The long-term consequences of previous hyperthyroidism. A register-based study of singletons and twins

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1. INTRODUCTION AND STUDY AIMS

Biochemically hyperthyroidism is defined by decreased levels of thyrotropin stimulating hormone (TSH) in combination with elevated levels of thyroxine (T4) and/or triiodothyronine (T3). The hyperthyroid state might be preceded by subclinical hyperthyroidism, which is defined by decreased levels of TSH, but with T4 and T3 levels within the reference range (1). Thyroid hormones affect all organ systems, and the effects on the cardiovascular system have been especially highlighted. Hyperthyroidism is often associated with sinus tachycardia (2) and, short-term hyperthyroidism is characterised by a hyperdynamic cardiovascular state (high cardiac output with low systemic resistance) (3). On the other hand, long-term exposure to elevated thyroid hormone levels may lead to diastolic dysfunction and heart failure (4). Moreover, thyroid hormones have a direct vascular effect and it has been suggested that elevated levels triggers endothelial dysfunction (5) and hypercoagulation (6).

Despite our pathophysiological knowledge, well described hyperthyroid phenotypes, as well as treatment modalities such as antithyroid medication, radioiodine and surgery, which have been known for more than 50 years (7), our knowledge regarding the long-term consequences of hyperthyroidism with respect to morbidity and mortality is poor. Studies considering hyperthyroidism and mortality are only partly comparable due to different hyperthyroid phenotype distributions, selections of cases based on different treatment modalities, a lack of relevant control-groups and the consideration of different confounders. Also, when considering hyperthyroidism and morbidity, the majority of studies are underpowered association studies with inconsistent confounding controls, hindering any firm conclusions.

An important overlooked confounder is genetic susceptibility. Since not only hyperthyroidism (8,9) but also various morbidities (10,11) and life-span (12,13) demonstrate familial clustering. It follows that, if some of the genes linked with mortality and/or morbidity are also involved in the development of hyperthyroidism, the observed associations between these conditions could, at least partly, be due to the presence of shared genetic factors (genetic confounding). Only the study of twin pairs discordant on exposure, and in particular discordant monozygotic twins, provides a useful approach to control for genetic confounding (14). Unfortunately, such studies are not available with regard to hyperthyroidism and mortality/morbidity.

Denmark has a long tradition of storing information on its citizens in nation-wide registers, mainly for administrative purposes. This has resulted in various databases holding information on demographics, health, and mortality, to name but a few (15). Separately, all twin births have been registered in The Danish Twin registry (DTR) (16). DTR comprises nearly 85,000 twin pairs all (in 2008) ascertained independently of zygosity. The identification number (CPR-number) assigned to all Danes allows individual record linkage between all databases, and turns those into an important research tool. Thus, a unique opportunity exists to study the long-term consequences of hyperthyroidism with respect to morbidity and mortality in both singletons and twins and overcome most of the limitations of existing studies.

The aim of this PhD-thesis was:

To study the long-term consequences of hyperthyroidism by exploring register-based data of singletons and twins on a nation-wide level

Four aspects were studied:
Is hyperthyroidism associated with an increased risk of mortality?
Is hyperthyroidism associated with an increased risk of morbidity?
Is an association between hyperthyroidism and mortality or morbidity influenced by the cause of hyperthyroidism?
Is an association between hyperthyroidism and mortality or morbidity influenced by genetic confounding?
This thesis is based on four papers (Paper I-IV), all of which were published in peer-reviewed journals. All papers will be discussed in a large context throughout the thesis, but a brief summary is given below:

Paper I: This meta-analysis, based on 7 studies investigating the association between hyperthyroidism and mortality, shows that hyperthyroidism is associated with an approximately 20% excess

mortality. However, the studies included are very heterogeneous with respect to the study designs, definition of hyperthyroidism and length of follow-up: this prevents any firm conclusion from being drawn.
Brandt F, Green A, Hegedüs L, Brix TH 2011 A critical review and meta-analysis of the association between overt hyperthyroidism and mortality. Eur J Endocrinol 165: 491-497

Paper II: This register-based cohort study, including 4,850 hyperthyroid singletons and 926 hyperthyroid twin individuals, demonstrates a 30% excess mortality associated with hyperthyroidism. While control for pre-existing co-morbidity had little impact on this result, genetic confounding cannot be ruled out completely.
Brandt F, Almind D, Christensen K, Green A, Brix TH, Hegedüs L 2012 Excess mortality in hyperthyroidism: the influence of preexisting comorbidity and genetic confounding: a Danish nationwide register-based cohort study of twins and singletons. J Clin Endocrinol Metab 97: 4123-4129

Paper III: This register-based cohort study, including 1,291 individuals identified with Graves´ disease and 861 individuals with toxic nodular goitre, demonstrates excess mortality associated with both phenotypes. However, Graves´ disease is associated with increased cardiovascular mortality of around 50%, which is significantly higher as compared to toxic nodular goitre.
Brandt F, Thvilum M, Almind D, Christensen K, Green A, Hegedüs L, Brix TH 2013 Graves´disease and toxic nodular goiter are both associated with increased mortality but differ with respect to the cause of death. A Danish population-based register study. Thyroid 23: 408-413

Paper IV: This register-based cohort study, including 2,631 hyperthyroid individuals, demonstrates a higher burden of somatic morbidity both before and after the diagnosis of hyperthyroidism. As seen for mortality, genetic confounding is likely to influence these findings.
Brandt F, Thvilum M¸ Almind D, Christensen K, Green A, Hegedüs L, Brix TH 2013 Morbidity before and after the diagnosis of hyperthyroidism. A nationwide register-based study. PLOS ONE 8; e66711

2. BACKGROUND

2.1. Epidemiology of hyperthyroidism

Hyperthyroidism is a common endocrine disorder, with a life-time risk of around 6,5% in Denmark (17). In Denmark, the incidence rate of hyperthyroidism is around 80/100,000 person-years, with a life-time risk of 2.4% in men and 10.5% in women (17). Hyperthyroidism is most often due to autoimmunity, like in Graves´ disease (GD), or autonomously functioning nodules, as in toxic nodular goitre (TNG) (18). GD is the dominant cause of hyperthyroidism in younger age but TNG increasingly outnumbers GD with advancing age (17). Regardless of its cause, the development of hyperthyroidism depends on a complex interplay between gender, age, genetics, and environmental exposures (19,20). Thyroid hormone levels (21), thyroid size (22), as well as thyroid autoimmunity (23), are all under genetic control. Based on twin research it has been estimated that up to 79% of the liability to develop GD and up to 82% of the likelihood of developing goitre is attributed to genetic factors (8,9).
Also, environmental factors are important, where the impact of iodine intake has been especially illuminated. In areas with mild to moderate iodine intake, hyperthyroidism is more common, while hypothyroidism dominates in areas with high iodine intake (24-26). In addition, the cause of hyperthyroidism is affected by iodine intake, as higher levels of iodine intake favor GD (27). Also, exposure to smoking, alcohol or industrially used chemicals may alter thyroid function. Smoking is associated with an increased risk of developing thyroid disease (28) and worsens the prognosis of GD (29). On the other hand, alcohol consumption might even protect against GD (30), while exposure to chemical agents like phthalates is inversely associated with thyroid hormone levels (31).

2.2. Hyperthyroidism and mortality

There is no doubt that the most severe form of hyperthyroidism - thyroid storm - if left untreated, is associated with a nearly 100% fatality rate (32). Whether milder forms of hyperthyroidism are also associated with increased mortality is still under debate. While three meta-analyses have failed to prove an association between subclinical hyperthyroidism and mortality (33-35), two newly published meta-analyses found subclinical hyperthyroidism to be associated with either cardiovascular (36) or all-cause mortality (37). 19 studies have evaluated the risk of mortality associated with overt hyperthyroidism (from now on referred to as hyperthyroidism) (38-56). Only eight of these - seven case-control studies (49-55) and one cohort study (56) - offer data on all-cause mortality, based on a unique study population with adequate sample size. Still, results are conflicting and the risk of mortality in patients with hyperthyroidism has not been evaluated in a meta-analysis. Some (49-54) but not all (55,56) studies report a significantly increased risk of all-cause mortality. This diversity might partly be explained from heterogenic study designs. While some studies only included radioiodine treated individuals (50,51,53,54,56), other studies included hyperthyroid individuals regardless of the treatment modality (49,52,55). Besides age and sex, only a few studies considered the impact of various co-morbidities or risk factors like smoking (49,50,52,53,55). However, most importantly, only two studies included age- and gender-matched, euthyroid control groups (52,53).
Our insight into the cause of death as well as the impact of the cause of hyperthyroidism (GD or TNG) on mortality is fragmented. On the one hand, three studies have linked hyperthyroidism to increased cancer mortality (49-51), but, on the other hand, a cohort study by Flynn et al., based on 4,660 individuals, failed to show such an association (55). This diversity also accounts for cardiovascular mortality, where only some (49-52,54), but not all studies (55), have shown an increased cardiovascular mortality associated with hyperthyroidism. Unfortunately, our knowledge regarding the cause of hyperthyroidism and mortality is also based on few studies. Metso et al. found only TNG but not GD to be associated with increased mortality (50). In contrast, Nyirenda et al. did not report an increased mortality in either GD or TNG patients (56).
Twin studies have indicated that genetic confounding is likely to influence the association between e.g. body mass index, physical activity or education level and mortality (57,58,59). Since both hyperthyroidism and mortality are to some degree inherited (8,9,12,13), also the increased risk of mortality associated with hyperthyroidism in some studies could be explained from genetic susceptibility. Since no previous study has evaluated this, we can only speculate on the impact of genetic confounding.

