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THYROID HORMONES AND BREAST CANCER
– PROSPECTIVE STUDIES ON INCIDENCE, MORTALITY
AND PROGNOSTIC FACTORS

Thyroid Hormones and Breast Cancer

– *Prospective studies on incidence, mortality and prognostic factors*

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To my boys – for all the stolen hours…

Kring tinget i sig själv, de vises sten,
är sinnevärlden blott ett brokigt sken.

Vem törs dock rycka undan slöjan kring
den rena verkligheten, tingens ting?
Hjalmar Gullberg, 1935

Title and subtitle
THYROID HORMONES AND BREAST CANCER – PROSPECTIVE STUDIES ON INCIDENCE, MORTALITY AND PROGNOSTIC FACTORS

Abstract

The aim of this thesis was to investigate in a prospective design, thyroid hormones in relation to breast cancer risk, mortality and prognostic factors.

The association between total triiodothyronine (T3) levels and breast cancer risk was studied in 2185 women with 146 incident breast cancer cases. The association of free T3 and free thyroxin (T4) levels in relation to breast cancer risk was studied in 676 breastcancer cases and 680 controls. It was found that: Total T3 levels in postmenopausal women are positively associated with the risk of developing breast cancer in a dose-response manner. Free T4 levels also appear to be positively associated with a higher risk of breast cancer.

The association between thyrotropin (TSH) levels and breast cancer risk was studied in 2696 women with173 incident breast cancer cases. The association between TSH and thyroid peroxidase antibodies (TPO-Ab) and breast cancer risk was studied in 676 breastcancer cases and 680 controls. It was found that: Women with a high level of TPO-Ab have a slightly lower risk of breast cancer, whereas TSH levels are not associated with breast cancer risk.

The association between total T3 and breast cancer mortality and prognostic factors in breast cancer was studied in 2185 women where 26 died from breast cancer. It was found that: Total T3 levels are positively associated with breast cancer-specific mortality, which is not related to a general effect on all-cause mortality. Total T3 levels have a positive association to negative prognostic factors in breast cancer such as the occurrence of lymph node metastases, and negative oestrogen and progesterone receptor status.

It is concluded that thyroid hormone levels are positively related to breast cancer risk and prognosis.

Key words: T3, T4, TSH, TPO-Ab, breast cancer risk, incidence, mortality, prognostic factors

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Signature _____   Date __January 16, 2014_____

Contents

List of Papers

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals.

I   Tosovic A, Bondeson AG, Bondeson L, Ericsson UB, Malm J, Manjer J: Prospectively measured triiodothyronine levels are positively associated with breast cancer risk in post-menopausal women. *Breast Cancer Research* 2010, 12(3):R33.

II   Tosovic A, Bondenson AG, Bondeson L, Ericsson UB, Malm J, Manjer J: Prospectively measured thyroid hormones and thyroid peroxidase antibodies in relation to breast cancer risk. *International Journal of Cancer* 2012, 131(9):2126–2133.

III   Tosovic A, Bondeson AG, Bondeson L, Ericsson UB, Manjer J: Triiodothyronine levels in relation to mortality from breast cancer and all causes: a population-based prospective cohort study. *European Journal of Endocrinology* 2013, 168 483–490.

IV   Tosovic A, Bondeson AG, Bondeson L, Ericsson UB, Manjer J: Triiodothyronine levels in relation to prognostic factors in breast cancer: a population-based prospective cohort study. (*Submitted*).

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Abbreviations

BMI	Body mass index
BRCA1	Breast cancer gene 1
BRCA2	Breast cancer gene 2
CI	Confidence interval
CV	Coefficient of variation
E2	Estradiol
ER	Estrogen receptor
HR	Hazard ratio
HRT	Hormone replacement therapy
IRMA	Imunnoradiometric analysis
MDCS	Malmö Diet and Cancer Study
MPP	Malmö Preventive Project
NHG	Nottingham grade
NIS	Sodium-iodide symporter
OC	Oral contraceptive
OR	Odds ratio
PGR	Progesterone receptor
RR	Relative risk
SD	Standard deviation
T3	Triiodothyronine
T4	Tetraiodothyronine
TBG	Thyroxine binding globulin
TPO	Thyroid peroxidase
TPO-Ab	Thyroid peroxidase antibody
TR	Tyroid hormone receptor
TSH	Thyroid stimulating hormone
TSHR	Thyroid stimulating hormone receptor
TSHR-Ab	Thyroid stimulating hormone receptor antibody

Introduction

Both thyroid disorders and breast cancer are common in postmenopausal women; this has given rise to the question of whether the relationship is causal or just involves covariation. Thyroid disorders have been reported to affect 10% of individuals over a lifespan [1] and breast cancer is the most common female cancer worldwide [2]. Thus, a causal association between the two would have a large clinical impact.

Supporting the association between thyroid disorders and breast cancer, and making it biologically plausible, are the functional similarities between the thyroid and mammary gland. The functions of these organs overlap in the area of uptake and utilization of iodide. The thyroid requires iodide for hormonogenesis, and in the breast, iodide is needed in breast milk as a source of neonatal nutrition [3]. For the transport of iodide, both organs use the sodium-iodide symporter (NIS), which may indicate the presence of the same antigens [3, 4].

Experimental studies have shown that thyroid hormone receptors influence both normal breast cell differentiation and breast cancer cell proliferation, and that thyroid hormones have oestrogen-like effects on breast cancer cell lines [5–7]. These findings indicate a possible linkage between thyroid status and breast cancer. However, although this field has been investigated for over a century, the results remain inconclusive.

The majority of epidemiological studies investigating the potential association of thyroid disorders and breast cancer have been cross-sectional, comparing levels of thyroid hormones and antibodies, or thyroid size in breast cancer patients versus healthy controls [8–21]. In addition, several record-linkage studies have been performed, using cohorts of patients with thyroid disorders, which investigated the risk of subsequent breast cancer [22–29]. On the other hand, in spite of the potentially substantial clinical relevance, there has been only limited research on thyroid hormones and clinical outcome, namely, survival following breast cancer [16, 28, 30, 31]. Likewise, the investigation of thyroid hormones and thyroid disorders in relation to breast cancer aggressiveness in epidemiological or experimental settings has not been substantial [9, 11, 29, 32–34].

The results of epidemiological studies have been contradictory, illustrating one of the difficulties in interpreting cross-sectional studies, namely, that breast cancer patients are examined following diagnosis, so causality is difficult to assess. Likewise, it is difficult to compare studies on thyroid conditions in their broad sense with studies where thyroid hormone levels and antibodies have been measured.

The aim of the work presented in this thesis was to investigate thyroid hormones and antibodies in relation to the subsequent risk of breast cancer disease, mortality from breast cancer and association with breast cancer aggressiveness. To strengthen the possible association, the studies described herein were designed in a prospective manner with thyroid hormones measured in patients prior to diagnosis.

The thyroid
Anatomy and physiology

The name of the thyroid gland is derived from the Greek word *thyreoeides*, meaning shield-shaped, owing to its position in the front of the thyroid cartilage "shielding" the larynx. In the 17th century, the thyroid gland was named by the anatomist Thomas Wharton, who believed that its function was to give women a beautifully shaped neck [35].

As it turns out, the role of the thyroid gland is much more substantial than just being an appendage to female aesthetics. It is the sole source of thyroid hormones, affecting the metabolism of virtually every organ in the human body and whose absence would not be compatible with life.
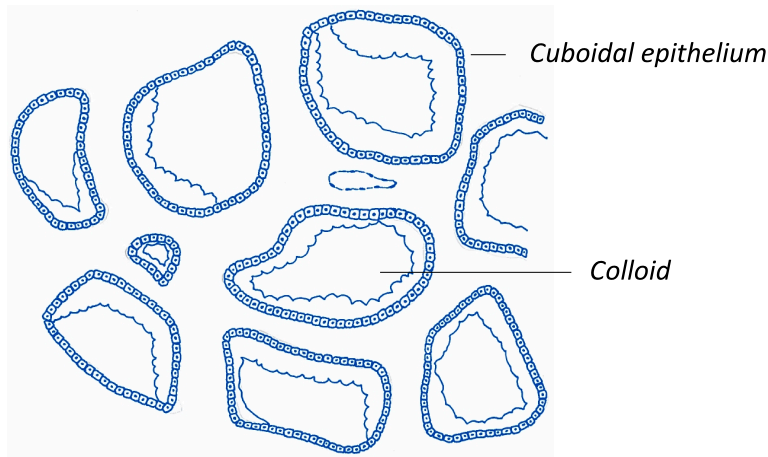
Fig 1. Histology of the thyroid gland

The embryological evolution of the thyroid gland occurs from the cephalic portion of the alimentary canal endoderm of the thyroglossal duct [36]. The thyroid tissue itself is composed of follicles consisting of a simple epithelial sphere whose lumen contains colloid. Follicular cells range from flat to low columnar. The thyroid is an extremely vascularised organ, having an extensive blood and lymphatic capillary network surrounding the follicles. It has a higher rate of blood flow per gram of tissue than any other organ. Endothelial cells of these capillaries are fenestrated, facilitating the passage of the hormones into the capillaries. The gland is considered hypoactive when the majority of follicular cells are flat, and active when the cells are columnar and follicular size and colloid content decrease [37].

The thyroid is the only secretory gland whose product is stored in substantial quantities. This accumulation occurs in the extracellular colloid, at a level sufficient to supply the organism for up to three months. Therefore, when its synthesis of hormones stops entirely, the effects of deficiency will not be observed for several months [38].

Thyroid colloid is composed of thyroglobulin, a high-molecular-weight glycoprotein.

Thyroglobulin is synthesized by the follicular cells and released into the follicle lumen.

An iodide pump is situated within the membrane of the basal region of follicular cells and actively transports circulating iodide, providing an essential element for hormone synthesis. It is stimulated by the thyroid-stimulating hormone (TSH), also known as thyrotropin [39].

The iodination of thyroglobulin takes place in the colloid. Iodide is activated by oxidation by the thyroid peroxidase enzyme into an intermediate that combines in the colloid with the tyrosine residues of thyroglobulin [40].

When stimulated by TSH, thyroid follicular cells take up colloid by pinocytosis. Proteases from the lysosomes split the peptide bonds between the iodinated tyrosine residues and the rest of the thyroglobulin, and triiodothyronine (T3) and thyroxine (T4) are liberated into the cytoplasm. T3 and T4 then cross the cell membrane and are discharged into the capillaries [38].
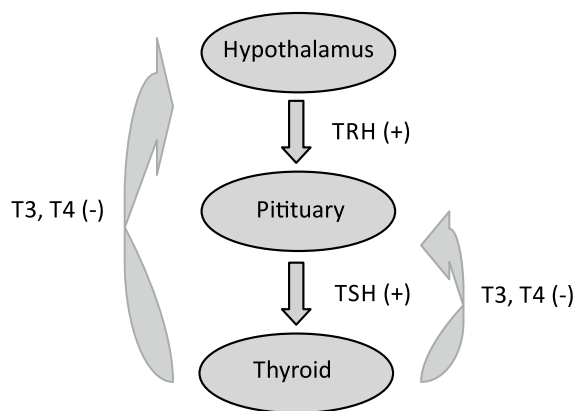
(-) = inhibition, (+) = stimulation

Fig 2. The thyroid feedback loop

Thyroid hormones

Thyroid hormones affect virtually all tissues in the human body and the net result of their actions is a general increase in metabolic activity [38]. To mention a few examples, thyroid hormones have both general and specific effects on growth, manifested mainly in growing children. They are also responsible for the development and maturation of the brain during foetal life. One particularly important effect is in myelination and neuronal differentiation. Deficiencies in maternal thyroid hormone levels result in mental retardation, deafness and spasticity of the child, and there is evidence that even mild maternal thyroid disorders are associated with reduced IQ in the offspring [41].

Thyroid hormones are also necessary for the growth of long bones in children and for skeletal maturation. Reduced levels of thyroid hormones slow longitudinal bone growth and endochondral ossification [42].

Thyroid hormones affect carbohydrate, fat and vitamin metabolism and cause an increase in the basal metabolic rate. The effects of T4 and T3 and those of the catecholamines epinephrine and norepinephrine are closely interrelated. They all increase the metabolic rate and stimulate the nervous system and heart. They also increase vasodilation and blood flow, as well as cardiac output and systolic blood pressure. Thyroid hormones also affect the rate and depth of respiration, increase brain activity, and influence muscle and other endocrine glands by increasing their secretion [38].

The production and utilization of thyroid hormones normally follow the principle, "of each and every one by ability, for each and every one by demand", which is applicable at the organ level as well as at the individual level [43]. Thyroid hormone synthesis is regulated by TSH, which is secreted from the anterior pituitary gland. The level of TSH is in turn inhibited by the free fraction of thyroid hormones in the plasma via a negative feedback loop. In normal conditions, the metabolic control is effective, but the system is complex and sensitive to disturbances caused by drugs and illness [44].

13

Thyrotropin

Thyrotropin, or TSH, is released by the pituitary gland. The secretion of TSH is regulated by a very sensitive feedback loop dependent on the circulating levels of the thyroid hormones. Like other pituitary hormones, TSH is a two-subunit glycoprotein. There is an alpha and a beta subunit. The beta subunit confers the specific binding properties and biological activity of TSH. TSH binds to a specific TSH receptor (TSHR) in the thyroid cell membrane, upon which the synthesis of T3 and T4 is initiated [44].

TSH has a biological half-life of 60 minutes and normal TSH secretion exhibits a circadian pattern, rising in the afternoon and evening, peaking after midnight and declining during the day [44]. The differences in measured levels of TSH depend on when throughout the day the sample is taken and can vary by up to a factor of two, which should be taken into consideration when repeated samples are drawn for comparison in the same individual. Furthermore, there are small variations in TSH during the daytime, as in other anterior pituitary hormones [43]. Even very small changes in concentrations of T3 and T4 give rise to logarithmically amplified variations in TSH serum concentrations via a negative feedback loop. However, the intra-individual variation in TSH concentrations is relatively small compared with the population-based reference intervals. Hence, individual TSH levels should if possible be compared to earlier measurements within the same patient [43]. Concerning long-term variation, tracking of individuals, that is, ranking of individuals over time, is quite stable for TSH [45].

