echter, deze verklaring is onwaarschijnlijk, omdat de TSH-waarden in onze studie vergelijkbaar zijn met de waarden van Yavuz *et al*.
Concluderend, we vonden geen effect op glucose- en vetmetabolisme van herstel van euthyreoïdie in patiënten die langdurig (>10 jaar) behandeld werden met TSH suppressieve therapie, wat mogelijk kan wijzen op adaptatie van het glucosemetabolisme na langdurige TSH suppressieve therapie.

VI. Autonome zenuwstelsel

In de literatuur zijn de consequenties van subklinische hyperthyreoïdie op het autonome zenuwstelsel niet duidelijk. Bovendien zijn de meeste studies uitgevoerd in heterogene patiëntenpopulaties waarin de duur en het beloop van de subklinische hyperthyreoïdie niet bekend zijn. Verder kan het gebruik van thyreostatica en β-blokkers ook invloed hebben op het autonome zenuwstelsel. Daarom hebben wij de eerste prospectieve, gerandomniseerde, placebo-gecontrolleerde studie verricht naar het effect van het herstel van euthyreoïdie op het autonome zenuwstelsel in patiënten met langdurige exogene subklinische hyperthyreoïdie (hoofdstuk 9).
We vonden dat subklinische hyperthyreoïdie werd geassocieerd met een lagere activatie van het autonome zenuwstelsel vergeleken met gezonde vrijwilligers, terwijl de activatie hoger was vergeleken met patiënten met hyperthyreoïdie.

De cathecholamine-excretie in de urine was hoger tijdens subklinische hyperthyreoïdie vergeleken met gezonde vrijwilligers, terwijl deze lager was vergeleken met hyperthyreoïdie.
Herstel van euthyreoïdie in patiënten met langdurige subklinische hyperthyreoïdie door TSH suppressieve therapie had geen effect op het autonome zenuwstelsel. Er werd geen verschil gezien in de cathecholamine-excretie in de urine, parameters van hartritme variabiliteit en de response op een mentale stress test tussen patiënten die TSH gesupprimeerd bleven en patiënten in wie euthyreoïdie werd hersteld. Hoewel de TSH-waarden van alle patiënten in de interventiegroep (herstel van euthyreoïdie) binnen de normale range kwamen met een daling van het FT4 en FT3, resulteerde het herstel van euthyreoïdie in patiënten die meer dan 10 jaar behandeld werden met TSH suppressieve therapie niet in veranderingen in de activatie van het autonome zenuwstelsel. Een verklaring zou kunnen zijn dat tijdens langdurige TSH suppressieve therapie irreversibele veranderingen of adaptatie ontstaat, zoals we hebben gezien in het glucosemetabolisme, of dat herstel van de setpoints van het autonome zenuwstelsel langer duurt dan 6 maanden.
In de patiënten die 8 weken behandeld zijn met schildklierhormoonsubstitutie na kortdurende hypothyreoïdie was het LF component significant hoger vergeleken met gezonde euthyreote vrijwilligers, terwijl er geen verschillen werden gevonden in het HF component of de LF/HF ratio. Andere studies hebben een sympatovagale onevenwichtigheid getoond tijdens (subklinische) hyperthyreoïdie met een verhoogde sympaticus activiteit en een verlaagde vagale tonus gekenmerkt door een verhoogde LF component en een verlaagde HF component resulterend in een verhoogde LF/HF ratio. Een mogelijke verklaring voor het feit dat het HRV spectrum kenmerken heeft van hyperthyreoïdie terwijl de gemiddelde TSH-waarde binnen de referentiewaarden valt, zou kunnen zijn dat de patiënten langdurig zijn behandeld met TSH supprimerende schildklierhormoonsubstitutie en dit kan geleid hebben tot irreversibele veranderingen of adaptatie van het autonome zenuwstelsel. Dit komt overeen met onze eerder genoemde studie. We merkten echter ook op dat er een significant verschil was in de hele lage frequentie component tussen patiënten en gezonde vrijwilligers, wat ook onze bevindingen van de HF en LF component kan hebben beïnvloed.
De response op een uitdaging van het autonome zenuwstelsel, die de meest prominente verschillen in het autonome zenuwstelsel lijkt te openbaren, werd ook onderzocht. We vonden geen verschil in de response op een mentale stress test, die een gevalideerde test is om het autonome zenuwstelsel te onderzoeken, tijdens schildklierhormoonsubstitutie vergeleken met gezonde vrijwilligers.
De impact van hypothyreoïdie op het autonome zenuwstelsel is onduidelijk. De huidige literatuur laat tegenstrijdige resultaten zien met een verhoogde sympaticus activiteit, een verlaagde sympaticus modulatie of een verhoogde vagale tonus. Daarom hebben we het effect van kortdurende gecontroleerde hypothyreoïdie en herstarten van schildklierhormoonsubstitutie op het autonome zenuwstelsel onderzocht door hartritme variabiliteit te meten in rust en na een mentale stress test (Hoofdstuk 10). We vonden een verlaagde LF/HF ratio met een verlaagde LF component en een verhoogde HF component tijdens hypothyreoïdie vergeleken met schildklierhormoonsubstitutie, wijzende op een sympatovagale onevenwichtigheid met een verlaagde sympaticus activiteit en een verhoogde vagale tonus tijdens hypothyreoïdie vergeleken met schildklierhormoonsubstitutie. Dit komt overeen met de literatuur. Er werden geen verschillen gezien in de response op een mentale stress test tijdens hypothyreoïdie vergeleken met gezonde vrijwilligers.
Samengevat, langdurige exogene subklinische hyperthyreoïdie heeft invloed op het autonome zenuwstelsel, gemeten met hartritme variabiliteit en de urine excretie van catecholamines. Zes maanden herstel van euthyreoïdie toonde geen veranderingen in het autonome zenuwstelsel, wat er op wijst dat langdurige TSH suppressieve therapie kan resulteren in irreversibele veranderingen of adaptatie.