2.3 Hyperthyroidism and morbidity

The finding of an increased mortality in some studies should intuitively indicate an excess morbidity associated with hyperthyroidism. Unfortunately, interpretation of studies linking hyperthyroidism to various morbidities is challenging. Hyperthyroidism is

common and therefore occurs frequently in conjunction with other diseases. This has resulted in a large number of publications of putative associations. However, these reports are often case reports or uncontrolled studies of small series, providing only limited evidence for or against an association (60).
Different pathophysiological changes related to an excess of thyroid hormone have been investigated. Thyroid hormones e.g. affect both skeletal muscles (61) and cardiac function (4). Accordingly, hyperthyroid patients temporarily have impaired cardio-pulmonary function (62-64), until euthyroidism is restored (64-67). It seems biologically plausible to expect a higher risk of e.g. cardiovascular morbidity associated with these pathophysiological changes. In particular, cardiac arrhythmia has been intensively studied. Atrial fibrillation is reported in 10-28% of patients with subclinical or overt thyrotoxicosis, compared to 0.5-9% of the background population (68-70). As cardiac arrhythmias are a risk factor for cerebrovascular events (71,72), it is no surprise that even mild forms of hyperthyroidism have been associated with an increased risk of stroke (73,74). Unfortunately, most studies are association studies, hindering firm conclusions being drawn on the temporal association between hyperthyroidism and other morbidities.
The detection of an increased frequency of cardiovascular disease (CVD) associated with hyperthyroidism does not necessarily indicate causality. Diagnostic procedures and/or the treatment of CVD may increase the risk of developing hyperthyroidism e.g. due to the use of iodine containing substances (i.e. x-ray contrast agents and amiodarone) (75,76). In addition, the interpretation of a potential association between hyperthyroidism and the diagnosis of cardiovascular disease may be complicated by misclassification of hyperthyroidism due to non-thyroidal illness (77) or an increased awareness of thyroid disease resulting in detection bias or confounding by indication (78). Also, genetic confounding could hamper the interpretation of data, since hyperthyroidism (8,9), CVD (10), as well as stroke (11) demonstrate familial and, to some degree, individual clustering.
Clearly, the same reservations reported for CVD hold true for other potential morbidity associations as well. Individuals diagnosed with GD and thyroid nodules have an increased risk of developing thyroid cancer (79) but positive associations between hyperthyroidism and other cancer sites have also been reported (80,81). It has been suggested that such an association could be explained by radioiodine therapy (82,83), but findings are inconsistent (44,84). However, anti-neoplastic treatment may also increase the risk of hyperthyroidism (85). Still, autoimmunity could be a possible pathophysiological link between hyperthyroidism and cancer: not only is the immune system involved in cancer development (86,87) but also in autoimmune thyroid disease. In fact, cancer patients seem to have a higher risk of thyroid immunity (88). In line with this, autoimmune conditions seem to coexist within individuals (89). In particular the link between type 1 diabetes mellitus and thyroid antibodies is well established (90-92): still, an association between hyperthyroidism and diabetes mellitus type 1 is questioned (93). On the other hand, hyperthyroidism and type 2 diabetes mellitus appear to be associated (94). Since the above mentioned findings are based on small study samples, they should be viewed with care: as a result, our knowledge regarding the consequences of hyperthyroidism with respect to somatic morbidity remains fragmented.

3. MATERIALS

The Danish population comprises around 5,300,000 inhabitants.

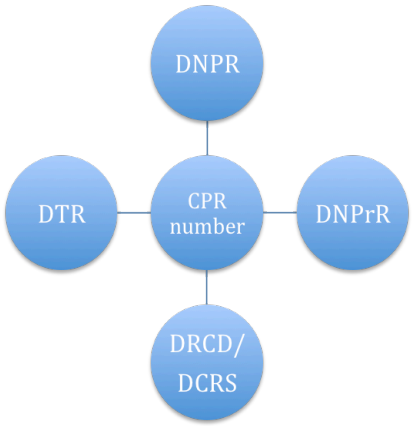

Figure 1. Linkage possibilities of utilised Danish medical databases using the central personal registration (CPR) number. The Danish Civil Registration System (DCRS), The Danish National Patient Registry (DNPR), The Danish National Prescription Registry (DNPrR), The Danish Registry Of Causes Of Death (DRCD) and The Danish Twin Registry (DTR)

Every citizen is given a personal identification number (CPR-number) at birth or immigration (95). The CPR-number is used in all contacts with public services, including pharmacies and hospitals. Mainly for administrative use, this information is stored in nation-wide registers. This, in combination with the CPR-number, allows record linkage on an individual level between different registers, opening up a unique possibility for epidemiological science (Figure 1). Data in this thesis is derived from The Danish Civil Registration System (DCRS), The Danish National Patient Registry (DNPR), The Danish National Prescription Registry (DNPrR), The Danish Registry Of Causes Of Death (DRCD) (15) and DTR (16). All of these registers are hosted at Statistics Denmark. The calendar period covered by each register is shown in Figure 2.

3.1 DCRS and DCRD

DCRS was established on April 2, 1968, and all Danish citizens were registered for administrative use (96,97). Thereafter, all live born children and immigrants have been registered. DCRS is based on the CPR-number and contains information on gender, date of birth and vital status, among others. It is generally accepted that the information recorded is of very high quality. DCRS is continuously used for administrative purposes, there is an ongoing validation, and registration is required by law. In addition, failure to supply information results in an inability to receive e.g. supplementary benefits or a tax deduction card (96).

DRCD was established in 1875 and covers all deaths among citizens dying in Denmark (98). DCRD holds information on date of death, manner of death (natural, accident, suicide, violence and uncertain), main cause of death and up to three contributory causes of death. Information is based upon the death certificates and coding for the cause of death is based on the 8th revision of the International Classification of Disease (ICD) until 1993 and the 10th revision thereafter. Even though new diagnostic techniques and changes in concepts of diseases may have affected the reported causes of death over time, the completeness of DCRD is valid, as death certificates are required by law in Denmark. Nevertheless, the quality of the diagnostic coding depends on the physicians completing the death certificates.

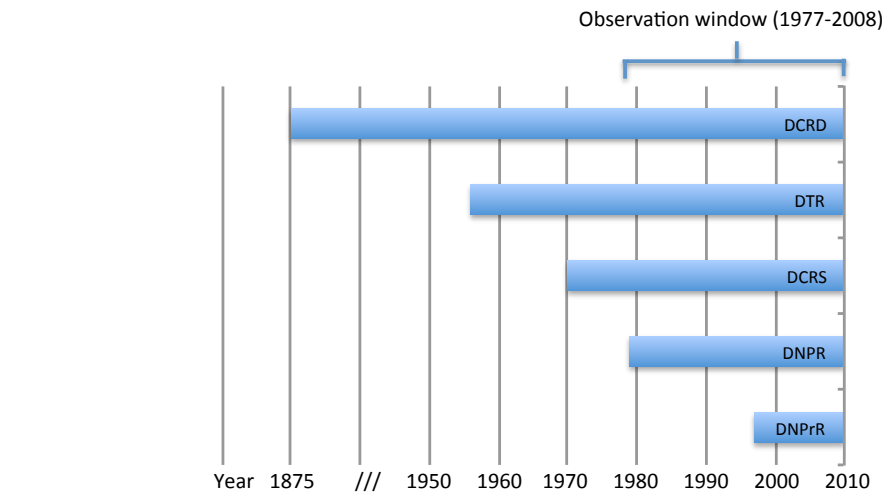

Figure 2. Calendar period covered by different data sources
The Danish Civil Registration System (DCRS), The Danish National Patient Registry (DNPR), The Danish National Prescription Registry (DNPrR), The Danish Registry Of Causes Of Death (DRCD) and The Danish Twin Registry (DTR)

Modified from study II

3.2 DNPR

DNPR contains information on all admissions to non-psychiatric hospitals since January 1, 1977, and all hospital contacts to emergency rooms and outpatient clinics since January 1, 1995 (99). Diagnostic codes include a principal diagnosis reflecting the primary cause of admission and up to 19 secondary discharge diagnoses based on the 8th revision of ICD until 1993 and the 10th revision thereafter. Diagnoses are assigned by a physician at the time of discharge and electronically transferred to the DNPR. Reporting to the DNPR is mandatory and data is used for financial reimbursement of the hospitals. In addition to the ICD-code, the register covers information on type of referral (in- or outpatient treatment), as well as the date and duration of treatment. The DNPR has a high accuracy regarding the type of admission (100) and is suitable as a sampling frame for longitudinal population based and clinical research (99). DNPR has previously been validated in respect to hyperthyroidism and misclassification occurred in less than 2 percent of cases (101).

3.3 DNPrR

Since 1994 information on drugs dispensed at Danish community pharmacies have been registered in the DNPrR (102). Coding for medical products sold with a prescription is according to the Anatomical Therapeutic Chemical (ATC) classification system. Besides the ATC code, the register covers information on date of dispensing, strength, and quantity (in defined daily doses). In Denmark, the national health security system covers all inhabitants and partially reimburses drug expenses. Data from DNPrR are transmitted directly from the cash register in the pharmacy and used in the calculation (made on an individual level) of the
expenses to be reimbursed. Due to the universal reimbursement, the system provides a strong economic incentive for recording all drugs dispensed: thus, the validity of information is high (102).

3.4 DTR

DTR was founded in 1953 and includes the information of nearly 85,000 twin pairs born from 1870 and until 2008 (16). Early birth cohorts have been identified from church books, while younger cohorts are identified from DCRS. Since 1968 the ascertainment of live-born twins is complete (16). Zygosity of same sex pairs has been classified by means of questionnaires consisting of four standard questions of physical similarity, a method with misclassification in less than 4% of cases (103). Because of identification independent of traits on a population basis, DTR is valid and especially suitable for studies to understand the influence of genetic and environmental factors. In line with this, a number of thyroid related conditions have been investigated using data from DTR (8,9,21-23,28).

4. METHODS

As already stated, this thesis is based on four papers, one meta-analysis (Paper I) and three register-based cohort studies (Paper II-IV). In the following section, the overall selection of studies included in the meta-analysis (Paper I), selection of cases and controls, comorbidity measurements, and statistical methods applied in paper II, III and IV are considered briefly. More detailed information can be found in the respective original papers.