There is a certain physiological variation in TSH associated with age, with higher levels in the elderly, especially in women [46]. Differences between geographic regions can also occur due to differences in climate, lifestyle and iodide intake. Factors inhibiting TSH secretion, besides the effect of high concentrations of circulating T3 and primary pituitary disorder, include acute inflammatory responses, such as in general illness, stress and severe depression. In addition, the use of certain drugs can be associated with low TSH concentrations, such as corticosteroids, β-adrenergic blockers and dopamine [47]. The TSH measurement is the primary serum analysis in diagnostics of thyroid disorders [46].

Thyroid-stimulating hormone receptor

The thyroid-stimulating hormone receptor (TSHR) is expressed on the cell membrane of thyroid epithelial cells, and is responsible for the regulation of thyroid growth and function [49]. The stimulation of the receptor by TSH results in increases in the uptake and transport of iodide, and the synthesis of T3 and T4, as well as thyroid cell hypertrophy. When chronic stimulation of the thyroid by TSH occurs, as in iodide deficiency, the entire gland hypertrophies, increases in vascularity and becomes a goitre [44]. In the autoimmune hyperthyroidism of Graves' disease, autoantibodies (TSHR-Ab) are directed towards the TSHR antigen [49] and there is activation of the receptor, resulting in increased release of T3 and T4. There are also antibodies that bind to the TSHR but lack the ability to stimulate it; instead, they block the binding of normal TSH, causing a decrease in thyroid hormone release and hypothyroidism [43]. Laboratory methods concentrate on measuring the total level of TSHR-Ab, regardless of their stimulatory or blocking effects. Positive TSHR-Ab titres have high specificity and sensitivity for the diagnosis of Graves' disease, but have also been found in 10% of patients with Hashimoto's thyroiditis [43].

Thyroxine and triiodothyronine

Thyroxine (T4) is more abundant than triiodothyronine (T3), with plasma concentrations

of 100 and 2 nmol/L, respectively. Circulating T4 is secreted by the thyroid, whereas only 10%–20% of T3 is. Instead, the majority of T3 originates from the conversion of T4 into T3 in the tissues. T3 persists in the blood for a much shorter period of time than T4 and has a half-life of 24 h, compared with 6–7 days for T4. The secretion of T3 from the thyroid increases with increased stimulation by TSH, as part of the physiological feedback loop, or in hyperthyroidism or subclinical hyperthyreosis [43]. In addition, the local conversion of circulating T4 at the tissue level is increasingly recognized as an important mechanism of regulation of thyroid hormone action [50].

T3 is the biologically active hormone, while T4 constitutes a circulating form of transport and buffer for T3. The majority of T3 and T4 are protein-bound and 0.3% of T3 is in its free form, while the corresponding proportion is 0.02% for T4. Around 80% of the hormones are bound to thyroxine-binding globulin (TBG), which as the name suggests has a much higher affinity for T4 than for T3. The remaining protein-bound hormones bind to transthyretin and albumin [43].

Studies have shown that there is a circadian rhythm for T3 but not for T4. Free T3 levels peak approximately 90 minutes after TSH levels. Free T3 shows a lower amplitude than TSH, which may be because the change in free T3 is related to TSH stimulation of thyroid hormone release from the thyroid and not from the peripheral conversion of free T4 to free T3 [51].

There is low short-term variation in free T3 and free T4, but it may be slightly higher for TSH [52]. Concerning long-term variation, tracking of individuals, namely, the ranking of individuals over time, is quite stable for thyroid hormones and TSH [45]. No significant variation in T3 concentration is seen throughout adult life, but after 70 years of age, there is a small decrease in the level of T3, partially due to the higher prevalence of illness [43].

Among exogenous conditions that influence the levels of thyroid hormones in euthyroid subjects are certain drugs, for example, heparin, which is associated with elevated levels of free T3 and free T4. Propranolol and iodide-containing drugs inhibit the conversion of T4 to T3, resulting in a lower level of T3, sometimes accompanied by a higher level of T4. The same mechanism is responsible for the low T3 levels in patients undergoing surgery or suffering from high fever.

Oestrogens, endogenous during pregnancy and exogenous during hormone replacement therapy (HRT), stimulate the conversion of T4 to T3 in the periphery, which elevates the plasma level of T3 [43]. Studies have shown that different types of deiodinase at the tissue level have a role in the regulation of thyroid hormone bioactivity, controlling the level of peripheral T3 production [49]. It is possible that oestrogens can influence the activity of deiodinases and in this way cause an increase in plasma T3 level.

Thyroxine-binding globulin

Thyroxine-binding globulin (TBG) binds 70% of T4. The physiologic function of this is not known, but it may serve to distribute T4 evenly to the tissues. Sustained increases or decreases in the concentrations of TBG and other thyroid-binding proteins in the plasma are produced by several normal and disordered physiologic states and medications [43]. When TBG concentration increases, the concentration of free thyroid hormones falls temporarily. This stimulates TSH secretion, which increases the production of free hormone. Eventually, the total plasma T4 and T3 are elevated, but the concentrations of the free hormones are normal and the patient is euthyroid.

Serum concentrations of TBG are similar in women and men, including postmenopausal women, indicating that this production is not affected by age or sex, hence the natural rates of oestrogen production [53].

Pregnancy, oestrogen-secreting tumours

15

and exogenous oestrogens increase the production and glycosylation of TBG, which decreases its clearance. Any orally administered oestrogen has this effect on TBG, given by itself or in conjunction with progesterone. In women, the oestrogen-induced rise in TBG has no physiological effect other than raising the total serum T4 concentration, but the free T4 concentration remains normal [53]. Glucocorticoids, on the other hand, decrease the level of TBG and total T4 and T3, but free T3/T4 and TSH remain normal.

Thyroid hormone receptor

The nuclear thyroid hormone receptor (TR) is, just like the oestrogen receptor (ER), a member of the steroid hormone receptor family of nuclear transcription factors [54].

There are two TR genes coding for multiple receptor isoforms. The TR genes TRα and TRβ have different patterns of expression in development and in adult tissues. TRα has one T3-binding isoform, TRα1, predominantly expressed in brain, heart and skeletal muscle. TRβ has three major T3-binding isoforms, with TRβ1 having the widest distribution and greatest T3 binding capacity [50]. The different receptor forms may help to explain both the normal variation in thyroid hormone responsiveness of various organs and the selective tissue abnormalities found in various thyroid resistance syndromes.

Recently, a structural protein of the plasma membrane, integrin, has been described to contain a binding site for thyroid hormone responsible for cellular events, such as cell division [48] and angiogenesis [55]. The functions of this integrin receptor seem to be distinct from those of the classical nuclear receptor for thyroid hormone [56, 57]. The actions of the cellular surface receptor have been described as non-genomic and associated with the activity of certain membrane ion transport systems and cell proliferation [57]. The integrin receptor has been found primarily in cancer cells,

dividing endothelial, vascular smooth muscle cells and osteoclasts, where it seems to enable T4 and T3 to stimulate cancer cell proliferation and angiogenesis [56].

Thyroid pathophysiology

Functional diseases of the thyroid present as hyperthyroidism and hypothyroidism. Diffuse or focal enlargement of the gland, goitre, has no simple correlation to resultant clinical manifestations [58].

Hypothyroidism

Hypothyroidism is a clinical entity resulting from deficiency of thyroid hormones or, more rarely, from their impaired activity at the tissue level. Hypothyroidism may be congenital or acquired, primary or secondary, chronic or transient. Primary hypothyroidism is caused by disease or treatment that destroys the thyroid gland or interferes with thyroid hormone biosynthesis. Autoimmune thyroiditis is the predominant cause of primary hypothyroidism in countries such as Sweden where severe iodine deficiency is non-existent. Another cause of primary hypothyroidism, chronic or transient, is previous radioiodine or surgical treatment of hyperthyroidism. In secondary or central hypothyroidism, which is very rare, there is a lack of TSH or TSH activity, due to a pituitary or hypothalamic cause [59].

Symptoms due to low thyroid hormone levels are associated with a decrease in overall metabolism. Hypothermia is common and patients are prone to gain weight in spite of normal energy intake. The effects on the nervous system in adults are slowed mentation and forgetfulness. Chronic hypothyroidism is associated with bradycardia, muscle weakness and hair loss. Owing to a decrease in gastrointestinal motility, constipation is also a common problem. In women, secondary effects of

decreased secretion of gonadotropins include amenorrhea and infertility [44].

Treatment of systemic symptoms of hypothyroidism involves the pharmacological substitution of the thyroid hormones by the oral administration of levothyroxine, which is the pharmaceutical counterpart of physiologic T4 [60].

Hyperthyroidism

Hyperthyroidsm is caused by increased thyroid hormone production in Graves' disease, toxic multinodular goitre and solitary toxic adenoma.

Graves' disease is one of the most prevalent autoimmune thyroid disorders leading to hyperthyroidism [61]. Sibling and twin studies indicate a strong genetic influence on the development of this disease.

Toxic multinodular goitre is more common in elderly patients from iodide-deficient areas. In areas of mild and moderate iodide deficiency, hyperthyroidism is the most common cause of thyroid dysfunction [62]. The mechanism of action is that iodide deficiency can lead to the development of multinodular goitre and then some of the nodules can become autonomous. It is this autonomous function that is responsible for increased production of thyroid hormones and hyperthyroidism. Thyroid autonomy is caused by somatic, activating mutations of genes regulating follicular cell activities [63].

Symptoms in hyperthyroidism are related to excessive expression of T3 and T4. A general increase in metabolism results in weight loss and hyperthermia. Lipolysis is increased and, owing to the activation of osteoblasts and osteoclasts, there is increased bone turnover and loss of bone mineral density. Owing to the thyroid hormone interaction with epinephrine and norepinephrine, there is an increased sensitivity of the tissues to catecholamines. Cardiac output is increased and pulse pressure elevated. The effects on the nervous system are those of nervousness, irritability and restlessness [44]. The

ophthalmopathy in Graves' disease is mainly related to the effects of TR antibodies on the eye muscle tissue, but is also due to the infiltration of lymphocytes and oedema [61].

There are three types of treatment for thyrotoxicosis; radioiodine, antithyroid drugs and surgery. Radioiodine is usually restricted to patients over the age of 50. Pregnancy is an absolute contraindication for radioiodine treatment and relative contraindications are ophthalmopathy and solitary thyroid nodules. A complication due to radioiodine therapy is permanent hypothyroidism, which is treated with lifelong levothyroxine supplementation [60]. Antithyroid drugs, in Sweden tiamazol and propylthiouracil, are suitable for pregnant patients, children and mild forms of thyrotoxicosis, as preparation before surgery and as a complement to radioiodine treatment.

Total thyroidectomy is the treatment of choice in young patients with severe forms of the disease owing to a high degree of disease recurrence after treatment with antithyroid drugs. It is also a suitable treatment for pregnant patients. Post-surgery, lifelong treatment with levothyroxine is necessary [60].

Autoimmune thyroid disease

The two major autoimmune thyroid diseases are Graves' disease and Hashimoto's thyroiditis. Both of these are characterized by the production of autoantibodies against surface antigens or products from the thyroid gland and clinically by hypo- or hyperfunction of the thyroid gland. The development of thyroid autoimmunity involves an interaction between genetic predisposition and environmental triggers such as iodine, medications, infection and smoking [64].

The most common autoantibodies are antibodies against the TSH receptor, thyroid peroxidase (TPO) and thyroglobulin. TPO is a membrane protein in the apical portion of active follicular cells and its concentration in the plasma is influenced by the activity of the fol-

licular cells and their decay. The prevalence of TPO-Ab increases with age and it is more common in women than in men. Depending on the method of analysis, it has been estimated that around 12% of the healthy population have an increased concentration of TPO-Ab in the blood [43]. The frequency increases with age and female sex and is often seen without any associated thyroid disorder. It is however a risk factor for developing autoimmune thyroid disease in the future.

TPO-Ab are present at increased levels in chronic thyroiditis, mainly in Hashimoto's thyroiditis and to a lesser degree in Graves' disease. TPO-Ab are also detected in most cases of post-partum thyroiditis and its presence has been associated with an increased risk of miscarriage. TPO-Ab can also sometimes be detected in pernicious anaemia, diabetes mellitus, rheumatoid arthritis, Addison's disease and Sjögren's syndrome [43].

Other autoantibodies

Other autoantibodies that commonly appear in autoimmune diseases of the thyroid are those against the TSH receptor (TSHR-Ab) and thyroglobulin (Tg-Ab) [49]. Binding of TSHR-Ab to follicular cell receptors has a stimulating effect similar to that of TSH. This results in hyperthyreosis and diffuse thyroid gland enlargement, known as Graves'/Basedow's disease [43].

Tg-Ab can be found at high concentrations in patients with thyroid malignancies and are usually monitored during follow-up after thyroid cancer treatment [43].

Subclinical thyroid disease

In subclinical hypothyroid disease, the individual has pathological levels of serum TSH and levels within the normal reference ranges for both T3 and free T4 [46]. The majority of individuals with subclinical hypothyroid disease also have positive TPO-Ab titres [65].

An aspect that has to be taken into consideration when discussing the prevalence of subclinical thyroid disease is the fact that there is little variation in serum T4 and T3 within individual subjects compared with the variation within the population [51, 52]. Hence a small change in thyroid hormone has physiological significance for the individual subject. Accordingly, a test result within laboratory reference limits is not necessarily normal for an individual. Because serum TSH responds with logarithmically amplified variation to minor changes in serum T4 and T3, abnormal serum TSH may indicate that serum T4 and T3 are not normal for an individual. As mentioned, a condition with abnormal serum TSH but with serum T4 and T3 within laboratory reference ranges is labelled subclinical thyroid disease. Studies indicate that the distinction between subclinical and overt thyroid disease (abnormal serum TSH and abnormal T4 and/or T3) is somewhat arbitrary [45]. For the same degree of thyroid function abnormality, the diagnosis depends to a considerable extent on the position of the patient's normal set point for T4 and T3 within the laboratory reference range. Hence, serum TSH outside the population-based reference range may indicate that serum T3 and T4 are not normal for the individual [45]. As such, individuals classified as having subclinical disease may in reality have an overt thyroid disorder.