Kortdurende gecontroleerde hypothyreoïdie werd geassocieerd met een sympatovagale onevenwichtigheid met een verminderde sympathicus activiteit. De cardiovasculaire reflex op (mentale) stress was echter onveranderd.

Klinische implementaties
Onze bevindingen wijzen op een adaptatie van het autonome zenuwstelsel na langdurige TSH suppressieve therapie, welke niet hersteld 6 maanden na herstel van euthyreoïdie. Daarom adviseren wij om de noodzaak van TSH suppressieve behandeling in patiënten met gedifferentieerd schildkliercarcinoom zorgvuldig te overwegen per patiënt.

VII. Kwaliteit-van-leven

Kwaliteit-van-leven kan beïnvloed zijn in patiënten met gedifferentieerd schildkliercarcinoom door enerzijds de diagnose en initiële behandeling en anderzijds de TSH suppressieve behandeling. Er zijn verscheidene studies gedaan naar dit onderwerp, echter de resultaten zijn niet eenduidig. Daarom hebben we kwaliteit-van-leven onderzocht in een groot cohort van genezen patiënten met gedifferentieerd schildkliercarcinoom gebruik makend van verschillende kwaliteit-van-leven vragenlijsten en hebben we deze resultaten vergeleken met de resultaten van gematchte gezonde vrijwilligers (hoofdstuk 11).
Onze bevindingen tonen een verminderde kwaliteit-van-leven in patiënten met genezen gedifferentieerd schildkliercarcinoom, wat mogelijk hersteld kan worden na jaren van follow-up. Een langere duur van genezing werd geassocieerd met betere scores op de verschillen kwaliteit-van-leven onderdelen. Voordelen van onze studie zijn het grote aantal patiënten geïncludeerd in de studie, het gebruik van verschillende kwaliteit-van-leven vragenlijsten en de vergelijking met gematchte gezonde vrijwilligers.
De gevolgen van subklinische hyperthyreoïdie op de kwaliteit-van-leven zijn ook onduidelijk. De studies die dit onderwerp hebben bestudeerd includeerden geselecteerde groepen patiënten met gedifferentieerd schildkliercarcinoom of patiënten met endogene subklinische hyperthyreoïdie waarin de duur en het beloop van de subklinische hyperthyreoïdie niet bekend zijn. In onze studie was kwaliteit-van-leven niet beïnvloed door veranderingen in TSH-waarden ten tijde van het onderzoek of gedurende de tijd sinds initiële behandeling.
Samengevat, kwaliteit-van-leven wordt beïnvloed in een groot cohort van genezen patiënten met gedifferentieerd schildkliercarcinoom vergeleken met gematchte gezonde vrijwilligers. Alleen een langere duur van genezing kan deze effecten normaliseren.