4.1 Search method and study selection (Paper I)

All studies included in the meta-analysis (Paper I) were identified based on a MEDLINE database search using the PubMed search engine with the MeSH–words hyperthyroidism or thyrotoxicosis and mortality or survival. Only abstracts written in English were considered for inclusion, while no restrictions considering the publication date, treatment modality, study design, study setting (hospital or primary health care), gender, or age were made. Based on this initial search, only case-control or cohort studies based on original data and with no overlap of study populations, published in

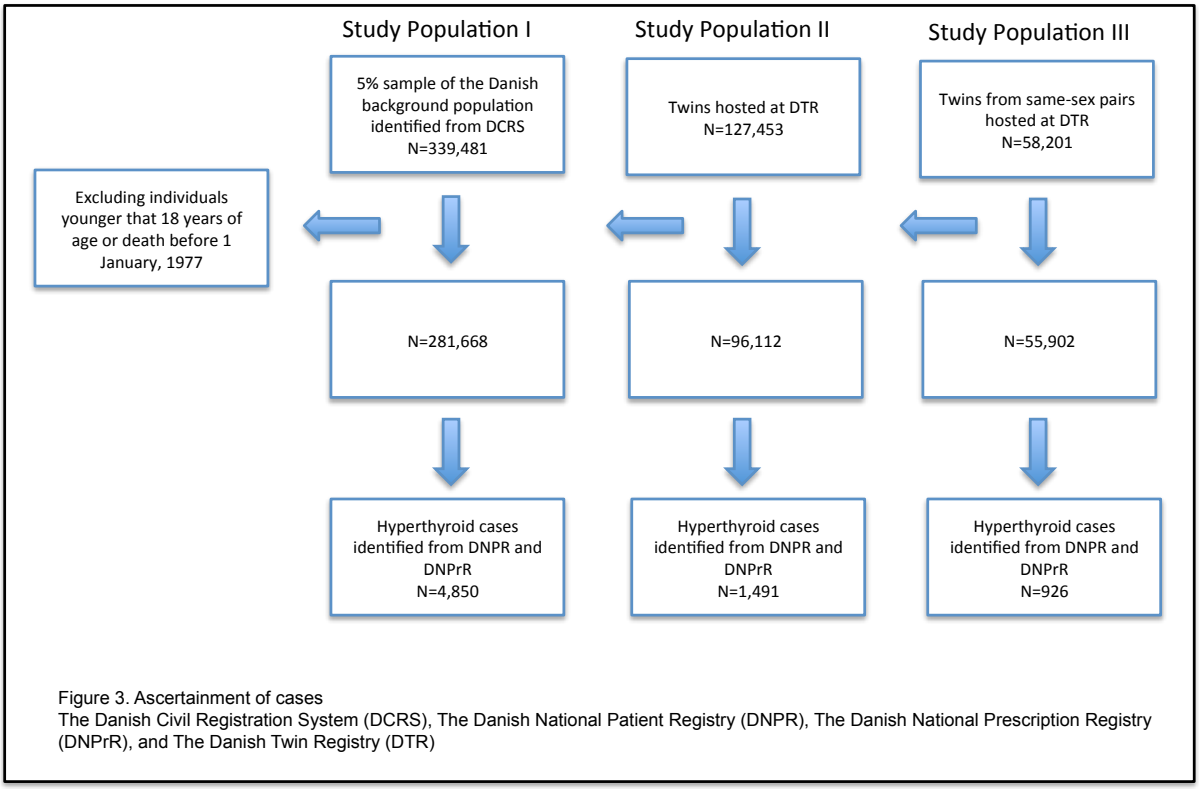

Figure 3. Ascertainment of cases
The Danish Civil Registration System (DCRS), The Danish National Patient Registry (DNPR), The Danish National Prescription Registry (DNPrR), and The Danish Twin Registry (DTR)

peer-reviewed journals, and addressing the question of whether clinically overt hyperthyroidism is associated with a change in mortality were eligible for the meta-analysis.

4.2 Definition of hyperthyroidism (Paper II-IV)
In the register-based studies information on thyroid status was obtained from DNPR and/or DNPrR. In DNPR, hyperthyroidism was defined by ICD-8 codes 242.00-242.99 (1977-1994) and the ICD-10 codes E05-E05.9 (1995-2008). GD was defined by the ICD-8 codes 242.08, 242.09, 242.00 or 242.01 as well as with the ICD-10 codes E05.0, H05.2 or H06.2. TNG was defined by the ICD-8 code 242.19 and the ICD-10 codes E05.1 or E05.2. Both principal and secondary discharge diagnoses from in- or outpatient treatments were included. In DNPrR, hyperthyroidism was defined by at least two dispensed prescriptions of anti-thyroid medication (ATC=H03B). Either first date of registration with a hyperthyroid diagnosis in DNPR or the first date of collecting anti-thyroid medication registered in DNPrR, whichever occurred first, was chosen as the date for diagnosis with hyperthyroidism (index-date).

4.3 Study populations (Paper II-IV)
Study populations were identified based on a 5% sample of the Danish background population identified from DCRS (n=339,481) and from all twins hosted at DTR (n=127,453). Hyperthyroid cases were ascertained as shown in Figure 3. After excluding all individuals younger than 18 years of age or those who were dead before January 1, 1977 (start of the DCRS), 4,850 singletons from the random 5% of the background population and 1,492 twins were identified with hyperthyroidism. From these hyperthyroid twins, 926 were from same-sex pairs and 625 were from same-sex pairs discordant for hyperthyroidism. Based on the 5% sample of the background population (singletons cases) and DTR (twins cases) cases were matched 1:4 with controls after the principles of density sampling (104), and three study populations were identified:

Studypopulation I: 4,850 hyperthyroid singletons matched with 19,400 non-hyperthyroid singletons from the 5% sample of the background population.
Studypopulation II: 1,492 hyperthyroid twins matched with 5,968 non-hyperthyroid twins hosted at DTR.
Studypopulation III: 625 same-sex twin pairs discordant for hyperthyroidism identified from DTR. Furthermore cases identified from DNPR in study population I, were stratified according to the cause of hyperthyroidism: including 1,291 incident cases of GD and 861 incident cases of TNG (Paper III and paper IV).

4.4 The Charlson Score (paper II-IV)
One of the most important predictors of health-related outcomes is the presence of co-morbidities (105). Therefore, to predict the risk of mortality and morbidity related to hyperthyroidism, risk-adjustment for comorbidity is essential. The Charlson Score (CS) includes 19 disease categories each assigned a weight (1 to 6) depending on their severity (Table 1). The CS is the sum of the weights for all conditions on an individual level. Each increment in the CS level has been associated with a 2.3-fold (95 percent confidence interval: 1.9, 2.8) increase in the 10-year mortality risk in a cohort of 685 breast cancer patients (106). Similar results have been reported for postoperative survival in patients with hypertension or diabetes (107). The CS has been validated for outpatients (108-110) and different morbidities (111-115) including non-malignancies like osteoarthritis (108), hypertension (114) and migraine (115). More

Table1: The Charlson Score

Weight	Clinical condition	ICD-8	ICD-10	ATC
Myocardial infarction	1	410	I21-I23	
Congestive Cardiac insufficiency	1	427.09-427.11; 427.19; 428.99; 782.49	I50; I11.0; I13.0; I13.2	B01; C01; C03; C07-C09; N021
Peripheral vascular disease	1	440-445	I70-I74; I77	
Dementia	1	290.09-290.19; 293.09	F00-F03; F05.1; G30	
Cerebrovascular disease	1	430-438	I60-I69; G45; G46	
Chronic pulmonary disease	1	490-493; 515-518	J40-J47; J60-J67; J68.4; J70.1; J70.3; J84.1; J92.0; J96.1; J98.2; J98.3	R032
Connective tissue disease	1	712; 716; 734; 446; 135.99	M05-M06; M08-M09; M30-M36; D86	
Diabetes mellitus, non-complicated	1	249.00; 249.04; 249.07; 249.09; 250.00: 250.06; 250.07; 250.09	E10.0-E10.1; E10.9; E11.0-E11.1; E11.9	A102
Stomach ulcer disease	1	530.91; 530.98; 531-534	K22; K25-K28	
Chronic, mild liver disease	1	571; 573.01; 573.04	K70.0-K70.3; K70.9-K71.9; K73-K76	
Hemiplegia	2	344	G81-G82	
Moderate or severe liver disease	2	403; 404; 580-583; 584; 590.09; 593.19; 753.10-753.19; 792	I12; I13; N00-N05; N07; N11; N14; N17-N19; Q61	
Diabetes mellitus, complicated	2	249.01-249.05; 249.08; 250.01-250.05; 250.08	E10.2-E10.8; E11.2-E11.8	
Malignant tumours	2	140-163; 170-194	C00-C75	
Leukaemia	2	204-207	C91-C95	
Lymphoma	2	200-203; 275.59	C81-C88; C90; C96	
Moderate or severe liver disease	2	070.00; 070.02; 070.04; 070.06; 070.08; 573.00; 456.00-456.09	B15.0; B16.0; B16.2; B19.0; K70.4; K72; K76.6; I85	
Metastatic malignant tumours	6	195-199.19	C76-C80	
AIDS	6	079.83	B21-B24	

1 paper III, 2 paper III and IV, Anatomical Therapeutic Chemical (ATC), International Classification of Disease (ICD)

importantly, even though there has been a shift in diagnostic criteria and a change in coding algorithms over time, the CS is still a valid prognostic indicator with a similar performance in predicting mortality regardless of whether they were based on ICD-9 (112) or ICD-10 (116). Consequently, the CS has been attributed a high predictive performance for mortality regardless of the study population and exposure to disease (117).

In all study populations (I-III), the CS was calculated on an individual level, based upon relevant disease groups registered in DNPR and/or DNPrR (Tabel 1). In Denmark, patients with mild diabetes, cardiovascular disease (i.e. hypertension) and lung diseases (i.e. chronic obstructive lung diseases) are often diagnosed and treated solely in primary care. To get full coverage of these individuals co-morbidities, users of anti-diabetics, cardiovascular drugs and users of drugs for obstructive airway disease, as identified from DNPrR, were classified as having diabetes, cardiovascular disease, or lung disease, respectively (Table 1). For subjects with hyperthyroidism, the CS reflects the time period from January 1, 1977 (the start of DNPR), until the index-date. In controls, the CS covers the time period from the start of DNPR until the index-date of the corresponding case.