Subclinical hyperthyroidism is caused by the release of excess thyroid hormone by the gland or exogenous thyroid hormone therapy [63]. Both overt and subclinical disease may lead to characteristic signs and symptoms and, although symptomatic, subclinical thyroid disease is classified as such due to the levels of thyroid hormones being within the reference range.

Treatment of subclinical hyperthyroidism can be considered in individuals above the age of 60 years, and in patients with cardiac symptoms or osteoporosis [60]. Indications for the treatment of subclinical hypothyroidism in Sweden are suspicion of symptoms related to

low levels of thyroid hormones, high TSH titres and high TPO-Ab titres [60].

Nonthyroidal illness syndrome

Nonthyroidal illness syndrome (NTIS), also known as low T3 syndrome or euthyroid sick syndrome, is described as a condition characterised by abnormal thyroid function test results in patients with acute or chronic systemic illnesses without underlying thyroid disease. The laboratory parameters of this syndrome include low serum levels of T3, with normal or low levels of T4 and normal or low levels of TSH [66].

The absence of an increase in TSH as a response to low T3 levels indicates the absence of negative feedback regulation. This may represent a useful adaptation of the body to counteract excessive catabolism observed during illness and can be viewed as part of the acute phase response [67]. Most of the biologically active T3 originates from the conversion of T4 to T3 in the periphery by deiodinases that have a tissue-specific distribution. Studies have shown that, in NTIS, there is a change in deiodinase expression that may either activate or inhibit the thyroid hormone action [67]. This is also considered a possible mechanism responsible for the low T3 levels observed in NTIS. Furthermore, the binding affinity of the hormones to TBG, transthyretin and albumin is diminished, which also results in the low T3 levels observed [43].

Epidemiology of thyroid disorders

When reviewing the epidemiology of thyroid diseases, difficulties arise due to problems of definition, selection criteria and different techniques used for the measurement of thyroid function. However, overt abnormalities in thyroid function are common in Europe and the USA, affecting 5–10% of individuals over a lifespan. Minor abnormalities in thyroid function with subclinical hypothyroidism or hyperthyroidism are even more common [45]. Worldwide, the most common cause of thyroid disorders is iodide deficiency. Deficient iodide intake leads to goitre development and hypothyroidism. In areas without iodide deficiency, the most common form of thyroid disease is autoimmune disease [1].

Hypothyroidism

In iodide-deficient regions such as mountainous areas in South-East Asia, Latin America and Central Africa, the prevalence of goitre is as high as 80% [40]. A review article by McGrogan of studies conducted in Scandinavia, Spain, the UK and the USA reports incidence rates of autoimmune hypothyroidism varying between 80/100,000 per year (men) and 350/100,000 per year (women) [68]. In iodide-sufficient areas such as Sweden, the prevalence of spontaneous hypothyroidism in women is about 3%, chronic lymphocytic thyroiditis being the most common cause [59]. In Sweden, a longitudinal population study of elderly people in Gothenburg showed the incidence rate of hypothyroidism in women to be 200/100,000 per year [69].

Hypothyroid disorders are more common in women than in men and more common than hyperthyroid disorders [68]. The prevalence of hypothyroid disorders is higher among the elderly in the community [70].

Hyperthyroidism

Hyperthyroidism is a common condition with a wide variation in the reported incidence due to differences in dietary iodine intake, ethnic origin and population structure. It is more common among women, affecting around 2% of women and 0.2% of men, with a worldwide incidence between 23 and 97/100,000 per year [62]. The most common form of hyperthyroidism is caused by increased thyroid hormone

19

production in Graves' disease, toxic multinodular goitre and solitary toxic adenoma [60].

Differences in degrees of iodide deficiency result in different thyroid disorders. In severely iodide-deficient areas, hypothyroidism is the most common disorder, whereas in areas with mild and moderate iodide deficiency, hyperthyroidism is a common cause of thyroid dysfunction. This is thought to be due to multifocal autonomous thyroid nodules [71]. The World Health Organisation (WHO) has declared Sweden to be iodide-sufficient [72] due to the iodination of dietary salt since 1936. The total incidence of hyperthyroidism in Sweden has been reported to be 27.6/100,000 inhabitants per year [62].

Subclinical thyroid disease

Subclinical hypothyroidism or mild thyroid failure is a common problem, with a prevalence of 3% to 8% in the population of the USA without known thyroid disease [73, 74]. The prevalence increases with age and is higher in women. After the sixth decade of life, the prevalence in men approaches that of women, with a combined prevalence of 10% [73]. Thyroid antibodies can be detected in 80% of patients with subclinical hypothyroid disease [64].

Common causes of subclinical hyperthyroid disease include Graves' disease, autonomous functioning thyroid adenoma and toxic multinodular goitre. Subclinical hyperthyroidism is defined by low or undetectable serum TSH levels, with normal free T4 and total or free T3 levels [75]. The reference intervals regarding the lower limit of TSH concentrations vary among investigators [76]. This fact along with different causes of subclinical hyperthyroidism, sex, age, iodine intake and the sensitivity of the methods used to measure serum TSH concentrations explains the variability in prevalence reported by different studies [77]. There is an inverse correlation between population iodine intake and the occurrence of thyroid autonomy, with prevalence as high as 15% in

20

the elderly living in iodine-deficient areas [78].

In a large population-based cohort study in Denmark, including residents of Copenhagen of 18 years of age and older who underwent thyroid screening, the prevalence of subclinical hypothyroidism was 2% and that of subclinical hyperthyroidism was 1% [79].

Thyroid disorders in Malmö

Malmö is a city in southern Sweden with 300,000 inhabitants from 170 different nationalities. It is an expanding city where 40% of the population today has a foreign background, the largest group of immigrants coming from Iraq. By comparison, 30 years ago, the proportion of foreign citizens in Malmö was less than 8% [80]. This has to be taken into consideration when interpreting the data on the incidence of thyroid disorders in Sweden. Concerning hyperthyroidism, the highest incidence in Sweden has been reported in Malmö, namely, 29.6/100,000 per year [81]. This finding is particularly interesting since, historically, in spite of the high prevalence of goitre in Sweden over a century ago, owing to its geographic location, Malmö was never a high-incidence area for this condition. However, the incidence of thyrotoxicosis in Malmö increased in 1988–1990, compared to 1970–74 [82]. It is believed that a change in smoking habits might have been partly responsible for the increase. The high incidence of hyperthyroidism in Malmö today might be related to ethnic differences, socioeconomic status and demographic structure. A study by Lantz *et al.,* reported a higher incidence of Graves' disease among residents of Malmö born outside of Sweden than in Swedes. However, the age-specific peak incidence in women was at 50–59 in both groups. It is unknown whether menopause plays a role in this age distribution [81].

Concerning hypothyroid disorders, 5%–7% of postmenopausal women in Sweden are treated for hypothyroid disorders [72]. There does not seem to be a similar trend concern-

ing hypothyroidism, with the highest proportion of hypothyroid patients in Malmö compared to the rest of Sweden. However, there have been few studies on hypothyroidism in Sweden and it is difficult to draw any conclusions as to differences in geographical distribution across the country.

Risk factors

Gender

Thyroid disorders are highly associated with gender and as much as 10 times more common in women than in men. The incidence rates of spontaneous hypothyroidism are 3.5 per 1,000 and 0.6 per 1,000 in women and in men, respectively. Spontaneous hyperthyroidism has a reported incidence rate of 0.4 per 1,000 in women and 0.1 in men [1].

Age

Thyroid disorders vary with age and hypothyroid disorders have a peak incidence in postmenopausal women [3]. The prevalence of hypothyroidism is higher among the elderly, with a reported prevalence as high as 7% in the age range of 85–89 years [83,84].

In terms of hyperthyroidism, the reported age-specific incidences vary among studies and depending on the underlying cause. Graves' disease has been reported to have a peak incidence in the age range of 20 and 49 by some studies, but in a Swedish study, a peak incidence at 50–59 years has been reported [81].

Iodide deficiency

When the levels of circulating iodide decrease due to inadequate intake, this is compensated for by increased secretion of TSH, which in turn stimulates the expression of the sodium/iodide symporter. The clearance of iodide by the thyroid is increased. This compensatory mechanism is sufficient until the intake of iodide falls below 50 µg/day, a level at which diffuse goitre starts to develop. The development of goitre and resulting hypothyroidism affect all age groups, but in postmenopausal women, iodide deficiency can result in toxic multinodular goitre [40]. The effects of iodide deficiency vary among individuals and populations, even in endemic areas, suggesting influences by other dietary compounds and genetic predisposition [40].

Smoking

There are several mechanisms by which smoking affects thyroid hormone levels. Smoking is associated with increased thyroid hormone secretion due to nicotine-induced sympathetic activation [85]. Furthermore, tobacco smoke contains thiocyanate, which is a potential goitrogen that acts by the inhibition of iodide transport [86].

Smoking is an established risk factor for Graves' disease and ophthalmopathy [87] and a potential risk factor in subclinical hypothyroidism by reducing thyroid hormone secretion. It may also exacerbate the peripheral effects of thyroid deficiency in overt hypothyroidism and evidence suggests that, in Hashimoto's thyroiditis, smoking may contribute to the development of hypothyroidism through an increase in thiocyanate levels [85].

Genetics

The genetic predisposition to Graves' disease is well established since it was reported that a third of siblings of Graves' disease patients developed autoimmune thyroid disorders and over half of asymptomatic children had thyroid antibodies in their blood [88]. It is believed that this predisposition is accounted for by multiple genes, with very modest individual effects [89].

Hashimoto's thyroiditis and Graves' disease have a complex aetiology that involves genetic and environmental influences. To date, seven genes have been shown to contribute to the aetiology of autoimmune thyroid disease. The
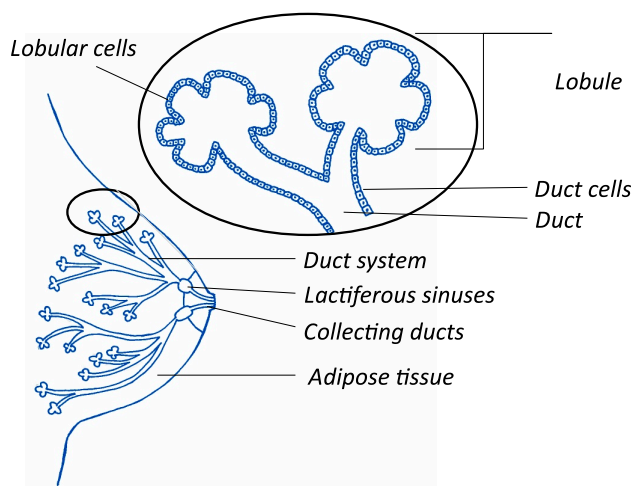
Fig 3. Schematic picture of the breast

first gene discovered, HLA-DR3, is associated with both Graves' disease and Hashimoto's thyroiditis. Other non-MHC genes described to be associated with autoimmune thyroid diseases are immune regulatory genes and thyroid-specific genes. It is believed that these genes interact with environmental factors such as infection and are likely to trigger disease through epigenetic mechanisms [90].

The breast

Anatomy and physiology

The breasts appear in the embryo as a pair of thickenings of the epidermis, the milk lines. These extend from the forelimb to the hindlimb on the ventral side of the foetus [91].

Each breast consists of 15–25 lobes, separated from each other by dense connective tissue and adipose tissue. Each lobe is a gland in itself with its own excretory lactiferous duct that emerges independently in the nipple [36]. The structure of the female breast varies according to age and physiologic status. Before puberty,

the breasts are composed of lactiferous sinuses and lactiferous ducts. The development during puberty is due to stimulation by ovarian oestrogens and includes proliferation of the lactiferous ducts and accumulation of adipose and connective tissue [36]. In the adult female, the mammary gland is composed of lobules that consist of alveoli and several intralobular ducts that empty into one terminal interlobular duct. The terminal interlobular ducts become the lactiferous duct that dilates near the opening of the nipple, forming the lactiferous sinuses [37]. These are lined by a double-layered epithelium that continuously covers the lactiferous ducts, intralobular ducts and finally the terminal duct lobular units. This epithelial lining is thought to originate from a multipotent stem cell and is separated from the surrounding tissue by a basal membrane [92].

Small alterations in the structure of the breast occur during the menstrual cycle, as cells of the ducts proliferate at about the time of ovulation, coinciding with the time at which circulating oestrogen is at its peak [37]. During pregnancy, the high quantities of oestrogens cause the ductal system of the breast to

grow and to branch. There is a proliferation of the alveoli that eventually become active milk secretory acini in lactation. In the breast milk, iodide is needed as a source of neonatal nutrition [3]. Similar to the thyroid gland, breast tissue uses the NIS to transport iodide, which is than catalysed by lactoperoxidases. However, the structure of this transport protein differs in the breast and the thyroid. NIS is not expressed in normal non-lactating tissue [93], and may represent a marker for breast malignancy since it has been reported that over 80% of human breast cancer samples expressed this symporter [93]. Apart from being a nutrient, there is no known role for iodide in the breast [3], although it has been suggested that it plays a protective role in the maintenance of healthy breast tissue [94, 95].

The development of the lobule-alveolar system is the result of the synergistic action of mainly oestrogen and progesterone, the breast being the only structure in the human body that undergoes such a striking structural and physiological change during the hormonal cycle of pregnancy [38]. During lactation, milk production is stimulated by prolactin, which is secreted due to the baby's suction of the lactating breast. Prolactin is secreted by the pituitary gland and regulated by this reflex mechanism in the hypothalamus. The suction reflex also stimulates the release of oxytocin, which in turn acts on the smooth muscle surrounding lactiferous ducts and sinuses, facilitating milk secretion [92]. After the cessation of lactation, the lobules regress and the breast size is diminished, although the increases in size and number of lobules are permanent. The breast is thus first fully evolved after the first pregnancy [96]. After menopause, involution of the breast is characterised by a reduction in size and atrophy of the secretory portions and, to a certain extent, the ducts. Russo *et al.,* proposed four stages in the remodelling of the female breast throughout a lifespan: lobular stage 1 in childhood, lobular stage 2 after puberty, lobular stage 3 during pregnancy and lobular stage 4

during lactation. The breast is then remodelled back into lobular stage 1 in postmenopausal females. It has been proposed that stages 1 and 2 are more susceptible to carcinogenic influences than stages 3 and 4 [96].