Klinische implementaties
Artsen moeten er op bedacht zijn dat, ondanks de goede prognose, het psychologische welzijn van genezen patiënten met gedifferentieerd schildkliercarcinoom beïnvloed kan zijn en dat professionele hulp moet worden aangeboden wanneer deze noodzakelijk is.

VIII. D2-Thr92Ala polymorfisme en schildklierhormoon

Er bestaat onenigheid over de functionele implicaties van het D2-Thr92Ala polymorfisme. Daarom hebben we de associatie tussen het D2-Thr92Ala polymorfisme, schildklierhormoonwaarden en de schildklierhormoon onderzocht in 2 separate groepen patiënten: patiënten zonder schildklier die behandeld zijn voor gedifferentieerd schildkliercarcinoom en patiënten met Hashimoto thyreoïditis (hoofdstuk 12).

Het D2-Thr92Ala polymorfisme was niet geassocieerd met schildklierhormoonwaarden en schildklierhormoon in de 2 separate groepen van patiënten in onze analyse, wat overeenkomt met de resultaten uit andere studies. Echter, Torlontano *et al*. berichtten dat homozygote dragers van het D2-Thr92Ala polymorfisme een hogere dosis schildklierhormoon nodig hebben. Dit verschil was aanwezig in de bijna-TSH suppressieve groep, maar niet in de TSH suppressieve groep. Beperkingen van deze studie zijn de afwezigheid van data over TSH-waarden in de bijna-TSH suppressieve groep en de analyse strategie, die primair gericht zou moeten zijn regressie analyses in plaat van categorieën naar TSH-waarden. Het is opmerkelijk dat Torlontano *et al*. geen verschillen in schildklierhormoonwaarden vond, wat er op wijst dat patiënten met het D2-Thr92Ala polymorfisme een hogere schildklierhormoon nodig hebben om dezelfde FT4 waarden te bereiken wat suggereert dat h*et Ala* allel de schildklierhormoon absorptie beïnvloed in plaats van de schildklierhormoon feedback. Bovendien werd deze associatie tussen het D2-Thr92Ala polymorfisme en schildklierhormoon alleen gevonden in de bijna-TSH suppressieve groep, wat een slecht gedefinieerde groep is met een wijde range in TSH-waarden waarin ook patiënten met normale TSH-waarden zitten.
In conclusie, er werd geen associatie gevonden tussen het D2-Thr92Ala polymorfisme en schildklierhormoon in patiënten met gedifferentieerd schildkliercarcinoom zonder schildklier en in patiënten met Hashimoto thyreoïditis.

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Curriculum vitae

Karen Anne Heemstra werd geboren op 7 februari 1982 in Weert. Zij behaalde in 2000 haar Gymnasium diploma aan de Philips van Horne Scholengemeenschap in Weert. Hierna verbleef zij een jaar in Bergen, Noorwegen op Fana Følkehøgskule, waar zij outdoor life en ecologie bestudeerde. In 2001 werd zij via de decentrale selectie geselecteerd om geneeskunde te mogen studeren aan de Universiteit Leiden. Tijdens deze studie volgde zij vakken aan het Karolinska Institutet in Stockholm, Zweden. In 2005 begon zij via het excellente studententraject met promotieonderzoek op de afdeling endocrinologie van het Leids Universitair Medisch Centrum onder begeleiding van Prof. Dr. J.W.A. Smit, Prof. Dr. J.A. Romijn, en Dr. E.P.M. van der Kleij-Corssmit. Zij verrichtte onderzoek naar de klinische effecten van schildklierhormoon op het metabolisme. In 2008 kreeg zij de Medical Student Achievement Award van de Endocrine Society. Tevens was zij winnaar van de S.E. de Jonghprijs. Zij behaalde haar artsenbul in maart 2008. Hierna werkte zij als arts-onderzoeker op de afdeling endocrinologie. Per 1 juli is zij begonnen met de opleiding tot arts-microbioloog in het Universitair Medisch Centrum Utrecht (Opleiders: Dr. A.J.L. Weersink, Prof. Dr. J. Verhoef).

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