4.5 Outcome (Paper II-IV)

The overall outcome was categorised into all-cause mortality, disease specific mortality or morbidity.

All-cause mortality was recorded from DCRS, while information on disease-specific mortality was recorded from DCRD. Death was analysed due to the most common causes (118,119), cardiovascular diseases (ICD-8 codes 390-458 and ICD-10 codes I00-I99), cancer (ICD-8 codes 140-207 and the ICD-10 codes C00-C97), lung diseases (ICD-8 codes 464-493 & 508-519 and ICD-10 codes J00-J99), and

diabetes mellitus (ICD-8 codes 249-250 as well as the ICD-10 codes E10-E14).
Morbidity was recorded from DNPrR and DNPR. Outcomes, categorised into CVD, rheumatic disease (RD), lung disease (LD), malignant disease (MD), diabetes mellitus (DM), and other diseases (dementia, gastric ulcer, liver disease, hemiplegia, kidney disease, liver failure and AIDS), were defined on the basis of the 19 disease groups covered by the CS (Table 1). For each individual, the first date of possible registration in each of these groups was identified. Accordingly, stratification for the first registration in each disease group before or after the date of diagnosis with hyperthyroidism was performed (index-date).

4.6 Statistics

4.6.1 Metal-analysis (Paper I)

From all studies included in the meta-analysis, the number of deaths and number of expected deaths were extracted. Summary Relative Risk (RR) estimates were calculated by the method of DerSimonian and Laird using a random effect model (120). The statistical heterogeneity was assessed by the squared-I value, which describes the total variation across study results attributable to heterogeneity rather than chance (a value above 25%, 50% and 75% being indicative of low, moderate and high heterogeneity, respectively) (121).

4.6.2 Register-studies (Paper II-IV)

In the register studies, the relationship between hyperthyroidism and mortality was evaluated by the Cox regression model (Paper II and III). Age was chosen as the underlying time variable. In both cases and controls, person years of follow-up were accumulated from the index-date and were terminated at the date of death, migration, or end of follow-up (December 31, 2008), whichever came first. The variable "pair" was used as a stratum variable, fixing the baseline hazard within a matched pair, while at the same time allowing this baseline hazard to vary freely between pairs. All analyses were adjusted for the degree of co-morbidity preceding the diagnosis of hyperthyroidism using the CS. Analyses were repeated in all three study populations.

The odds ratio (OR) for morbidity, prior to the diagnosis of hyperthyroidism, was evaluated in a logistic regression analysis that was adjusted for age and sex (Paper IV). The Cox regression model was explored to evaluate the risk of morbidity following the diagnosis of hyperthyroidism (Paper IV). Age was chosen as the underlying time variable and in both cases and controls, person years of follow up were accumulated from the index-date until the date of diagnosis with morbidity, migration, death or the end of follow-up (December 31, 2008), whichever came first. In all Cox analyses, the variable "pair" was used as a stratum variable while both Cox and regression analyses were adjusted for the degree of co-morbidity preceding the diagnosis of hyperthyroidism, using the CS.
All analyses were conducted using STATA version 11.0 (2009; Stata Corporation, College Station, TX, USA).

5. RESULTS

5.1. Meta-analysis (Paper I)

Based on a MEDLINE database search, 19 case-control or cohort studies published in peer-reviewed journals and addressing the question of whether clinically overt hyperthyroidism is associated with a change in mortality were identified (38-56). Following review, studies were excluded either because they only addressed cancer mortality (43): due to overlap with subjects from other studies (42,44,47): because they were reviews not providing original data (38,40,48): due to the inclusion of too few (n<10) hyperthyroid individuals to meaningfully allow calculation of the mortality risk (39,46): based on the inclusion of a control group which was also hyperthyroid (41): or, finally, because evaluation of thyroid status was based solely on serum TSH (45). Of the remaining eight studies (49-56), only seven could be pooled since one study did not provide the exact number of deaths (53). On the pooled data a meta-analysis revealed a significantly increased risk of all-cause mortality associated with hyperthyroidism (Relative Risk (RR) 1.21, 95% confidence interval (CI): 1.05-1.38; Table 2). This finding did not change significantly if only studies controlling for co-morbidity (49,50,52,55), studies performed at a hospi-

Table 2. Number of deaths/expected deaths and calculated Relative Risk of all-cause mortality

Author	Observed num. of deaths	Expected num. of deaths	RR (CI 95%)
Goldman et al., 1990 (49)	790	564	1.40 (1.28, 1.53)
Franklyn et al., 1998 (54)	3,611	3,186	1.13 (1.09, 1.17)
Hall et al., 1993 (51)	5,400	3,673	1.47 (1.42, 1.52)
Flynn et al., 2006 (55)	565	539	1.05 (0.94, 1.17)
Nyrienda et al., 2005 (56)	568	548	1.04 (0.94, 1.15)
Metso et al., 2007 (50)	1,390	1,299	1.07 (1.01, 1.13)
Osman et al., 2007 (52)	26	12	2.17 (1.11, 4.23)
Meta-analysis	12,350	9,821	1.21 (1.05, 1.38)[1]

1 I^2 = 96,9%, P=0.000

Table 3. Baseline characteristics of study population I-III

Study population	I			II	III
Hyperthyroidism	All-cause	Graves´ disease	Toxic nodular goitre	All-cause	All-cause
Mean age, yrs (range)	70 (23-106)	66 (23-102)	73 (24-104)	66 (23-99)	66 (26-99)
Mean age at diagnosis, yrs (range)	60 (18-99)	55 (18-96)	62 (18-96)	56 (18-94)	55 (19-94)
Females, %	83	80	85	83	83
CS[1] = 1, %	43	51	53	45	45

1 Charlson Score

tal setting (49-52,54,56) or studies only including radioiodine treated individuals (50,51,54,56) were pooled (Figure 4). Six studies showed data on cardiovascular mortality (49-52,54,55). After pooling these studies, hyperthyroidism was associated with significantly increased cardiovascular mortality (RR 1.27, 95% CI: 1.05-1.53; Figure 4). Importantly, regardless of criteria used for pooling original studies, the squared-I value was above 89%. This is much higher than the 50% generally viewed as a threshold (121). On the other hand, no evidence of publication bias was detected (Egger´s test, P=0.409) (122).

5.2. Characteristics of the study populations (I-IV)

The baseline characteristics of all cases are shown in Table 3. In general, twin cases (study population II and III) were younger and were diagnosed at a younger age than cases identified from the 5% sample of the background population (study population I). As expected, GD cases were younger as compared to TNG cases.

5.3. Mortality (Paper II-III)

Singletons from the random 5% sample of the Danish background population identified with hyperthyroidism (study population I) had an increased all-cause mortality compared with the control individuals, as reflected by a hazard ratio (HR) of 1.37 with (95% CI: 1.30-1.46; Table 4). In order to include only incident hyperthyroidism, all cases identified in 1977 (start of DNPR) and 1995 (start of DNPrR) were excluded, which did not affect the outcome (HR 1.41, 95% CI: 1.32-1.50). Neither stratification for sex nor adjustment for pre-existing co-morbidity as measured by CS changed the findings significantly. Even when more conservatively restricting the analyses to subjects without co-morbidity (defined as a CS = 0), hyperthyroidism remained associated with increased all-cause mortality (HR=1.20; 95% CI: 1.12-1.31). On the other hand, the data source used for identification of hyperthyroid cases influenced the outcome significantly. Risk estimates were smaller for cases ascertained from DNPrR compared to cases identified from DNPR (HR 1.09; 95% CI 1.01-1.18 and HR 1.29; 95% CI 1.21-1.32, respectively). After stratification for the cause of hyperthyroidism, both GD and TNG were associated with a significantly increased all-cause mortality, which did not change after adjustment for pre-existing co-morbidity (Figure 5). However, the cause-specific mortality varied between GD and TNG. GD was associated with increased cardiovascular mortality (HR 1.49, 95% CI: 1.25-1.77) and mortality from lung diseases (HR 1.91, 95% CI: 1.37-2.65), while TNG was only associated with significantly
increased cancer mortality (HR 1.36, 95% CI 1.06-1.75). Moreover, while there was no difference in all-cause mortality, GD was associated with a significantly higher cardiovascular mortality (HR 1.36, 95% CI: 1.10-1.76), when compared to TNG (Table 5). To investigate the impact of genetic confounding, the risk of mortality was investigated in the twin population. When handling the twin population as singletons (study population II), the risk of all-cause mortality associated with hyperthyroidism was similar to the risk calculated in the singleton population (HR 1.35, 95% CI 1.20-1.52). In the within-pair analyses of same-sex twin that were pairs discordant for hyperthyroidism (study population III), this did not change significantly (HR 1.43, 95% CI: 1.09-1.88). However, stratification for zygosity had a major influence on this finding. While hyperthyroidism was associated with increased all-cause mortality in dizygotic (DZ) twins (HR 1.80, 95% CI: 1.27-2.55), the effect was completely attenuated in monozygotic (MZ) twins (HR 0.95, 95% CI: 0.60-1.50).

5.4. Morbidity (Paper IV)

5.4.1 Overall association

In the random 5% sample of the background population (study population I) singletons identified with hyperthyroidism had a higher frequency of CVD, LD and DM, as well as the group of other diseases (Table 6). Stratification for the cause of hyperthyroidism (GD and TNG) did not change these findings significantly: however, TNG was also positively associated with RD (cases 6%, controls 3%, p<0.01).