Breast cancer

Breast cancer is a heterogeneous disease that comprises a diverse collection of malignant conditions in the breast [92]. However, when referring to breast cancer in these circumstances, adenocarcinomas of the breast are exclusively indicated [97]. Multiple aetiological factors contribute to breast cancer risk and breast cancer is a disease with a wide spectrum of genetic alterations and phenotypic heterogeneity [98]. The prognosis of breast cancer also varies depending on the developmental stage of the breast tissue at diagnosis [ibid.].

Virtually all breast cancers are adenocarcinomas [97]. The cancers are bilateral or sequential in the same breast in 5%–10% of cases [99]. Curiously, breast cancer is more common in the left breast than in the right and the majority of the cancers arise in the upper outer quadrant, which influences the pattern of nodal metastasis [58]. Accordingly, metastasis occurs first in the ipsilateral axillary lymph nodes passing the sentinel lymph node. Distant metastasis occurs to the bone, lungs, liver and brain, making the disease incurable [92].

Diagnosis

Triple diagnosis is the gold standard in the diagnosis of breast cancer, whether it presents as a lump in the breast or is discovered by mammography screening. Triple diagnosis constitutes a clinical examination of the breast, mammography and needle biopsy. All findings that are discovered clinically and by mammography and raise a suspicion of malignancy are subjected to triple diagnosis, for which the sensitivity approaches 100% [92].

23

In Malmö, there has been particular interest in breast cancer for half a century. Since 1977, weekly multidisciplinary conferences have taken place, where the management of women diagnosed with breast cancer is discussed [100]. Throughout the period 1976–1986, the Malmö Mammographic Screening Trial (MMST) was conducted to investigate the effect of mammography screening on survival and mortality in breast cancer; the data were evaluated in 1988 [101]. In 1990, a general mammography screening service was started in Malmö according to national guidelines, and currently women between 40 and 70 years of age are invited to participate [102].

Classification

The staging of invasive tumours is usually according to the TNM classification: size of the tumour (T), axillary lymph nodes (N) and distant metastasis (M). Stage together with grade is the most important determinant of survival in breast cancer [103].

The histological classification of tumours of the breast according to the WHO in 2003 comprises multiple tumour types [99]; among the epithelial tumours, invasive ductal and lobular carcinomas are the two most frequently occurring. Invasive ductal carcinoma is seen in 50%–80% of cases and invasive lobular carcinoma in 5%–15%. Histological grade is often determined by the Nottingham grade (NHG), a scoring system for tumour aggressiveness based on mitotic count, tubular formation and the degree of nuclear atypia. NHG is an independent determinant of survival [92].

Classification based on the expression of different receptors is of predictive value concerning targeted therapy and of prognostic value. Frequently studied receptors are ERα, progesterone receptor (PGR) and human epidermal growth factor 2 receptor (HER2). Approximately 70% of breast tumours express ER and/or PGR. ER is the primary transcription factor driving oncogenesis in receptor-positive breast cancers; it is both a target of, and a predictor of response to, antioestrogen therapy [104]. HER2 is a growth factor receptor and targeted therapy is available for tumours expressing HER2. Tumour aggressiveness can further be studied by assessment of the proliferation marker Ki-67 [105].

Treatment

Surgery is the primary treatment for breast cancer. Sentinel-node biopsy and axillary nodal surgery are preceding the choice of endocrine, chemotherapy and/or radiation treatment [92]. Whether mastectomy is required or breast-conserving surgery is the treatment of choice depends on the size of the tumour, and its location and multifocality [106]. Adjuvant endocrine therapy is the standard for the majority of receptor-positive patients. Endocrine therapy is sometimes given in conjunction with chemotherapy to obtain maximal treatment effect.

Chemotherapy can be given as neo-adjuvant, adjuvant or palliation depending on the individual and breast cancer characteristics. Furthermore, all patients with HER2-positive tumours should receive adjuvant or neoadjuvant trastuzumab therapy [92].

Radiation therapy is given as palliative therapy or as a complement to surgery in tumours over 5 cm and for patients with nodal metastasis as well as after breast-conserving surgery [ibid].

Epidemiology of breast cancer

Worldwide, breast cancer is the most common cancer diagnosed among women and it is the leading cause of cancer death [107]. Global differences in incidence rates are affected both by changes in risk factor prevalence and trends in breast cancer diagnosis. In most developed countries, mammography screening was introduced in the 1980s and led to an increase in

Breast cancer mortality, 1987–2012, all women age 15–74 år. Age standardised mortality rate/ 100 000.

Fig 4. Breast cancer mortality

breast cancer incidence. Earlier detection and more advanced treatment for breast cancer have in turn led to a decrease in mortality in some developed countries [ibid.].

Breast cancer incidence increases with age and the incidences of premenopausal breast cancer are similar globally; however, there is fivefold variation in the rates of postmenopausal breast cancer incidence worldwide [92]. The highest incidence rates are seen in north-west Europe and North America and the lowest in Asia [97]. Studies of immigrants show that the rates of the host country are mirrored by the immigrants within one or two generations, suggesting the influence of environmental and lifestyle factors [97]. In most countries however, incidences have been increasing in the past few decades. The most rapid rises have been seen in developing countries, where breast cancer risk has historically been low relative to the rate in

industrialised countries. Changes in reproductive patterns, increased obesity in postmenopausal women and HRT could partly explain this increase[107].

Sweden

Incidence

In 2011, the reported age-standardised incidence of breast cancer in Sweden was 320/100,000 in the age group 50–74 years. During 2011, there were a total of 8,382 reported cases, constituting 30% of all cancer in women. The age-standardised incidence of breast cancer in women has increased by 1.4% annually for the last 20 years, but the increase in a recent 10-year period was slightly less pronounced, with an annual change of 1.2% [108].

Regionally, the highest incidence of breast
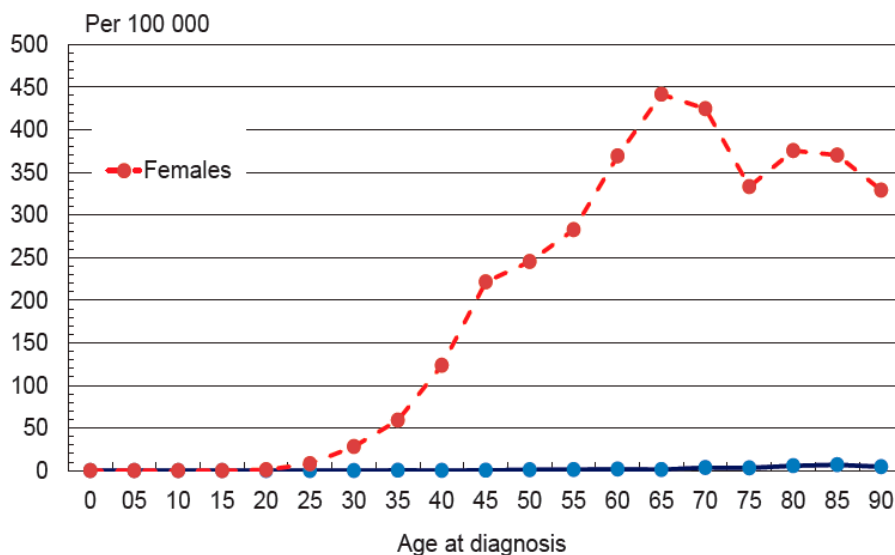
Fig 5. Breast cancer incidence with regard to age

Source:
The National Board of Health and Welfare.
Cancer incidence in Sweden 2011 (108).

cancer in 2011 was reported in the south of Sweden, namely, 247/100,000, compared with the lowest rate in the region of Uppsala/Örebro, 208/100,000 [ibid.].

Mortality

In spite of the increase in breast cancer incidence over recent decades, mortality due to breast cancer has been decreasing in Sweden since the 1980s [109]. This decrease might be explained by the earlier stage at diagnosis due to mammography screening [110, 111] and improved breast cancer treatment, although this remains controversial [112,113].

Sweden is divided into six separate healthcare regions and, in 2012, the mortality due to breast cancer ranged from 20/100,000 in the North Region to 28/100,000 in the West Region, in terms of age-standardised mortality (age-standardised using the Nordic population) [114]. The age-adjusted mortality rate in Skåne (Malmö being a city in the south of Skåne) in

2012 was reported to be 27.6/100,000, similar to that in Stockholm (26.8/100,000) [109].

Survival

Most recurrences of breast cancer appear within five years of diagnosis [92]. The five-year survival rate has increased substantially since the 1960s, when it was about 65%, to 89% in 2004–2010 [115]. Differences in survival of breast cancer among the different healthcare regions in Sweden are small, ranging from 87% to 93% [ibid.].

Risk factors

Genetics

A family history of breast cancer in a first-degree relative is an established risk factor and the risk is substantially higher when a woman's mother or sister had breast cancer at an early age.

Approximately 5%–10% of all breast cancers are attributed to genetic factors [97].

Mutations in the high-penetrance breast cancer susceptibility gene 1 (BRCA1) or breast cancer gene 2 (BRCA2) are responsible for 2%–5% of all breast cancers and give a 20-fold greater risk of breast cancer compared with that in the general population [97]. These genes are also associated with relatively aggressive breast cancer subgroups [116]. The remaining susceptibility for hereditary breast cancer seems to be polygenic in nature, involving a large number of low-penetrance genes and single-nucleotide polymorphisms [117]. Ongoing genome-wide association studies continue to identify breast cancer susceptibility loci and explore gene-environment interactions; however, bringing order to the search for low-penetrance alleles is challenging due to the multiple-comparisons problem [118].

Age, age at menarche and menopause

Age is a very important risk factor for cancer disease in general, breast cancer included.

In Sweden, the incidence of breast cancer increases with age, particularly steeply until the age of 45 where it reaches a plateau between the ages 45 and 55, and then again increases steeply until peaking at the age of 65. The plateau around the age of 50 is near the age of menopause, which strongly suggests a role for reproductive hormones in the aetiology of breast cancer [97]. In 2011, the peak incidence of breast cancer in Sweden was in the age range of 65–69; beyond the age of 70, the incidence decreased [108].

Risk of breast cancer is also associated with age-linked factors such as age at menarche, age at first childbirth and age at menopause. Age at menarche is associated with both premenopausal and postmenopausal breast cancer, the risk being reduced by 5%–20% for each year that the onset of menarche is delayed [119]. For each year that the menopause is postponed, a

3% increase in risk has been reported [ibid.]. A potential explanation for this pattern is that earlier menarche and later onset of menopause increase the lifetime exposure to higher levels of female sex hormones, with a subsequent increase in breast cancer risk.

Parity

Parous women have a lower risk of breast cancer than nulliparous ones, but the association is dependent on the age at first childbirth. Parity is an independent determinant of breast cancer risk [120] and also associated with the number of childbirths, being reduced with each subsequent full-term pregnancy [121].

Oestrogen

Evidence shows that the risk of breast cancer increases with increased endogenous oestrogen levels in postmenopausal women [122,123]. This relationship has not been proven in premenopausal women, possibly due to the complexity of variation of oestrogen levels during the menstrual cycle [97].

Exogenous oestrogens in the form of oral contraceptives (OC) have been extensively studied and over 50 epidemiological studies have evaluated the relationship between OC and breast cancer risk. In a large pooled analysis by the Collaborative Group on Hormonal Factors in Breast Cancer [124], no overall relationship was observed between duration of use of OC and breast cancer, but current and recent users had an increased risk of 1.24. This modest increase in risk applies to younger women, with OC use being restricted to fertile females, and this group has a low absolute risk of breast cancer to begin with.

In postmenopausal women, the association of exogenous oestrogens and breast cancer is more distinctive. It is well established that current use of combination HRT is associated with an increased risk of breast cancer and that this risk is higher than with use of oestrogen therapy

alone. This is specifically true for a longer duration of use, namely, 15 years or longer [125]. However, despite increasing the risk of breast cancer development, the use of HRT has also been associated with less aggressive breast cancer forms [ibid.].

Obesity and height

Obesity is a well-established risk factor for breast cancer in postmenopausal women without HRT treatment [126, 127]. After menopause, adipose tissue is the major source of oestrogen and obese postmenopausal women run a higher risk of breast cancer [126]. In premenopausal women, however, a higher body mass index (BMI) seems to have a protective effect [128].

Height has been associated with a modest increase in breast cancer risk, possibly reflecting higher energy intake during adulthood [97].

Breastfeeding

A meta-analysis by The Collaborative Group on Hormonal Factors in Breast Cancer showed that, for each additional 12 months of breastfeeding, the risk of breast cancer was decreased by 4.3%. In Western countries, the duration of breastfeeding is usually short, which may contribute to the increase in incidence of breast cancer [120]. Furthermore, a study of the duration of breastfeeding in Swedish women showed a positive association with more aggressive breast cancer subgroups [129].

Lifestyle factors

There is no substantial evidence that the composition of one's diet is associated with breast cancer risk; the evidence that does exist is merely suggestive that the dietary amount of total fat might increase breast cancer risk in postmenopausal women [130]. However, drinking alcohol appears to be positively associated with breast cancer [131]. In a literature review by Fredeinreich *et al.,* a total of 87 cohort studies and case-

control studies specific to different types of physical activity and breast cancer were studied. The finding was a 25% reduction of breast cancer risk amongst women in the most physically active group, compared with that in the least physically active women [132].

Other risk factors

Other well-confirmed risk factors for breast cancer are as follows:

Benign proliferative breast disease.
There seems to be a positive association between atypical hyperplasia, ductal and lobular, and the subsequent development of breast cancer [133].