5.4.2 Prior to the thyroid diagnosis

The register-based design allowed stratification in the periods before and after the diagnosis of hyperthyroidism. Individuals with hyperthyroidism had an increased risk of CVD (OR 1.65; 95% confidence interval (CI): 1.23-1.87), RD (OR 1.19; 95% CI: 1.05-1.46), LD (OR 1.53; 95% CI: 1.29-1.60), DM (OR 1.43; 95% CI: 1.20-1.72), and other diseases (OR 1.49; 95% CI: 1.23-1.79), prior to the diagnosis of hyperthyroidism (Table 6). Evaluating the same disease categories but censoring diagnoses made within 365 days prior to the diagnosis of hyperthyroidism in order to evaluate potential confounding by indication (78) did not change this finding. Also, stratification for the cause of hyperthyroidism did not significantly change the findings as singletons from the random 5% sample of the background population both with GD and TNG had an increased risk of CVD (ORGD 1.44; 95% CI: 1.06-1.96, ORTNG 2.20; 95% CI: 1.70-2.82), RD (ORGD 1.68; 95% CI: 1.06-2.65, ORTNG 2.39; 95% CI: 1.36-4.19), LD (ORGD 1.55; 95% CI: 1.27-1.89, ORTNG 1.38; 95% CI: 1.06-1.79), and DM (ORGD 1.91; 95% CI: 1.34-2.73, ORTNG 1.64; 95% CI: 1.06-2.52).

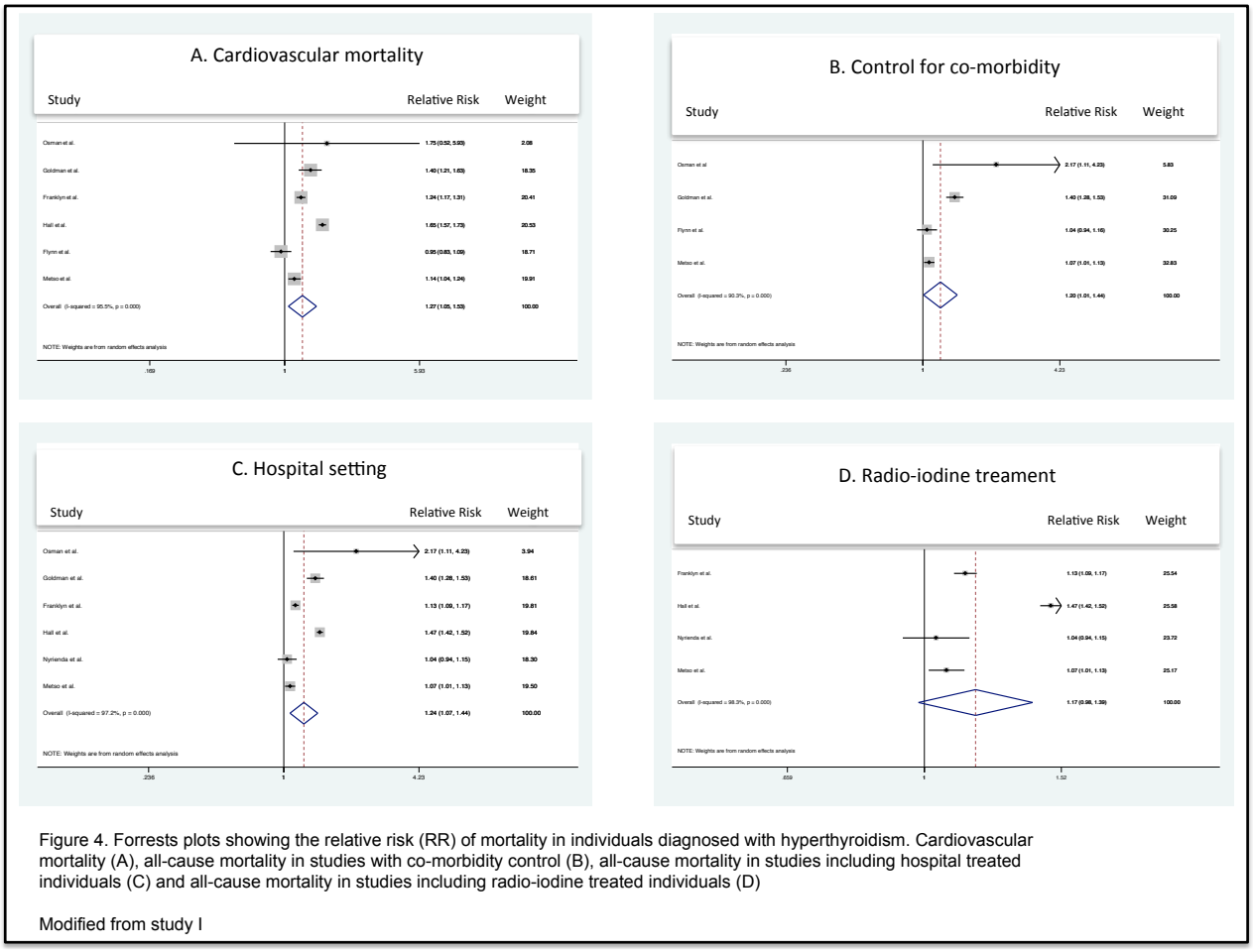

Figure 4. Forrests plots showing the relative risk (RR) of mortality in individuals diagnosed with hyperthyroidism. Cardiovascular mortality (A), all-cause mortality in studies with co-morbidity control (B), all-cause mortality in studies including hospital treated individuals (C) and all-cause mortality in studies including radio-iodine treated individuals (D)

Modified from study I

5.4.3 Following the thyroid diagnosis

Following the diagnosis of hyperthyroidism, case individuals had a significantly increased risk of being diagnosed with CVD (HR 1.34; 95% CI: 1.15-1.56), LD (HR 1.28; 95% CI: 1.10-1.49), DM (HR 1.46; 95% CI: 1.16-1.84), and other diseases (HR 1.47; 95% CI: 1.17-1.84). Except for CVD (HR 1.07; 95% CI: 0.89-1.29), and other diseases (HR 0.99; 95% CI 0.74-1.33), these results did not change significantly when censoring diagnoses made within 365 days following the diagnosis of hyperthyroidism (HRLD 1.30; 95% CI: 1.09-1.54, HRDM 1.37; 95% CI: 1.06-1.78). After stratification for the cause of hyperthyroidism, singletons identified with both GD and TNG had a significantly higher risk of being diagnosed with CVD (HRGD 1.93; 95% CI: 1.46-2.56, HRTNG 1.72; 95% CI: 1.26-2.34), DM (HRGD 1.76; 95% CI: 1.14-2.70, HRTNG 1.62; 95% CI: 1.00-2.65), and other diseases (HRGD 1.90; 95% CI: 1.17-3.07, HRTNG 1.88; 95% CI: 1.18-2.99). After pooling all disease categories, hyperthyroid individuals from disease discordant same-sex twin pairs (study population III) also had a higher risk for morbidity (OR 1.13, 95% CI: 0.82-1.56) as compared to their co-twin. Again, zygosity had a major impact on this result as hyperthyroid individuals from DZ pairs still had an increased risk of morbidity (OR 1.30, 95% CI: 0.89-1.90) that completely attenuated in MZ pairs (OR 0.78, 95% CI 0.42-1.45).

6. DISCUSSION

The results provide interesting insights into the long-term consequences of hyperthyroidism in respect to both mortality and morbidity. Firstly, irrespective of its cause, hyperthyroidism is associated with an approximately 30% increased all-cause mortality: this was true for both GD and TNG. On the other hand, GD and TNG differ with respect to cause-specific mortality, as e.g. GD is associated with a higher cardiovascular mortality. Secondly, irrespectively of its cause, hyperthyroidism is positively associated with CVD, LD and DM. Stratification for the period before and after the diagnosis of hyperthyroidism revealed an increased risk of being diagnosed with CVD, LD and DM both before and after the diagnosis of hyperthyroidism. In contrast to what we found for mortality, stratification for the cause of hyperthyroidism yield similar results for both GD and TNG in respect to morbidity. Thirdly, based on the findings from the intra-pair twin analyses of monozygotic and dizygotic twins, genetic confounding is likely to affect these results at least to some degree.

6.1 Hyperthyroidism and mortality

Five of the seven studies included in the meta-analyses showed significantly increased all-cause mortality associated with hyperthyroidism (49-52,54). After pooling all seven studies, hyperthroidism

Table 4. Hazard ratio of mortality in all-cause hyperthyroid individuals from the 5% sample of the background population (study population I)

Gender	Hazard ratio of mortality			
	All cases		Only incident cases	
	Non-adjusted	Adjusted[1]	Non-adjusted	Adjusted[1]
All	1.37 (1.30-1.46)	1.28 (1.21-1.36)	1.41 (1.32-1.50)	1.31 (1.22-1.39)
Male	1.31 (1.14-1.49)	1.12 (0.98-1.29)	1.32 (1.34-1.54)	1.14 (0.98-1.32)
Female	1.39 (1.31-1.48)	1.32 (1.24-1.41)	1.43 (1.34-1.54)	1.35 (1.26-1.45)

1 Adjusted for preexisting co-morbidity as measured by the Charlson Score

was associated with a 20% increase in all-cause mortality. However, the squared-I value was 96,9%, indicating a statistically significant difference with respect to the size of the risk estimates between the seven studies (123). Intuitively, this inhomogeneity is due to various definitions of hyperthyroidism,
the inclusion of different treatment modalities and inconsistency in considering confounders. However, pooling more homogenous studies did not change the risk estimates for mortality nor the squared-I values. Due to this inconsistency of previous studies, we evaluat
ed the association between hyperthyroidism and mortality in a population-based nation-wide sample of the Danish population and showed hyperthyroidism to be associated with a 30% increased risk of mortality. In contrast to previous studies, the register-based design allowed the inclusion of a wide spectrum of hyperthyroid cases based on discharge diagnoses or the prescription of anti-thyroid medication. Additionally, we introduced a standardised and validated scale based on extensive coverage of co-morbidity (106,107,124). Surprisingly, we found that pre-existing co-morbidity had only a minor impact on size and strength of the association between hyperthyroidism and mortality. The fact that even subjects without pre-existing co-morbidity (CS=0) before the diagnosis of hyperthyroidism had a significantly increased risk of mortality even indicates a direct association between hyperthyroidism and mortality.