Breast density.
Breast density, defined as mammographic density and parenchymal patterns, is an independent risk factor for breast cancer. The relative risk (RR) for women with high-density breasts is 4.4 compared with those with low density [134].

Smoking.
The subject of smoking as a risk factor has been somewhat controversial. Study reports have not been conclusive and there have been some with suggestive evidence concerning long-term smoking prior to the first childbirth [97]. In a recent report from the International Agency for Cancer Research (IARC), it has been stated that smoking can be a risk factor for breast cancer [130]. The possible effect of smoking on breast cancer risk is probably rather small, but could be indirectly associated with socioeconomic index and differences in survival.

Socioeconomic index.
Generally, for most malignancies, there is a higher risk of cancer diagnosis in the population with a lower socioeconomic index. However, for breast cancer, the opposite applies. Females with a higher socioeconomic index

have a higher risk of developing breast cancer, the incidence being 260/100,000 compared with 210/100,000 for females with a low socioeconomic index [108]. This may be due to differences in risk factors such as age at first childbirth and number of children and, possibly, more extensive use of HRT in the group with the highest socioeconomic index. However, women with a higher socioeconomic index have a better prognosis of survival than the group with a low socioeconomic index, which may be due to differences in time to diagnosis and lifestyle factors.

Prognostic and predictive factors

Survival after the diagnosis of breast cancer is in itself dependent on several prognostic and predictive factors. Prognostic factors are used to evaluate the risk of recurrence, the most important prognostic factor being TNM stage, with NHG as an important complement [135]. Other prognostic factors are the overexpression of HER-2, which is associated with an increased risk of recurrence [126], proteolytic enzymes responsible for tumour invasion [136] and proliferation-associated factors like Ki-67 [105]. Other prognostic factors are age at diagnosis, vascular invasion and oestrogen and progesterone status.

Predictive factors are in turn used to predict the best adjuvant treatment for each individual patient. ER and PGR status are important predictive factors for both primary and metastatic breast cancer and determine sensitivity to endocrine therapy [137]. Likewise, the overexpression of HER2 is used to evaluate the benefit of treatment with monoclonal antibodies [138].

Thyroid disorders and breast cancer

An association between thyroid diseases and breast cancer has been discussed for over a century. Already in 1896, Beatson undertook an attempt to cure breast cancer patients with an extract from the thyroid gland, unfortunately without great success [139]. This issue has continued to intrigue scientists since then and, during the past 50 years, many epidemiologic and experimental studies have been performed.

Experimental studies and biology

Several experimental studies concerning thyroid hormones/receptors and breast cancer have been conducted. TR is found in the breast tissue and is believed to have a function in the development and differentiation of the normal breast [13]. Studies have shown that thyroid hormones seem to have oestrogen-like effects in breast cancer, and that TR influences breast cancer cell proliferation [5–7]. Evidence also suggests that T3 induces cell proliferation by activating the same signal transduction pathways as oestradiol (E2), which controls cell cycle progression and associated gene transcription. In breast cancer cell lines, oestrogen and T3 induce cell proliferation in a dose-dependent manner. Oestrogen stimulates cell proliferation more than T3, but the two in combination act in synergy, producing an effect greater than just the additive effect of each hormone alone. Furthermore, T3 seems to regulate the gene expression of both ER and PGR in breast cancer cell lines [140]. In conclusion, these studies indicate that there seems to be an interaction between thyroid hormone and oestrogen in breast cancer development.

There are substantial changes in the expression of TR in breast cancer cell lines when comparing benign breast disease, carcinoma in situ and invasive carcinoma. It has been shown that the level of TR expression gradually decreased when comparing benign breast diseases to invasive cancers [6]. Furthermore, a recent experiment in mice showed that hypothyroidism has an enhancing effect on invasiveness and the formation of metastasis by breast cancer cells

independently of the cellular expression of TR [33]. These findings indicate that the action of thyroid hormones on breast cancer cells is multifactorial and executed in a more complex manner than by a single pathway of action.

Other experimental studies have investigated the role of iodine in breast cancer [141]. In an early study, animal models showed that iodine deficiency can lead to breast atypia and an increase in the incidence of malignancy [142]. It has also been stated that iodine is important in the maintenance of healthy breast tissue, although the mechanisms of action are not known [94, 95]. It has been suggested that iodine alters gene expression, resulting in suppression of the effect of oestrogen on breast cancer cell lines [143], but that this occurs in a thyroid-independent manner. As mentioned earlier, only the lactating and cancerous mammary gland expresses NIS and has the ability to accumulate iodine [3]. This may indicate a role for iodine in breast cancer, but there is very little evidence that confirms this hypothesis at present.

Thyroid hormones and breast cancer risk

Epidemiological studies have not been able to shed much light on the issue of an association between thyroid hormones and breast cancer risk. Results have been contradictory and there is still no agreement on the nature of the association between levels of thyroid hormones/ thyroid disorders and breast cancer risk.

Several large record-linkage studies using cohorts of patients with thyroid disorders to investigate the risk of subsequent breast cancer with more than 1,000 breast cancer cases found no association between thyroid conditions and breast cancer [22–27]. However, information on thyroid conditions in these studies was usually obtained from registers and thyroid hormone levels were not measured. Thus, the possibility that sub-clinical and overt thyroid conditions may play a role in breast cancer pathogenesis cannot be ruled out.

Low levels of thyroid hormones and breast cancer

Historically, starting with Beatson, a trend towards the association of hypothyroidism and breast cancer has been predominant. Indeed, one of the few prospective studies on this issue found a positive association between low T4 level and breast cancer risk, but there was no association with TPO-Ab [144]. This is somewhat contradictory since chronic lymphocytic thyroiditis is the most common cause of hypothyroidism in western countries, and an association would be expected concerning the presence of high TPO-Ab titres in autoimmune disease. Autoimmune disease beeing a common cause of hypothyroidsm. Low thyroid hormone levels were found in breast cancer patients in several earlier cross-sectional studies [12, 14, 15]. Such studies reported on the prevalence of hormone levels in breast cancer patients, and it is difficult to assess the causal effect of the exposure on the outcome. A possible explanation of the findings of low thyroid hormone levels in breast cancer patients is nonthyroidal illness syndrome and not hypothyroidism. In contrast to these findings Cristofanilli et al. investigated more than investigated more than 1,000 breast cancer patients and found a negative association between hypothyroidism and breast cancer [29].

High levels of thyroid hormones and breast cancer

A positive association between elevated thyroid hormone levels and breast cancer has been reported in several studies [9, 11, 13]. In two of these [11, 13], subclinical hyperthyroid hormonal patterns were observed and, in one [13], the association was only seen in postmenopausal women. These women had an increased thyroid hormone E2 ratio and it has been speculated whether such an imbalance may promote tumour growth. However, E2 is the predominant oestrogen during the female reproductive

years, while oestrone is predominant during menopause. It is therefore not surprising that the ratio of thyroid hormone/E2 was found to be increased since the level of E2 is low overall in postmenopausal women.

Thyrotropin and breast cancer

Concerning TSH, most cross-sectional studies have found no association between TSH levels and breast cancer [9, 11, 12, 16–18, 145]. However, some found that high TSH levels were positively associated with breast cancer risk [14, 19], but the only prospective analysis to date found a negative association [144]. One study found that TSH is low in cases in postmenopausal women but high in premenopausal ones [13], indicating that menopause status may modify the potential association between thyroid hormones and the risk of breast cancer.

Antibodies and breast cancer

Other cross-sectional studies in which thyroid hormones and TPO-Ab were measured in breast cancer patients found no association between thyroid hormones and breast cancer, but an increased prevalence of TPO-Ab [16, 17, 20, 145] and, interestingly, better survival in breast cancer patients positive for TPO-Ab [16].

In addition, associations of increased thyroid hormone levels, increased TPO-Ab levels and breast cancer have been reported [8]. Again, in cross-sectional studies, it is unclear whether the presence of TPO-Ab in serum from patients with breast cancer is related to an increased risk following TPO-Ab-related conditions or if it is a general autoimmune response to the malignancy [146]. Sarlis performed a meta-analysis on Hashimoto's thyroiditis and breast cancer and found no association [147]. Au contraire, a recent meta-analysis of 28 studies reported significant evidence of an increased risk of breast cancer in patients with autoimmune thyroiditis [148].

Thyroid conditions, breast cancer survival and mortality

Only a few studies on survival after breast cancer diagnosis in patients with thyroid disorders have been conducted. Goldman *et al.,* who conducted the only study on thyroid conditions and breast cancer mortality, found no excess risk of breast cancer death in women with thyroid disorders [28].

Smyth *et al.,* followed 195 breast cancer patients and found that positive TPO-Ab status was associated with a favourable survival, namely, a lower risk of recurrent disease and death [16]. Fiore *et al.,* also found that positive TPO-Ab status was associated with lower mortality [30]. On the other hand, a small study by Jiskra *et al.,* could not confirm these findings [31]. However, as described earlier, there seems to be an increased prevalence of TPO-Ab in breast cancer patients, which in turn appears to be a beneficial prognostic factor. Even so, none of these studies investigated thyroid function in relation to breast cancer-specific mortality in a 'breast cancer healthy' population.

Recently, there have been several meta-analyses and reviews on the potential association between thyroid conditions and all-cause mortality. Overt hyperthyroidism was associated with a slightly increased risk of all-cause mortality in a meta-analysis, including more than 30,000 patients [148]. In addition, subclinical hyperthyroidism was positively associated with all-cause mortality in a meta-analysis of seven cohort studies using the general population as a reference [149]. Interestingly, another meta-analysis on ten prospective studies found no statistically significant association between subclinical hyperthyroidism and all-cause mortality [150]. Moreover, some of the studies also included patients treated with supplementary thyroid hormone, which may reflect an exogenous exposure effect.

Thyroid conditions and breast cancer aggressiveness

The literature on thyroid disorders and breast cancer aggressiveness, that is, subtypes of breast cancer, is very limited. To our knowledge, there have been only three epidemiological studies on the subject. Lemaire *et al.,* reported a prevalence of higher thyroid hormone levels in breast cancer patients but found no relationship between thyroid function and breast cancer aggressiveness. Cengiz *et al.,* showed that metastatic lymph nodes and vascular invasion were more common in patients with thyroid pathology, but there was no relationship between thyroid disorder and histopathological grade [11]. Finally, Farahati found no relationship among thyroid hormones, TSH and breast cancer aggressiveness, but a lower frequency of distant breast cancer metastasis in patients with positive TPO-Ab titres [32].

Accordingly, the search for an association between thyroid conditions and breast cancer risk, survival, aggressiveness and mortality continues. This subject is far from depleted and further prospective studies could contribute to the discovery of a possible association, with clinical implications for the treatment of both thyroid disorders and breast cancer.

Study aims

The aim of the studies described in this thesis is to investigate, via a prospective design, thyroid hormones in relation to the risk of breast cancer and breast cancer-associated mortality.

The specific aims are as follows:

1. To test the hypothesis that T3 and T4 levels increase the risk of breast cancer.
2. To examine TSH and TPO-Ab levels in relation to the risk of breast cancer.
3. To test the hypothesis that total T3 levels are related to increased breast cancer mortality.
4. To examine the relationship of total T3 levels and prognostic factors in breast cancer.

Material and methods

In order to conduct our studies, we chose to use two different patient cohorts. For papers I, III and IV, we used The Malmö Preventive Project (MPP) as the basis for our study population and, for paper II, The Malmö Diet and Cancer Study (MDCS).

The city of Malmö

Malmö is a city in southern Sweden with 300,000 inhabitants of 170 different nationalities. At present, 40% of the population has a foreign background [151]. By comparison, 30 years ago, the proportion of residents with a foreign backround in Malmö was less than 8% [152]. This is an important feature to consider since the inhabitants of Malmö in the 1980s–1990s constitute our population at risk.

In 1976, mammography was introduced in a screening trial [101] and, in 1990, a general mammography screening service started in Malmö according to national guidelines [153]. The women of Malmö between the ages of 40 and 74 are now invited to mammography screening, rather than between the ages 50 and 74 years as was the case previously [102]. At present, around 73% of women participate in mammography screening in Malmö; the participation rate is higher in older women (55–65 years), at 80% [154].

In 1994, Malmö General Hospital became Malmö University Hospital. In 2010, Skåne University Hospital was formed by the merger of Malmö University Hospital and the University Hospital in Lund. During the study period, Malmö General Hospital was the only hospital in Malmö involved in the diagnosis and treatment of breast cancer patients, with no referrals to other institutions.

The Malmö Preventive Project

The Malmö Preventive Project was established in 1974 at the General Hospital of Malmö under the initiative of Professor Bertil Hood. It was directed against cardiovascular diseases, diabetes mellitus and alcohol abuse. The residents of Malmö were invited to participate in a health survey and entire birth cohorts, men and women, born between 1926 and 1949, were examined until 1992 when the department closed. Approximately 70% of invited subjects participated, of whom 10,902 were women [155].

All participants completed a questionnaire on socio-demographic information, lifestyle habits and medical history. Questions on reproductive factors, use of OC and hormonal replacement therapy were only included for women screened from April 1983 onwards (8,051 subjects). No information on the type of HRT was obtained. BMI was assessed by a trained nurse upon baseline examination. Routine blood tests were taken in the morning after an overnight fast. A subject was considered to have a previous history of goitre if the question, "Have you been treated for goitre?" was answered in the affirmative. From the baseline questionnaire, no information was available on the type of treatment for thyroid conditions [156].

All subjects were informed about the present studies by newspaper, as required by the Ethical Committee at Lund University (Dnr 652/2005 and Dnr 501/2006), and they were offered the opportunity to be excluded from this work.

Laboratory analyses, papers I, III and IV

The serum samples were analysed for T3 and TSH in women born in 1928 and 1941 and examined in 1983–84. In women born in 1935 (examined in 1990–92), TSH levels were measured in all subjects, but T3 was not. In the group of women born in 1935, T3 was only analysed in those with pathological TSH values, a history of thyroid disease or an enlarged thyroid gland at examination. In addition, the attending physician could also decide to analyse T3. The basis for the decision to analyse T3 in an individual subject was not recorded systematically.