Although literature favours hyperthyroidism to be associated with increased mortality, different causes of hyperthyroidism might result in higher or lower risk estimates. As previously pointed out, the most frequent forms of hyperthyroidism are GD and TNG (18). Clearly, there are major differences in the epidemiology and the clinical features of these phenotypes (70): GD is an autoimmune condition with possible extra-thyroidal involvement and primarily affecting younger individuals, while TNG describes autonomy of the thyroid gland and primarily affecting older individuals (125). As not only the degree of hyperthyroidism but also the duration of hyperthyroidism and even autoimmunity may affect the prognoses, GD and TNG are likely to differ in respect to mortality. Surprisingly, only two studies have evaluated the all-cause mortality associated with different phenotypes of hyperthyroidism. In contrast to our finding of an increased all-cause mortality associated with both GD and TNG, Metso et al. reported only TNG to be associated with increased all-cause mortality (50), while Nyrienda et al. did not report increased all-cause mortality in neither GD nor TNG (56). Importantly, both of these studies included only radioiodine treated patients and patients were not randomised to this therapy, but were assigned this based on on a number of reasons, such as the severity of disease and/or patient/physician preference (50,56). In contrast, we used a more robust method in the present study to obtain the complete coverage of GD and TNG from discharge diagnoses in both inpatients and outpatients, independent of the treatment modality, and consequently hinder selection bias.

Only a few studies have analysed the association between hyperthyroidism and disease-specific mortality. None of these studies stratify for the cause of hyperthyroidism. By utilising information-from DNPR and based on death certificates registered at DRCD, we were able to stratify not only for the cause of hyperthyroidism but also for the causes of death. After stratification for the cause of disease, we found only GD but not TNG to be associated with a statistically significant increased cardiovascular mortality. Of course this finding could pertain to the higher age of TNG individuals. Cardiovascular morbidity is more frequent in older age (126) and is thus more likely to affect the TNG study population. Consequently, the detection of increased CVD in the TNG population could be hindered due a high frequency of CVD in this age group giving rise to low power in our analyses. Nevertheless, GD had a significantly higher cardiovascular mortality when compared to TNG, even after adjustment for the age difference. It follows that,
even though the exact pathophysiological mechanism related to the different cardiovascular prognosis in GD and TNG requires further investigation, it is likely that e.g. autoimmunity, age at onset, as well as severity of hyperthyroidism, is attribute to the different outcomes.

No previous study has evaluated the risk of mortality from lung diseases attributed to hyperthyroidism. After stratification for the cause of disease we found only GD but not TNG to be associated with a higher risk of lung disease mortality. This finding could not only be explained by the co-existence of immune related diseases (89), but also by shared risk factors like smoking (127). The attributable risk of smoking in GD has been reported to be approximately twice as high as in TNG (45% vs. 28%, respectively) (128). It follows that the higher risk of lung disease mortality in the GD group could be related to a greater proportion of smokers when compared to the TNG group. Intuitively, as smoking is an important risk factor for a number of cancer diseases (129), this should also induce higher cancer mortality in GD individuals. However, we found only TNG but not GD to be associated with increased cancer mortality. Unfortunately, no previous study has evaluated the risk of cancer mortality associated with hyperthyroidism stratified for the cause of disease. However, at least four studies have evaluated the risk of cancer mortality associated with all-cause hyperthyroidism. In contrast, with the findings by Flynn et al. (55), three studies have reported increased cancer mortality in hyperthyroid individuals (42,49-50). As two of these studies only included radioiodine treated cases, selection bias and confounding (radiation) could hinder the interpretation of results (42,50). Unfortunately, our register-based design did not allow us to speculate on the possible influence of radioiodine treatment. However, a meta-analyses showed no increased cancer risk in hyperthyroid cases treated with radioiodine (130).

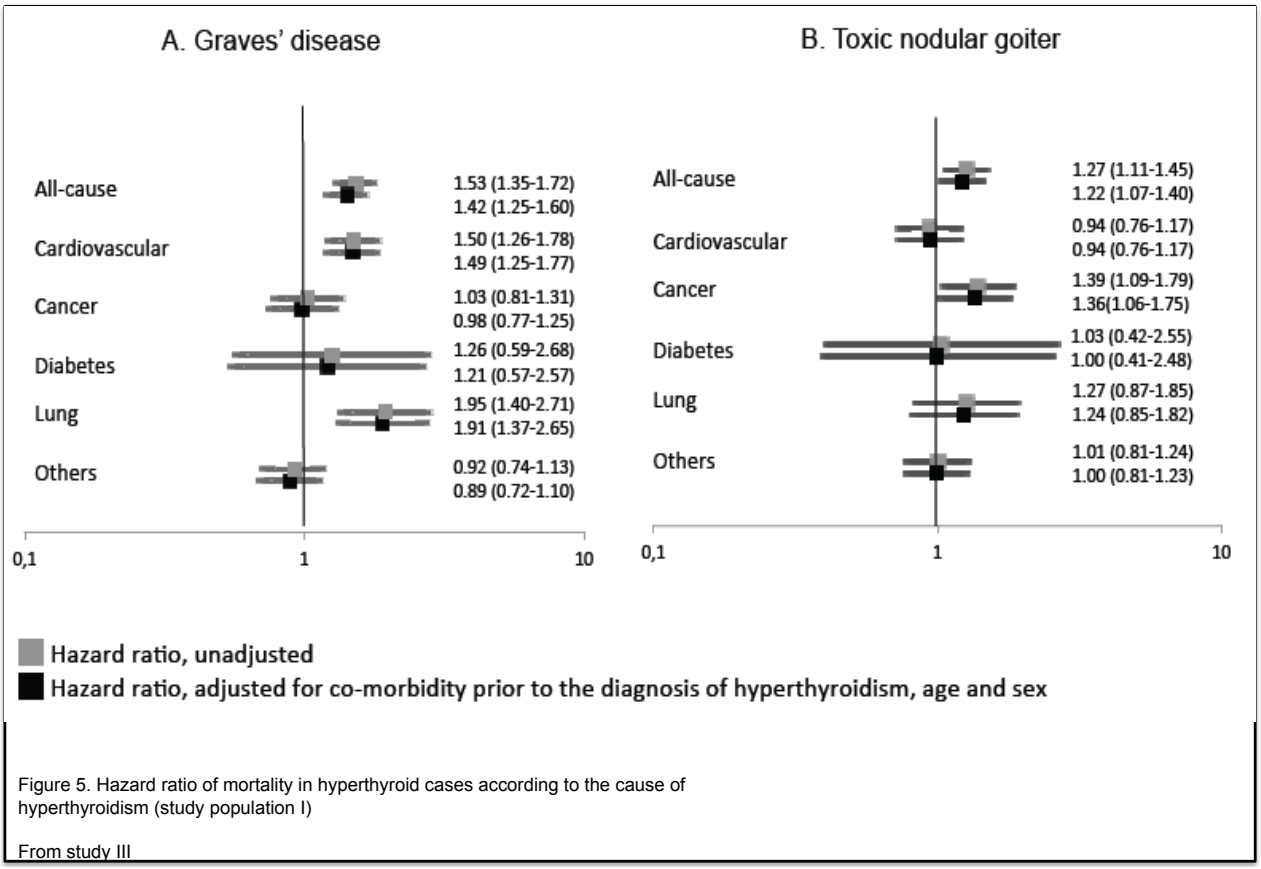

Figure 5. Hazard ratio of mortality in hyperthyroid cases according to the cause of hyperthyroidism (study population I)

From study III

Despite the finding of a positive association between hyperthyroidism and various disease-specific mortalities, it must be questioned whether such a relation is causal. Clearly, the optimal study for investigating whether the observed association between hyperthyroidism and mortality is causal, would be a life-long follow-up study of an entire birth cohort. However, such studies are not available and are rarely feasible. It follows that other means, using less advantageous designs, to build up evidence for or against cause must be employed. In practice, it is widely accepted to differentiate between causal and non-causal relations by evaluating the following: strength, consistency, specificity, temporality, dose-response relation and biologically plausible (131) – the so-called Bradford Hill criteria. As already pointed out, there is a strong and consistent association between hyperthyroidism and at least all-cause mortality. Based on the described physiological changes associated with hyperthyroidism an association is biological plausible, and since death is the final outcome, temporality is predefined. Unfortunately, no systematic evaluation of the dose-response relation between hyperthyroidism and mortality exists. However, assuming that the most severe form of hyperthyroidism is more likely referred to a hospital setting, while milder forms are treated in primary care, our findings indicate a possible dose-response relation. We found higher risk estimates for mortality when including hyperthyroid cases identified from DNPR, which represents treatment in a hospital setting, than when hyperthyroid cases were identified from DNPrR, which indicates treatment in primary care. Accordingly, the association between hyperthyroidism and mortality is strong, consistent, biologically plausible and most likely dose-dependent. In combination, these features indicate a causal relationship between hyper-

Table 5. The Hazard ratio of mortality, adjusted for pre-existing comorbidity and age, in individuals diagnosed with Graves´ disease in comparison to individuals diagnosed with toxic nodular goitre

Cause of death	Graves´ disease vs. toxic nodular goitre
All-cause	1.07(0.93-1.23)
Cardiovascular	1.39(1.10-1.76)
Cancer	0.78(0.58-1.05)
Diabetes Mellitus	0.94(0.88-1.00)
Lung	1.19(0.77-1.85)
Others	0.83(0.63-1.08)

thyroidism and at least all-cause mortality.

6.2. Hyperthyroidism and morbidity

It is unknown whether the finding of a higher mortality associated with hyperthyroidism can be explained by the increased thyroid hormones per se, or by intermediary factors such as an increased burden of morbidity prior or subsequent to hyperthyroidism. We found a higher frequency of CVD, LD, DM, and RD in hyperthyroid cases as compared to their euthyroid controls. Unfortunately, due to the small study samples and inaccurate endpoints used in earlier studies a direct comparison with the existing literature is not meaningful. As an example, case reports have raised the possibility of positive associations between hyperthyroidism and heart failure (132), hyperglycaemia (133) as well as asthma (134). In addition, a number of studies have used surrogate markers of the possible associated disease, rather than the clinical disease itself. Hyperthyroidism has been associated with increased levels of antinuclear antibodies (135,136), antiendomysial antibodies (137) and islet cell antibodies (138). In addition, much evidence is based on association studies neglecting the temporality of a possible association (60). Clearly, these studies provide only weak evidence for a link between various morbidities and hyperthyroidism. However, to build up evidence for or against a causal relation between hyperthyroidism and various morbidities, the temporal relation between hyperthyroidism and the disease of interest should at least be addressed.

After stratification for the period before and after the diagnosis of hyperthyroidism, our data showed that hyperthyroid individuals have an increased risk of being diagnosed with CVD, LD, DM and RD, prior to the diagnosis of hyperthyroidism. Following the diagnosis of hyperthyroidism, there was an increased risk of being diagnosed with CVD, LD, and DM. These findings could be interpreted as a causal association between CVD, LD and DM on the one hand and hyperthyroidism on the other hand, or vice versa. However, it is important to point out that the observed associations, in some cases could be influenced by the diagnostic procedure and/or treatment of a specific morbidity. For example, iodine-containing anti-arrhythmias (75), and iodine-containing diagnostic drugs (139) may induce hyperthyroidism.

Table 6. Temporality and the impact of co-morbidity in hyperthyroidism (study population I)

Disease category	Overall association			Odds ratio[2]	Hazard ratio[3]
	Cases[1]	Controls[1]	P-value		
Cardiovascular disease	677 (26%)	1,992 (19%)	<0.001	1.65 (1.23-1.87)	1.34 (1.15-1.56)
Lung disease	780 (30%)	2,409 (23%)	<0.001	1.53 (1.29-1.60)	1.28 (1.10-1.49)
Diabetes mellitus	283 (11%)	795 (8%)	<0.001	1.43 (1.20-1.72)	1.46 (1.16-1.84)
Rheumatic disease	123 (5%)	418 (4%)	0.100	1.19 (1.05-1.46)	1.39 (0.92-2.09)
Malignant disease	370 (14%)	1,329 (13%)	0.050	1.16 (0.99-1.36)	1.18 (0.97-1.42)
Other diseases[4]	361 (14%)	1,040 (10%)	<0.001	1.49 (1.23-1.79)	1.47 (1.17-1.84)

[1]Number of individuals with a first time hit of the respective disease category

[2] Odds ratios before the diagnosis of hyperthyroidism

[3] Hazard ratios after the diagnosis of hyperthyroidism, adjusted for the Charlson score

[4] Dementia, gastric ulcer, liver disease, hemiplegia, kidney disease, liver failure and AIDS

Data given in bold represent significant findings

Also, shared environmental factors might explain the increased risk of morbidity both before and after the diagnosis hyperthyroidism. As an example, smoking is a risk factor for hyperthyroidism (28), but also for LD (140).

Despite the observation that some anti-neoplastic drugs may induce hyperthyroidism (85) and smoking is a risk factor for cancer (141), we found no positive association between hyperthyroidism and cancer. As the immune system may be involved in the link between hyperthyroidism and cancer, one would, at least theoretically, expect a higher cancer risk associated with GD than TNG (86). However, after stratification for the cause of hyperthyroidism, we found neither GD nor TNG to be associated with cancer before or after the diagnosis of hyperthyroidism. This is in contrast to the findings of another register-based study, reporting an increased cancer risk in GD (142). Remarkably, the study population was nearly 20 years younger than ours, and there was no increase in the cancer incidence in individuals older than 50 years of age (142). It follows, therefore, that the higher age of our population as well as low power may hinder the detection of increased cancer risk associated with GD. In addition, the fact that we also did not stratify for the cancer site may have affected our results. While thyroid cancer and e.g. breast cancer are increased in GD (79-81), trends towards a lower risk of e.g. lung and stomach cancer have been reported (142). As not all cancer sites show the same prognosis, this could also explain our finding of increased cancer mortality associated with TNG but not with GD.

For CVD, our findings are at first eye sight in conflict with our findings regarding cardiovascular mortality. Cardiovascular mortality was significantly increased in GD when compared to TNG. On the other hand, both GD and TNG showed significantly increased CVD both before and after the diagnosis of hyperthyroidism. As for cancer, this could pertain to our definition of CVD, which may not be precise and specific enough. Cardiac valve degeneration, as an example, mostly occurs in GD and other autoimmune thyroid disorders (70). In contrast, TNG, more frequently than GD, has been found to be associated with cardiac arrhythmias and heart failure (70). These CVDs may have a very different prognosis. Still, the exact pathophysiological mechanisms related to the differences in

cardiovascular mortality in GD and TNG require further investigation.

Uncontrolled trials have estimated the prevalence of autoimmune thyroid disease (ATID) to vary between approximately 5-10% in patients with DM type 1 (143-145).

This has been interpreted as a positive association between ATID including GD and DM type I (146).

In line with this, we found both hyperthyroidism and GD to be associated with DM before and after the diagnosis of hyperthyroidism.

Shared pathogenesis is an obvious explanation for the coexistence of hyperthyroidism and other autoimmune disease.

Despite this, we found only a significant association with rheumatic diseases before, but not after, the diagnosis of hyperthyroidism. However, this is not necessarily in conflict with the literature, although an increased prevalence of rheumatoid arthritis associated with thyroid dysfunction has been suggested (89). In our definition of rheumatic diseases, we include a wide range of rheumatic diseases, including osteoarthrosis, which is a none-autoimmune condition affecting older people and is much more common than e.g. rheumatoid arthritis (147).

Clearly, possible biases leading to the detection of increased morbidity - both before and after the diagnosis of hyperthyroidism - could be information bias due to non-thyroidal illness (77) as well as detection bias (78). In order to minimise this, we applied censoring of diagnoses made 365 days before or after the diagnosis of hyperthyroidism. This only affected the risk of CVD after the diagnosis of hyperthyroidism, which in contrast to the analysis without censoring, was no longer statistical significant when using this time frame. Although CVD like atrial fibrillation or heart failure, which are both frequent cardiac complications (70), most likely remit after the restoration of euthyroidism (70), the association between hyperthyroidism and CVD could partly be due to detection bias. Whether this also applies to the other disease groups remains unclarified. However, the fact that the 365 days censoring of registration of morbidity did not significantly change our results argues against such an effect.

6.3. Twin analyses

By means of nation-wide twin cohorts, it has been possible to provide valid data regarding the influence of genetic factors in the aetiology of thyroid disease (148,149). The comparison of concordance rates between monozygotic and dizygotic twins provides irrefutable evidence of and a genetic component of around 75% in e.g. GD (8). Also, human lifespan is modestly heritable, with up to 30% of the variance attributed to genetic factors (150,151). In addition, risk factors associated with mortality such as smoking (152), and various morbidities (10,11) are also to some degree inherited. Even though we demonstrate statistically significant associations between hyperthyroidism and mortality/morbidity, the heritability of both the exposure, in this case hyperthyroidism, and the outcome, in this case mortality and morbidity, raises the possibility that the associations are confounded by shared genes and as a consequence are not causal. Twin studies are unique in that they provide powerful control for genetic and shared environmental confounding (14). Two members of a twin pair share aspects of their early environmental experienced due to their common rearing. In addition, MZ twin share 100% of their genes and DZ twins share on average 50% of their genes. Therefore, within DZ pair analyses allows control for common environmental influences and partly for genetic influences, and within MZ pair analyses allows control for both shared genes and early environmental factors. Thus, if there was a causal relation between the exposure and the outcome, an effect would be seen within both DZ and MZ pairs. If, on the other hand, an effect would be due to the co-inherence of both the exposure and the outcome, genetic confounding would be evident and risk estimates from the background population would partly attenuate within DZ pairs and completely attenuate within MZ pairs (153).

We introduced a multistep method of analysis to evaluate the impact of genetic confounding on the association between hyperthyroidism and mortality. In an unpaired analysis of twins (study population II), we showed an increased risk of all-cause mortality consistent with our analysis of the background population (study population II). Thus, assuming a similar effect on an individual level in both singletons and twins, the within pair analyses (study population III) of an increased mortality in the hyperthyroid twin, compared with the non-hyperthyroid sibling, indicates causality between hyperthyroidism and mortality. However, after stratification for zygosity, hyperthyroidism was associated with increased mortality within DZ-pairs, but not within MZ-pairs. These findings suggest that genetic factors may totally account for the apparent association between hyperthyroidism and mortality (14). Still, the confidence interval for MZ pairs contains the overall estimates from the singletons analyses and, consequently, there is no significant difference between these estimates. In addition, the estimates even increased within DZ pairs in comparison to the singleton population, while lower values were to be expected if genetic confounding is present. Therefore, we cautiously interpret our findings as being in line with the association between hyperthyroidism and mortality being explained, at least to some degree, by genetic factors. Unfortunately, no specific genes that might affect both the development of hyperthyroidism and mortality have been identified. It is likely that a wide range of genes play a role in both pathways. As an example, genetic confounding has recently been suggested in the association between obesity and mortality (57). In this case, a variant of the FTO gene could be an example of a potentially interesting genetic component, because it is a common genotype both linked to obesity and type 2 diabetes mellitus (154).

If hyperthyroidism and the investigated morbidities cross-trait, the finding of an increased risk of morbidity associated with hyperthyroidism could also be due to genetic confounding. Unfortunately, the number of twins included in the intra-pair analysis was too few to allow stratification according to the type of morbidity. After pooling all morbidities as one group, we found a higher risk of morbidity associated with hyperthyroidism. As for mortality, stratification for zygosity had a major impact on this result: while DZ-pairs still had an increased risk of morbidity the effect of hyperthyroidism completely attenuated within MZ pairs. Although these results are less specific and should be interpreted with caution, we cannot exclude the possibility of genetic confounding.