Blood samples were taken after an overnight fast with the patient in a supine position. T3 was measured by a double-antibody radioimmunoassay (reference interval 0.9–3.2 mmol/l) [157]. TSH was also measured by a double-antibody radioimmunoassay [157] in 1983–84 (period I, reference interval <8 mIU/l), whereas immunoradiometric analysis (IRMA) was used in 1990–92 (period II, reference interval 0.4–4.0 mIU/l). Data on coefficients of variation (CV) from the laboratory analyses were unfortunately not available. However, reported within-laboratory CV for T3 at the time was in the range of 9%–11% according to a large European analysis including 150 laboratories [158].

Study population and follow-up, papers I, III and IV

Among 10,902 women, reproductive data including menopausal status were assessed in 8,051 subjects. Serum TSH was measured in 2,944 of these (women born in 1928, 1941 and 1935). Those with prevalent breast cancer (n=46), goitre (n=196) or both of these conditions (n=6) were identified and excluded from the studies.

Finally, 2,696 women constituted our study population. Information on T3 was available in 2,185 of these women.

In paper I, incident breast cancer cases, invasive and in situ, were retrieved up until 31 Dec, 2006, by record linkage with The Swedish Cancer Registry (until 31 Dec, 2005) and, owing to a one-year delay in registration, also from its regional branch, The Southern Swedish Regional Cancer Registry (cases diagnosed in 2006). Among 2,696 women, there were
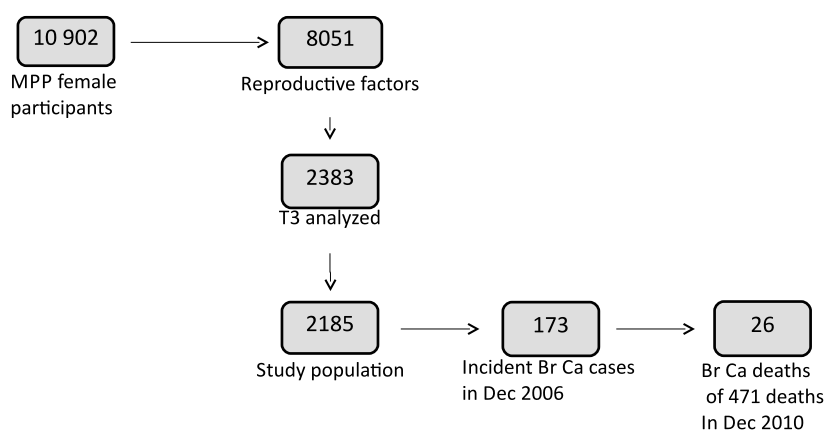
Fig 6. Study population, MPP

173 incident breast cancer cases. Information on vital status was retrieved from the Swedish Cause-of-Death Registry. Mean follow-up was 19.3 (5.08) years and total follow-up was 51,989 person-years.

In paper III, each of 2,185 women was followed from baseline until the end of follow-up, 31 Dec, 2010, or until death. The primary endpoint was breast cancer as the underlying cause of death. In addition, death from other cancers, death from other causes and death from all causes were included in the main analysis. Information on vital status and cause of death was retrieved from the Swedish Cause-of-Death Registry up until 31 Dec, 2010, which was the end of follow-up. Mean follow-up was 24.1 years (standard deviation (SD): 5.3) and total follow-up included 52,579 person-years.

In paper IV, in 2,185 women, tumour endpoints were retrieved by record linkage with the Swedish Cancer Registry and the Southern Swedish Regional Tumour Registry until the end of follow-up, 31 December, 2010. The primary endpoints were NHG, tumour size, lymph node status and ER and PGR status. Following baseline examination, 171 women in the study population were diagnosed with

breast cancer (149 invasive, 21 cancer in situ and 1 with missing information on the type of breast cancer) during a mean follow-up of 23.3 years (SD 6.2). Total follow-up included 50,807 person-years. Information on vital status was retrieved from the Swedish Cause-of-Death Registry up until 31 December, 2010.

Tumour grading and staging, paper IV

The evaluation of tumour samples diagnosed until December 2004 was originally performed as part of a previous study [159]. The tumour samples were re-evaluated by three senior pathologists. Histological grade was assessed according to the Nottingham classification as previously described [135]. Information on tumour characteristics in breast cancers diagnosed from January 2005 until December 2010 was obtained from the original pathology reports. Lymph node status and tumour size were obtained from pathology reports. Size was divided into two groups with a cut-off of 20 mm, the size that discriminates T1 from T2 tumours in the TNM classification [160].

Receptor status, paper IV

Information on ER and PGR status in tumours diagnosed before 30 April, 1997, was obtained by re-examination of collected tumour tissue by two senior pathologists using an immunohistochemical method. For tumours in the present study diagnosed after this date, information on receptor status was obtained from the original pathology reports as the immunohistochemical method had then been implemented into clinical practice. Receptor staining in more than 10% of the tumour cells was regarded as positive.

The Malmö Diet and Cancer Study

The Malmö Diet and Cancer Study, a population-based prospective cohort study, was set up to study the association between dietary factors and cancer incidence [82]. All women and men in Malmö born between 1923 and 1950 were invited. Recruitment was carried out between 1991 and 1996 and 41% of eligible subjects participated. In total, 17,035 women completed the baseline examination [161].

The MDCS baseline examination included a dietary assessment, a self-administered questionnaire, anthropometric measurements and the collection of blood samples [162]. The collection of blood samples among the participants was dispersed throughout the day and the individual times of collection were not registered. The blood and serum samples were stored at –80 °C [163]. A subject was considered to have a previous history of goitre if the question, "Have you been treated for goitre?" was answered in the affirmative. Potential and established risk factors for breast cancer available from the questionnaire were educational level, socioeconomic index, alcohol consumption, smoking status, marital status, place of birth, age at menarche, OC, number of children, menopausal status and HRT among peri/

postmenopausal women. HRT was assessed as current use according to an open-ended question. Menopausal status was defined using information on previous surgery and menstrual status; the classifications of pre-, peri- and postmenopausal women were as described in detail elsewhere [123]. The questionnaire also assessed medications and previous disease [162].

The Ethical Committee in Lund, Sweden, approved the MDCS (LU 51-90) and the present study (Dnr 652/2005 and Dnr 23/2007).

Laboratory analyses, paper II

Analyses were performed at the Department of Clinical Chemistry, Malmö University Hospital, according to the instructions of the manufacturers. Following venipuncture in non-fasting subjects, blood was separated into serum and buffy coat and stored frozen at –80 °C. Samples were stored for between 14 and 19 years and had been through two freeze-thaw cycles before the present analysis of free T3, free T4, TSH and TPO antibodies. Measurement was performed with the Beckman Access ® Immunoassay System on a UniCel™DxI800 from Beckman Coulter Inc. (Brea, Ca, USA). TPO-Ab status was classified as "positive" or "negative" using a cut-off level of 9 kIU/L.

Study population and follow-up, paper II

Patients with breast cancer, invasive and in situ, were retrieved by record linkage with The Swedish Cancer Registry up until 31 December, 2005, and owing to a one-year delay in reporting, with The Southern Swedish Regional Tumour Registry for the period 1 January, 2006, to 31 December, 2006, which was the end of follow-up. Vital status was collected from the Swedish Cause-of-Death Registry. There were 576 prevalent breast cancer cases at baseline, and 766 incident cases were diagnosed during follow-up. Two incident cases
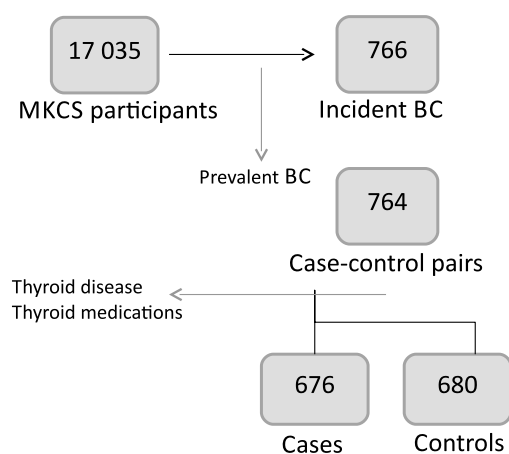
Fig 7. Study population, MDCS

had not donated blood at baseline; hence, there were 764 eligible incident cases of breast cancer. Mean age at diagnosis among these 764 cases was 64.0 years (SD: 8.0 years) and mean time from baseline examination to diagnosis was 7.0 years (SD: 3.8 years).

Originally, as part of a previous study, cases and controls were matched [164]. Incidence density matching, using age as the underlying timescale, was used to select one control for every case matched on calendar time at inclusion (+/−15 days), menopausal status (pre- vs. peri-/post-) and age at inclusion (62 years). In total, 760 case-control pairs were exactly matched according to the above criteria. Owing to the exclusion of individuals with insufficient amounts of serum provided and the replacement of three original controls, 1,483 unique individuals were included in the study, corresponding to 1,528 observations. For the present study, in order to maximise the total number of subjects included in the statistical analyses, matching was abandoned and analyses were adjusted for the matching factors instead. In addition, subjects who reported a previous history of thyroid disease and/or use of thyroid medications were

excluded. Finally, the present analysis included 676 breast cancer cases and 680 controls.

Statistical methods

In order to illuminate possible dose-response relationships among exposure and outcome, we chose to divide our analytes into quartiles or tertiles. Simultaneously, so as to avoid missing possible associations due to a limited number of cases, we conducted parallel analysis of the analytes as continuous variables. A malignant disease, like breast cancer, might influence the thyroid hormone panorama already during early development of the disease, before the cancer diagnosis. Therefore, cases diagnosed two to three years after baseline examination and the rest of the cohort with less than two or three years of follow-up were excluded from the study population during sensitivity analysis.

Statistical methods, papers I, III and IV

In paper I, the investigated exposure was T3 and TSH. Since no associations were found

with regard to TSH, further studies, namely, papers III and IV, investigated only T3 as exposure.

Since T3 was not measured in the whole group born in 1935, to avoid selection bias, sensitivity analysis was performed in paper I. This was achieved by repeating the analysis of T3 excluding the group born in 1935. Likewise, owing to the effect of oestrogen on TBG concentration, and hence the level of T3, an additional analysis was executed in paper I that excluded women treated with oral oestrogens.

Quartile cut points for T3 and TSH were based on the distribution among all women in the study cohort. In paper I, in which TSH was analysed, separate cut points were used for samples analysed before and after the change of method of analysis. In paper IV, tertile cut points were used due to the limited number of cases.

In paper I, the incidence of breast cancer per 100,000 person-years was calculated in different quartiles of T3 and TSH, whereas in paper III, breast cancer death was analysed in relation to different T3 quartiles, in which breast cancer-specific mortality was calculated per 10,000 person-years.

To calculate RR and hazard ratios (HR), Cox's proportional hazards analysis was used with a confidence interval (CI) of 95%. The assumption of proportional hazards was met as tested by log-minus-log curves. Possible confounding factors were introduced as covariates in subsequent, adjusted analyses (see below).

Breast cancer may be associated with different risk factors in peri/postmenopausal versus premenopausal women. As such, all analyses in all the papers were repeated for pre- and peri/postmenopausal women separately. Furthermore, obesity is associated with increased breast cancer risk in postmenopausal women [165], so, in papers I and II, the analyses were additionally stratified for BMI (BMI < 25 vs. ≥ 25).

The potential interaction between T3 and menopausal status was tested using an interaction term in the Cox analysis. A p-value <0.05 was considered indicative of a statistically significant interaction. This analysis was performed in papers I and III. In the same manner, the potential interaction between T3 and TSH and breast cancer risk was additionally analysed in paper I.

Confounders, Malmö Preventive Project

Established and potential risk factors for breast cancer were recognised as confounders and adjusted for in papers I and IV. In paper III, mortality-related factors were adjusted for.

In paper I, age was entered as a continuous variable and all other factors: menopause status, use of HRT, number of children, use of oral contraception, age at menarche, smoking status, alcohol consumption, BMI, height, education level and marital status, were introduced as categorical variables.

In paper IV, the limited number of breast cancer cases in each subgroup did not allow inclusion of all covariates in the same model, but in relation to invasive breast cancer, age and one additional factor at a time were included in the final model. In this way, OC affected the HR most, with an increase of the HR by 0.14, whereas the other covariates had barely any change of effect (0.00–0.02). Hence, the only adjusted model used for all different subgroups was the one including age at baseline and OC.

In paper III, T3 levels were investigated in relation to factors known to be associated with subsequent mortality, namely, age at baseline, education level, alcohol consumption and BMI. Kendall's Tau-b test was used, giving correlation coefficients (t_b) and corresponding p-values. Smoking was compared in relation to T3 quartiles using a chi-squared test. Bonferroni's correction was performed for p-values in pairwise chi-squared comparisons multiplying the p-value by the number of comparisons. A two-sided p-value <0.05 was regarded as statistically significant. Missing categories were not

included in these tests. Analyses were adjusted first for age at baseline and in a final model for all potential confounders. The limited number of breast cancer deaths did not allow inclusion of all covariates in the same model, but in relation to this endpoint, age and one additional factor at a time were included in the final model. In this way, a maximum of three variables were included in each model. In tables, only the model with the largest change from the crude HR for T3 was reported. After stratification for menopause status, the analyses only allowed the inclusion of one additional covariate, and we considered age at baseline to be most important.

Statistical methods, paper II

The cohort was divided into quartiles and decentiles based on the serum levels of free T3, free T4, TSH and TPO-Ab in controls. For free T3, free T4 and TSH, the first category was used as a reference. TPO-Ab status was classified as "positive" or "negative" using a cut-off level of 9 kIU/L. Owing to a large number of low values and a highly skewed distribution, TPO-Ab was divided into decentiles using dec 1–5 as the reference group, and not dichotomised as described below. To investigate a potential threshold effect, quartiles were used to dichotomise T3, T4 and TSH values into groups of high versus low.