6.4. Methodological considerations

The unique Danish identification number as well as the nation-wide health registers provide excellent research tools making the “entire country a cohort” (155). Unsurprisingly, this has made researchers name Denmark “The Epidemiologist´s Dream” (156). Utilising these possibilities, this thesis is besides one meta-analysis, based on three prospective studies (Paper II-IV). Clearly, the main advantage of the register-based design is the ability to investigate the long-term consequences of incident hyperthyroidism in a nation-wide setting with validated control of co-morbidity and long follow-up time. However, two types of errors can affect epidemiological studies (157): random and systematic errors. While we minimise

the risk of random errors due to the study size, systematic errors, or bias, are unaffected by the cohort size. They can be classified into three general categories: confounding, selection bias, and information bias (157).

Confounders are extraneous factors that correlate with both the exposure, in this case hyperthyroidism, and the outcome, in this case mortality or morbidity (157). The most obvious confounders like age and sex are controlled for by design. Unfortunately, we had no access to smoking data, and thus we were unable to control for smoking as a confounder. However, the fact that others have controlled for smoking, and still found an increased risk of mortality associated with hyperthyroidism (38,49,53), strengthens our overall conclusions and reduces the risk of confounding. Still, we cannot rule out that our detection of increased LD associated with hyperthyroidism may be due to smoking. However, by introducing the CS, we use a standardised and validated scale to measure pre-existing co-morbidity. Further more, mortality analyses were partly restricted to individuals without any pre-existing co-morbidity, with no change in the overall results.

Selection bias is distortion that results from procedures used to select subjects and from factors that influence study participation (157). Information bias is caused by measurement errors in the needed information (157). In all studies singletons from a random 5% sample of the Danish population identified from DCRS and twins registered at DTR with a hyperthyroid diagnosis in DNPR or DNPrR were included as cases. The overall outcome as well as the CS was measured from DCRD, DNPR, and DNPrR. In respect to the cases, selection or information bias could occur due to incomplete registers or erroneous registration in the registers. Even though reporting to DNPR is mandatory, coding for co-morbidity is optional (99). As hyperthyroidism is unlikely the primary cause of inpatient treatment and outpatient treatment has only been registered since 1995, this could lead to an underestimation of cases. On the other hand, if registered in DNPR, the hyperthyroid diagnosis is valid, with misclassification in less than 2% of cases (101). Also, the level of completeness in DNPrR is very high, and has been reported to be above 96% (158). This, in line with the fact that anti-thyroid medication is sold solely as a prescript drug, ensures high accuracy in the selection of cases. Information bias with respect to the outcome is also limited. We included data on morbidity not only based on DNPR but also due to drugs registered in DNPrR. Consequently, individuals were classified with e.g. lung disease, even though they had never been referred to a hospital setting but treated solely by a general practitioner. Mortality data are based on DCRS and DCRD, which are publically used databases with mandatory registration (96-98). However, for DCRD there is no validation to the diagnostic coding, which could introduce information bias regarding the cause of death. However, such an effect is likely to affect both cases and controls, and is thus limited by the design.

A special type of selection bias is the so-called Berkson´s bias, which could influence our results in paper IV (78). It refers to the phenomenon that the detection of one disease could lead to higher rates of detection of other, not necessarily related, diseases. In order to minimise this detection bias, we applied censoring of diagnoses made 365 days before or after the diagnosis of hyperthyroidism. We cannot be certain that this procedure completely eliminated such bias, but the fact that our results did not change significantly, whether analysed with or without 365 days censoring suggests that detection bias is not a major concern. In addition, the selection of controls due to the principles of density sampling and thus allowing the same individual both as a control and as a case, are likely to minimise the impact of detection bias.

Despite the accuracy of the health-registers, there have been a change in the diagnostic criteria and incidence of hyperthyroidism over time. In Denmark, a voluntary iodine fortification program was initiated in June 1998 and altered to a mandatory fortification in July 2000. Before this, Denmark was an area of mild to moderate iodine deficiency (159). Probably as a result of the increased iodine intake, the use of anti-thyroid medication (160), surgery and radioiodine therapy (161) has increased. Clearly, this change in iodine intake and the following change in phenotype distribution might have influenced our findings. However, we found both an increased risk of mortality if evaluating on hyperthyroid individuals diagnosed since 1977 (DNPR) and if evaluating only individuals diagnosed since 1995 (DNPrR), indicating that the change in iodine intake over time is not crucial to our overall interpretation of data. In addition, due to the matching of cases and controls by age and sex by the principles of density sampling, other time-dependent environmental effects are also limited by design.

A very important issue is the generalisability of the results. Our main study population is based on a random 5% sample of the Danish population. Clearly, our results can be extrapolated to the Danish population of the same birth cohorts. However, due to changes in risk factors, such as iodine intake and the prevalence of smokers, generalisability regarding other birth cohorts might be complicated. Also, the genetic liability of both thyroid disease and the outcome hinders comparison with other ethnic groups. However, various studies performed in different decades including cases of various ages are in line with at least our mortality data. This indicates that both ethnic- and time-dependent environmental factors only influence our data to a minor degree. With this being said, this phenomenon is not unique to our study, but a general issue in all medical research.

7. CONCLUSIONS

Studies addressing the long-term consequences of hyperthyroidism are few in numbers and have generated conflicting data. After conducting meta-analyses, we found hyperthyroidism to be associated with an increased mortality of approximately 20%. However, the high heterogeneity of the included studies hampers interpretation of data in respect to causality.

In the Danish background population, we found hyperthyroidism to be associated with a nearly 40% increase in all-cause mortality, which did not change significantly after control for pre-existing co-morbidity. Stratification for the cause of hyperthyroidism showed that GD and TNG differed with respect to the cause of death. GD was associated with an increased cardiovascular mortality and mortality due to lung disease, while TNG was only associated with increased cancer mortality. Despite this difference, both GD and TNG were positively associated with an increased risk of morbidity. Stratification for the period before and after the diagnosis of hyperthyroidism, yield similar results for both GD and TNG: an increased risk of CVD, LD and DM both before and after the thyroid diagnoses.

Uniquely to our studies, we repeated our calculations in monozygotic and dizygotic same-sex twin pairs discordant for hyperthyroidism. The effect of hyperthyroidism on mortality/morbidity was completely attenuated in the monozygotic twins indicating that the observed associations to some degree are due to the presence of genetic confounding.

8. FUTURE PERSPECTIVES

Future studies should address some of the limitations of our studies. Information on biochemical severity, duration of hyperthyroidism, treatment modality and effect, as well as smoking habits would clearly improve our insights into the long-term consequences of hyperthyroidism. In addition, the evaluation of even larger cohorts would allow stratification not only for the cause of hyperthyroidism but also a more detailed evaluation of morbidity groups. The observed increase in mortality associated with hyperthyroidism is not only due to increased somatic morbidity, but could also pertain to a higher burden of psychiatric morbidity. However, knowledge regarding the association of psychiatric morbidity and hyperthyroidism is limited. Some studies (101,162-165), but not all (166,167), have demonstrated an increased risk of psychiatric morbidity associated with hyperthyroidism. Clearly, evaluation of the association between hyperthyroidism and various psychiatric morbidities utilizing the Danish possibilities of register-based science would contribute new insights into our understanding of the medical consequences of hyperthyroidism.

Implications of hyperthyroidism on daily life are unquestionable, as patients have significantly reduced quality of life (168). Even though this logically correlates with significant social consequences for affected individuals, this has only partly been investigated. Based on 215 GD patients with orbitopathy, a high degree sick leave and work disability has been reported (169). Uniquely, Danish health register would allow studying the impact of hyperthyroidism on sick leave and work disability, but also on educational level and income to name but a few.

SUMMARY

Thyroid hormones affect every cell in the human body, and the cardiovascular changes associated with increased levels of thyroid hormones are especially well described. As an example, short-term hyperthyroidism has positive chronotropic and inotropic effects on the heart, leading to a hyperdynamic vascular state. While it is biologically plausible that these changes may induce long-term consequences, the insight into morbidity as well as mortality in patients with previous hyperthyroidism is limited. The reasons for this are a combination of inadequately powered studies, varying definitions of hyperthyroidism and outcome as well as sporadic control for confounding such as co-morbidity. In addition, since hyperthyroidism and various morbidities (and mortality) as well as a number of environmental risk factors are under genetic influence, a possible co-inheritance could, at least theoretically, cause misinterpretation of data due to genetic confounding.

In order to limit the shortcomings of previous studies, the unique possibility of nationwide register-based science in Denmark was utilised. The Danish personal identification number (CPR number), assigned to all Danes, allows individual record linkage between all nation-wide medical databases. Based on The Danish Civil Registration System and The Danish Twin Registry, in combination with The Danish National Patient Registry and The Danish National Prescription Registry, two study populations were identified. The first included 4,850 hyperthyroid and 19,400 non-hyperthyroid control individuals (matched for age and sex), all identified from a random 5% sample of the Danish background population (n=339,481). In the second study population, 625 same-sex twin pairs, discordant for hyperthyroidism, were included. For each individual, the degree of co-morbidity was evaluated by the Charlson score, based on information from The Danish National Patient Registry and The Danish National Prescription Registry. The register-based approach allowed evaluation of the risk of mortality and morbidity attributed to hyperthyroidism in a large study population with long follow-up (>10 years) as well as control for co-morbidity and genetic confounding.

The results provide a number of interesting insights into the long-term consequences of hyperthyroidism. Firstly, hyperthyroidism is associated with an approximately 30% increased all-cause mortality. The all-cause mortality does not differ between Graves´ disease (GD) and toxic nodular goitre (TNG). However, GD and TNG differ with respect to cause-specific mortality. In fact, GD individuals have a higher cardiovascular mortality as compared to TNG individuals. This finding was not affected by co-morbidity, whereas the presence of genetic-confounding could not be ruled out.

Secondly, hyperthyroidism is positively associated with cardiovascular disease (CVD), lung disease (LD) and diabetes mellitus (DM). Stratification for the period before and after the diagnosis of hyperthyroidism revealed an increased risk of being diagnosed with CVD, LD and DM both before and after the diagnosis of hyperthyroidism. Although the design used does not allow a stringent distinction between cause and effect, the findings indicate a possible direct association between hyperthyroidism and these morbidities, or vice versa.

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