Unconditional logistic regression analysis was used to calculate odds ratios (OR) with 95% CI for breast cancer in different categories of the studied factors. Before the analytes were analysed as continuous variables, the distribution was tested using a Kolmogorov-Smirnov test. None of the variables had a normal distribution, so they were transformed into their natural logarithms before entering the logistic regression analysis.

To explore potential modifying factors, all analyses were repeatedly stratified for menopausal status and BMI.

Confounders, Malmö Diet and Cancer Study

To avoid confounding by the abandoning of matching, adjusted analyses were performed adjusted for matching factors (age at baseline, time of baseline examination and menopausal status). Further analyses were performed adjusted for potential and recognised risk factors for breast cancer, such as education level, socio-economic index, alcohol consumption, smoking status, marital status, country of birth, age at menarche, use of oral contraception, number of children, HRT use and BMI.

Results

Results, paper I

Overall, the risk of breast cancer was statistically significantly higher in the fourth T3 quartile compared with that in the first quartile (HR = 1.87, 1.12–3.14). This association was even stronger for postmenopausal women, also showing a marked dose-response pattern (p-value for trend <0.001). For the second, third and fourth quartile, HR = 3.26 (0.96–11.1), 5.53 (1.65–18.6) and 6.87 (2.09–22.6). No such associations were seen in premenopausal women.

Overall, there was no statistically significant association between serum TSH level and breast cancer. Neither was there any statistically significant interaction between T3 and TSH in the whole cohort, in premenopausal or peri/postmenopausal women. Adjusted RR were very similar to crude RR with regard to both T3 and TSH.

In the sensitivity analysis, when women born in 1935 were excluded from the analysis, all results were similar. Likewise, when women treated with oral oestrogens (HRT and OC) were excluded from the analysis, the results did not differ.

Results, paper II

Triiodothyronine and thyrotropin

There were no statistically significant associations with regard to free T3 levels or TSH levels and breast cancer risk in the quartile, decentile or continuous analysis. There were also no statistically significant associations after stratification for menopause status or BMI. All ORs were similar after adjustment for potential confounders.

Thyroxine

In the dichotomised , OR= 1.40 (1.10–1.77) and continuous, OR= 2.48 (1.12–5.50) analysis, there were statistically significant positive associations between free T4 and breast cancer risk. There were also statistically significant positive trends over quartiles and decentiles (p = 0.02 and 0.046).

The quartile analysis suggested a positive association between free T4 and breast cancer risk in postmenopausal women. This was, however, not statistically significant and not confirmed in the dichotomised or continuous analysis.

Thyroid peroxidase autoantibodies

There was a statistically significant negative association between TPO-Ab and breast cancer risk in the logarithmical continuous analysis, OR= 0.95 (0.90–0.998). This association was also present although not statistically significant in the decentile analysis. After stratification, there was a statistically significant association between TPO-Ab and breast cancer risk in premenopausal women, OR=0.31 (0.10–0.92).

Results, paper III

In the continuous analysis, we observed a positive association between total T3 levels and the risk of death from breast cancer, age adjusted HR= 2.80 (1.26–6.25). This association with mortality due to breast cancer was only apparent among postmenopausal women, HR= 3.73 (1.69–8.22) compared with premenopausal ones. However, the terms for interaction between total T3 levels and menopausal status did not reach statistical significance, p= 0.12. In the quartile analysis, the risk of breast cancer death was higher in the fourth T3 quartile compared with the first quartile HR= 3.61 (1.08–12.1). The risks of death from cancers other than breast cancer, causes other than breast cancer, and all causes were positively associated with total T3 levels in the unadjusted analyses. However, following adjustment for age, and subsequently for all potential confounders, these estimates were close to unity.

Results, paper IV

The third T3 tertile had a statistically significant association with "all" breast cancers in the analysis adjusted for age and OC, HR= 1.61 (1.07–2.43). This was also confirmed in the continuous analysis, and the association was even stronger in postmenopausal women, RR= 2.88 (1.90–4.37).

There was a statistically significant association between the highest T3 tertile and NHG II tumours, RR=2.13 (1.06–4.30), and this association was even stronger in postmenopausal women.

Concerning tumour size, the tertile analysis showed a high risk for large tumours, RR= 3.17 (1.20–8.36) but this association was weaker in the continuous analysis adjusted for age, and in the analysis adjusted for age and OC, the positive association was lost. Instead in the continuous adjusted analysis, there was a positive statistically significant association between T3 levels and smaller tumours. Furthermore, after stratification for menopausal status there was a statistically significant positive association with small tumours in postmenopausal women.

There was a statistically significant positive association in a dose response pattern between

T3 tertiles and the risk of a positive nodal status, RR = 4.53 (1.60–12.83). The association was confirmed by the continuous analysis and in the analysis including only postmenopausal women.

T3 levels in the highest tertile were associated with a high risk of tumours with a negative ER status, RR= 3.52 (1.32–9.41) and negative PGR status, 3.52 (1.42–8.75).

Discussion

In the study based on The Malmö Preventive Project (paper I), we found a statistically significant association between total T3 and breast cancer risk in postmenopausal women in a dose-response manner. In the study based on The Malmö Diet and Cancer Study (paper II), we found that free T4 levels were positively associated with the risk of breast cancer, which was most pronounced in overweight women. We also found that high TPO-Ab levels were associated with a slightly lower risk of breast cancer. There was no clear association between TSH and breast cancer risk. In paper III, we found that total T3 levels were positively associated with high breast cancer-specific mortality and that this was not related to a general effect on all-cause mortality. In line with this, we found in paper IV that high T3 levels were associated with the occurrence of lymph node metastases, and a negative oestrogen receptor status as well as a negative progesterone receptor status.

Methodological issues

Selection bias

It may be questioned whether the breast cancer cases in our two cohorts are representative of the entire breast cancer population. The cohorts mainly comprised middle-aged women. In Malmö Preventive Project, 70% of the women invited participated, and in Malmö Diet and

40

Cancer Study, 41%. As we have no information about exposure to the studied risk factors in women outside these cohorts, observed incidence rates may not be applicable to all age groups or to the general population. However, as there was a wide distribution of thyroid hormones, TSH and TPO-Ab levels, it was possible to make internal comparisons between subjects with low and high values. Hence, we consider that our RR estimates were not considerably affected by selection bias.

In Malmö Preventive Project, the method of analysis for T3 remained the same throughout the study and all analyses were performed using a standardised collection of blood samples. Information on T3 was not available for all women born in 1935 in the study population. Our analyses, however, showed no difference in the risk of breast cancer in relation to T3 when the group born in 1935 was excluded. Hence, there was probably no major selection bias due to the inclusion of only some subjects with information on T3 in that period.

Detection bias

Subjects with elevated thyroid hormone levels and a possible diagnosis of hyperthyroidism may already be within the healthcare system and, as a result, the diagnosis of breast cancer might be established earlier than for euthyroid subjects. However, general mammography screening was introduced in Sweden in 1991 and accounts for the majority of detection of breast cancer cases [166]. It is also unlikely that thyroid status affects participation in mammography screening. It should also be noted that most women with thyroid hormone values within the fourth quartiles in these studies still had levels within the normal range, and these women probably did not have any symptoms of thyroid disease. It is thus unlikely that the results in this study were due to a detection bias.

Completeness of follow-up

Incomplete follow-up may have affected the results. However, the Swedish Cancer Registry and the Swedish Cause-of-Death Registry have been validated and found to have a completeness of about 97.3% in 2008 [167]. Moreover, the accuracy of cause of death due to malignancies has been shown to be higher than 90% in the Swedish Cause-of-Death Registry [168].

Misclassification

There is well-known circadian and seasonal variation in T3 and TSH levels [51]. Tracking of individuals, that is, ranking of individuals over time, is, however, quite stable [45]. True variation over time would most likely have led to an un-differential misclassification of T3, free T3 and TSH, and, hence, would have attenuated the observed risks. This is one possible explanation for the differences between the results based on the Malmö Preventive Project and the Malmö Diet and Cancer Study. In the Malmö Preventive Project, T3 was measured in blood samples drawn in the morning after an overnight fast. In the Malmö Diet and Cancer Study, there was no information on when during the day the blood samples were drawn. This may be less important for free T4 as it has been shown to have a considerably less marked circadian rhythm than T3 [51]. Short-term variation is low for free T3 and free T4, but may be slightly higher for TSH [52], which makes it difficult to rule out that such variation attenuated the true risks associated with TSH. Concerning long-term variation, tracking of individuals, namely, ranking of individuals over time, has been shown to be quite stable for thyroid hormones and TSH [45]. In any case, true variation over time would most likely have led to an un-differential misclassification and, hence, would have attenuated the observed risks.

Another aspect concerning misclassification is the change of method of analysis of TSH in the Malmö Preventive Project. The method changed between 1983 and 1990, as did the reference values. This was taken into consideration and adjusted for in our statistical analysis by using separate cut points for samples analysed before and after this change.

In the Malmö Diet and Cancer Study, we used prospectively collected blood samples that had been stored for between 14 and 19 years. First, it may be questioned whether frozen samples differ from fresh samples and, second, whether prolonged storage affects observed levels. This has been investigated by Mannistö *et al.,* who found that free T4, TSH and TPO Ab levels were very similar when analysed in frozen versus fresh serum, but for free T3, these levels were slightly higher in frozen/thawed samples than in fresh serum [169]. Concerning storage time, the same authors reported that free T3, free T4 and TSH levels were not affected by storage for 14 to 18 years, but TPO-Ab levels were only stable for up to 14 years, after which there was a slight increase [170]. That is, freezing/thawing and storage time may have affected the absolute levels of free T3 and TPO-Ab in the study based on the Malmö Diet and Cancer Study. However, there is nothing to suggest that samples from cases and controls are different, and it is not likely that relative comparisons such as OR would have been affected.

Statistical power

In these studies, 95% CI were used, which means that there is a 5% risk that the null hypothesis is correct in spite of a statistically significant finding. In paper III, the number of deaths due to breast cancer was very limited. CI were wide and the statistical power was relatively low. This led to imprecision in the estimates related to T3 and breast cancer mortality. This was specifically apparent in the quartile analysis for which the CI was very wide and the overall p-value did not reach statistical significance. Moreover, statistical power was an even more serious problem in analyses stratified for menopausal status.

41

Likewise, in paper IV, the limitation was the small number of breast cancer cases with separate endpoints, namely, prognostic factors. This resulted in wide CI and low statistical precision, leading to imprecision in the estimates. For instance, the lack of association between T3 and breast cancer histological grade III could have been due to a type II error. Furthermore, statistical precision was an even more serious problem in analyses stratified for menopausal status, and these results, with very wide CI, should be considered with caution.

Confounders

Established and potential risk factors for breast cancer and factors related to mortality were adjusted for as mentioned above.

One specific methodological aspect was the use of total T3 as a marker of T3 status. Most T3 in circulation is bound to three transport proteins: TBG, transthyretin and albumin. An increased TBG concentration leads to higher levels of total T3, which is important to consider in studies of breast cancer risk because exogenous oestrogens, namely, HRT and OC, which are risk factors for breast cancer, cause increased TBG binding capacity [53]. In order to rule out the association between total T3 levels and breast cancer risk as only an effect of differences in the use of HRT and OC, we adjusted the analyses based on the Malmö Preventive Project for HRT and OC. Moreover, in paper I, the analyses in postmenopausal women were repeated excluding women who had used HRT or OC. Hence, both adjustment for HRT/OC and exclusion of subjects using these medications resulted in similar risk estimates as were seen in the main analysis. This finding, together with the notion that there is no known association between between normal endogenous oestrogen levels and TBG [53], pregnancy beeing an exception as well as oestrogen-secreting tumours, makes it unlikely that the results based on the Malmö Preventive Project were affected by differences in TBG levels. The women

in our studies were all above the age of 40 and unlikely to be pregnant.

In the baseline questionnaire used to collect data from the participants in the Malmö Preventive Medicine Project, there were no questions on comorbidities or medication that may affect thyroid function, for example propranolol, salicylates and phenytoin. This is a limitation of the studies, but the beneficial effect of salicylates on breast cancer risk is controversial [171] and there is no known association between propranolol and phenytoin and breast cancer risk. Another limitation is the lack of specific information on thyroxine use from the baseline questionnaire. This was handled by excluding women who stated that they had undergone any treatment for *goitre*, which probably limited the interference of unreported thyroxine use with the results based on Malmö preventive Medicine Project. However, our studies did not allow any assessment of the risk of breast cancer following thyroxine therapy.

In paper III, the number of deaths due to breast cancer was very limited, so not all confounders could be included at the same time in the final model for this endpoint. However, the models including age at baseline and one additional factor yielded statistically significant positive associations between T3 levels and breast cancer death; this indicates that these factors did not seriously confound the observed association. Concerning deaths from cancers other than breast cancer, deaths from causes other than breast cancer and all-cause mortality, there were a substantially larger number of events, so we consider that the lack of strong associations was not merely the result of poor statistical power, that is, type II error.

An additional problem related to the limited number of breast cancer cases in the different subgroups in paper IV was that not all confounders could be included at the same time in the final model for each respective endpoint. However, when tested in the main analysis including all breast cancer cases, each factor at a time and the factor of age at baseline, gave very

little change of effect: at most, a 0.02 change in HR. Only the factor that was finally included, namely, OC, had a more substantial change of effect: 0.14. This indicates that the lack of adjustment for all factors did not seriously confound the observed HR.

Findings and literature

Triiodothyronine and breast cancer risk

Experimental studies have shown that T3 plays a role in the complex pathogenesis of breast cancer. The mechanisms of action are not yet fully understood, but seem to be associated with both the nuclear T3 receptor [5–7] and its surface counterpart, the integrin receptor [56, 57], which are both present in breast cancer cells. It has also been reported that T3 acts in synergy with oestrogen, promoting breast cancer cell proliferation, and that it regulates the expression of ER and PGR [140].

Paper I is the first prospective study on T3 levels in relation to breast cancer risk. It was found that T3 levels in postmenopausal women are positively associated with the risk of breast cancer in a dose-response manner [172]. Although epidemiological studies can never establish a causal relationship between exposure and outcome, the great benefit of conducting a prospective study is that it enables interpretation of the results unbiased by reverse causality. In contrast, in cross-sectional studies, reverse causality may very well play a role.

From a review of the literature, a large number of epidemiological studies have been performed over the last 50 years on benign thyroid conditions in relation to breast cancer [22–27, 29, 147, 173, 174], and at least seven studies included more than 1,000 breast cancer cases [22–27, 29]. The majority of these studies were cross-sectional, many used patient cohorts treated with thyroxine and their results were contradictory. It is thus difficult using these observations to establish a potential

causal relationship between thyroid disorders and breast cancer.

Another category of previous studies measured thyroid hormones in cases and controls. For example, Turken *et al.,* examined 150 cases and 100 controls; the results indicated that high T4 levels were associated with breast cancer [8]. However, of the few previous prospective studies, one found a negative association between T4 levels and breast cancer risk [144], another found no association between TSH and breast cancer [174] and we reported in paper I that prediagnostically measured T3 levels are positively associated with breast cancer risk in postmenopausal women [172]. The finding that T3 levels are positively associated with breast cancer risk has also been reported by some cross-sectional studies [9, 11, 13, 15], among them the largest study to date including 226 cases and 166 controls [9]. Contrary to this, several reports have suggested an association between low T3 [12, 14, 15] and/or low T4 levels [12] and a high risk of breast cancer. Finally, some studies did not show any associations at all with regard to T3/T4 and breast cancer [16, 17, 20]. A problem in previous cross-sectional studies is that breast cancer per se may change thyroid hormone levels. That is, if a nonthyroidal illness syndrome due to breast cancer leads to lower levels of T3, this would lead to a spurious association between low hormonal levels and a high risk of breast cancer. As such, it is difficult to evaluate a potential association between thyroid hormonal levels and breast cancer using results from previous studies, which may also explain why previous studies showed contradictory results.

Our second prospective study, investigating the association of free T3 and free T4 with breast cancer risk in Malmö Diet and Cancer Study, showed unexpected results compared with the findings in paper I [179]. One possible explanation for this is the circadian rhythm of T3 and the inconsistency in timing of blood sample collection in Malmö Diet and Cancer Study, as described earlier. Another reason

might be the short half-life of free T3 (~ 30 h) compared with the much longer half-life of free T4 (6–7 days) [43], making the use of free T3 as a marker of thyroid status less reliable than free T4 and total T3. However, our results call for further investigations.

Triiodothyronine and mortality

Paper III reports the first prospective study on T3 levels in relation to breast cancer mortality [175]. It shows that pre-diagnostic total T3 levels are positively associated with the risk of breast cancer-specific death and that this is not related to a general effect on cancer death or all-cause mortality.

The potential association between thyroid hormones and outcome following breast cancer has been discussed for more than a century. However, we found no studies published during the last 50 years on the topic of survival. Goldman et al., [28], who performed the only study on thyroid conditions and breast cancer mortality, found no excess risk of breast cancer death in women with thyroid disorders. However, in terms of mortality, no study has investigated thyroid function or T3 levels in relation to breast cancer-specific mortality in a 'breast cancer healthy' population.

All-cause mortality was associated with relatively high total T3 levels in the crude analysis in paper III, but adjusted for age and other potential confounders, all estimates were close to unity. There have recently been several meta-analyses and reviews on the potential association between thyroid conditions and all-cause mortality. Overt hyperthyroidism was associated with a slightly increased risk of all-cause mortality (RR = 1.21:1.05–1.38) in a meta-analysis by Brandt et al., [148], including more than 30,000 patients. All the included studies were adjusted for age, but none for education, BMI or alcohol consumption, and only some for smoking. Considering subclinical conditions, subclinical hyperthyroidism was positively associated with all-cause mortality in a

meta-analysis of seven cohort studies (HR = 1.41:1.12–1.79) using the general population as a reference [149]. Interestingly, another meta-analysis including ten prospective studies found no statistically significant association between subclinical hyperthyroidism and all-cause mortality [150]. This study excluded subjects with goitre, and even in the highest quartile, most subjects were within the reference limits for T3. All in all, this makes it difficult to compare our results to studies based on overt thyroid conditions. Some of the previous studies also included patients treated with T4; that is, these studies may reflect an effect caused by exposure to exogenous hormones.

Triiodothyronine and prognostic factors in breast cancer

Paper IV reports the first prospective study on serum T3 levels in relation to prognostic factors in breast cancer [34]. It shows a positive association between higher serum concentrations of total T3 and larger tumours in the main analysis, but an inverse association after stratification for menopause, a positive association with the presence of lymph node metastases and negative ER and PGR status. Thus, the findings indicate that the association between higher total T3 levels and increased mortality in breast cancer, as demonstrated by us in paper III, can be explained by both a higher incidence and more aggressive forms of breast cancer.

There have been very few studies on relationships with breast cancer aggressiveness. We found no prospective studies on the association of T3 levels and prognostic factors in breast cancer. One cross-sectional study on thyroid conditions and breast cancer aggressiveness found no relationship to the histopathological grade, but a higher frequency of metastatic lymph nodes and vascular invasion in patients with thyroid pathology [11]. This finding is in line with a recent experimental study that showed increased invasiveness and formation of metastasis in breast cancer of hypothyroid

mice [33]. In contrast, an early cross-sectional study by Lemaire *et al.,* showed no relationship between thyroid function and TNM stage of breast cancer [9]. However, the present study excluded subjects with goitre, and even in the highest quartile, most subjects were within the reference limits for T3. This makes it difficult to compare our results to studies based on overt thyroid conditions.

We found that total T3 levels were positively associated with invasive breast cancer in general, in both the tertile and the continuous analyses. NHG is an independent determinant of survival in breast cancer. When using NHG as the endpoint, we found a statistically significant association with T3 only in grade II. Whether this merely reflects a larger number of cases – and therefore higher statistical power in this group – or depends on certain tumour characteristics being more common in grade II tumours remains an open question.

There was a positive association between the third T3 tertile and large tumours (>20 mm), but not for small tumours. In addition, after stratification for menopause status, the association with large tumours disappeared. Instead, the association with small tumours became statistically significant in postmenopausal women. The continuous analysis showed a similar, high risk for both small and large tumours, although this was only statistically significant for small tumours. Taken together, these findings suggest that there may be an association between high thyroid hormone levels and large tumours, but the even stronger association with small tumours in postmenopausal women is intriguing and, together with the fact that CI were generally wide, these findings need to be confirmed. Given an overall association with large tumours, this could be explained by the proliferative effect of T3 on breast cancer cell lines reported in experimental studies [5, 6, 140, 176], but further studies specifically on tumour size would be of value.

All analyses showed a positive association with the presence of lymph node metastases.

The finding that total T3 levels are associated with more aggressive tumour forms may indicate the positive association in breast cancer mortality reported in paper III.

Furthermore, in paper IV, there was an overall positive association between serum T3 levels and ER-negative breast cancer. This implies that T3 may also affect breast cancer by some other mechanism(s), in addition to ER stimulation. An interesting possibility involves TRα and TRβ and their isoforms, which have been demonstrated by various methods to be expressed in breast cancer cells [177, 178]. Another observation of interest in this context involves the HER2 gene, which is of fundamental importance in the current evaluation and treatment of breast cancer. This gene is located close to the gene for TRα on the long arm of chromosome 17, and amplification of the HER2 gene is often accompanied by co-amplification of the gene for TRα [178]. In addition, point mutations in breast cancer that may affect tumour behaviour have also been detected in the genes encoding TRα and TRβ [177].

Thyroxine and breast cancer risk

In paper II, there was a positive association between free T4 and breast cancer risk [179]. We also found that the association between high prediagnostic levels of free T4 and breast cancer may be particularly strong among women with a BMI of more than 25. Obesity is associated with increased breast cancer risk in postmenopausal women [165] and oestrogen levels are higher in obese women than in those of normal weight [180]. It may be that high free T4 potentiates the association between obesity and breast cancer risk, as it has been suggested that oestrogens and thyroid hormones may act on the same receptors [140].

Thyrotropin and breast cancer risk

In neither paper I nor paper II did we find any association between TSH and breast cancer

45

risk. In paper I, the number of cases was limited, CI were wide and the statistical power was relatively low. There is a possibility that the lack of association between TSH and breast cancer was due to a type II error. The lack of association between TSH and breast cancer risk was however confirmed in paper II. In accordance with this, in a previous prospective study, Hellevik *et al.,* found no association between TSH and breast cancer risk [174]. However, another smaller prospective study found a negative association [144].

Concerning cross-sectional studies, at least two have reported a positive association between high TSH levels and breast cancer risk [14, 19], but most others found no association [9, 11, 24, 16–18, 145]. The findings on TSH in the present studies are in line with the majority of previous work in that there was no association between thyrotropin levels and breast cancer risk.

Thyroid peroxidase antibodies and breast cancer risk

There was a negative association between TPO-Ab levels and the risk of breast cancer in paper II. Autoimmune thyroid diseases have been shown to be positively associated with breast cancer risk in several cross-sectional studies [8, 20, 145], but the only previous prospective study, including only 15 cases, showed that the prediagnostic presence of TPO-Ab was not related to the subsequent risk of breast cancer [144].

Our prospective study showed a negative association between TPO-Ab and breast cancer risk. Patients with autoimmune thyroiditis in time become hypothyroid, with pathological levels of TPO-Ab that are maintained for years. Likewise, elevated levels of TPO-Ab prior to autoimmune thyroid disease indicate a risk of developing thyroiditis in the future. This could be associated with a protective effect against breast cancer and would be in line with the findings in paper II.

Conclusions

- Prospectively measured total T3 and free T4 levels are positively associated with breast cancer risk.

- Prospectively measured high levels of TPO-Ab are associated with a slightly lower risk of breast cancer, while thyrotropin levels do not influence the breast cancer risk.

- Prospectively measured total T3 levels are positively associated with breast cancer-specific mortality, unrelated to a general effect on all-cause mortality.

- Prospectively measured total T3 levels are positively associated to negative prognostic factors in breast cancer, as the occurrence of lymph node metastases, and negative ER and PGR status.

Implications and future studies

Based on the population at risk in Malmö Preventive Project, our first study showed that total T3 was positively associated with breast cancer risk in postmenopausal women. We did not see this association in our second study, based on the Malmö Diet and Cancer Study, when we investigated free T3 and risk of breast cancer. It would be interesting to see if the consequent use of total T3 or free T3 in both cohorts would produce different results.

In papers III and IV, we showed that total T3 was positively related to mortality of breast cancer and to negative prognostic factors, such as the occurrence of lymph node metastases, and negative ER and PGR status. Both these studies were based on a relatively small number of cases and the findings ought to be replicated, preferably based on a larger material.

If T3 levels are in fact related to more aggres-

sive forms of breast cancer and an increase in breast cancer mortality, an important issue for future studies would be to evaluate the widespread, long-term use of levothyroxine treatment in TSH-suppressive doses for benign thyroid disease [181, 182], and the management of women with subclinical hyperthyroidism [75]. Such studies will contribute with important evidence on whether or not to treat mild and/or subclinical thyroid disorders. Furthermore, judging from recent observations in various types of malignant tumour disease, this line of research may also lead to new strategies for the treatment of breast cancer [183, 184].

Svensk sammanfattning

Insjuknandet i sköldkörtelsjukdomar är liksom bröstcancer vanligast hos kvinnor efter klimakteriet och ett eventuellt samband har diskuterats i över ett sekel. Experimentella studier har visat att sköldkörtelhormoner påverkar både utveckling av normala bröstceller och tillväxt av bröstcancerceller. Det finns också belägg för att sköldkörtelhormon och östrogen binder till samma receptor i bröstcancerceller och har en samverkande effekt på bröstcancercellens tillväxt.

De senaste 50 åren har det publicerats ett flertal kliniska och epidemiologiska studier om sköldkörtelsjukdomar och sköldkörtelhormoner och deras eventuella samband med bröstcancer. Resultaten från dessa studier har varit motstridiga och de är svårtolkade då majoriteten av undersökningarna är tvärsnittsstudier som mätt hormonnivåer eller påvisat sköldkörtelsjukdom hos patienter som redan har bröstcancer. Sådana studier undersöker med andra ord både exponering och effekt på samma gång, vilket gör det svårt att bedöma eventuella orsakssamband. För att svara på frågan ifall exponeringen föregår effekten, d.v.s. om en viss typ av sköldkörtelrubbning medför en ökad risk att insjukna i bröstcancer, får man istället genomföra mer tidskrävande s.k. prospektiva kohortstudier. Sådana innebär att en kohort – en grupp individer – som inte har sjukdomen från början, i detta fall bröstcancer, delas in i undergrupper beroende på exponering, i detta fall olika nivåer av sköldkörtelhormon. Sedan följer man individerna i kohorten över tid för att se om utvecklingen av sjukdom skiljer sig mellan de exponerade och oexponerade. Kohortstudier ger på så sätt den bästa informationen om sjukdomsorsaker och det mest direkta måttet på risken att drabbas av en sjukdom.

Det övergripande syftet med denna avhandling var att just genom prospektiva kohortstudier undersöka förhållandet mellan sköldkörtelhormoner och 1) risken att insjukna i bröst-

cancer, 2) risken att utveckla en prognostiskt ogynnsam typ av bröstcancer och 3) risken att dö på grund av bröstcancer.

Till delarbete I, III och IV användes data från Malmö Förebyggande Medicin. Detta projekt startade 1974 vid Malmö allmänna sjukhus (numera SUS, Malmö), under ledning av professor Bertil Hood. Man bjöd in män och kvinnor födda 1926-1949, och då projektet avslutades 1991 hade 22000 personer deltagit, varav 10902 kvinnor. I projektet ingick ett frågeformulär om livsstil och socioekonomiska faktorer, deltagarna undersöktes och det togs blodprover. Blodnivåerna av sköldkörtelhormon hade mätts hos 2185 kvinnor som inte redan haft bröstcancer eller genomgått behandling för struma. Fram till utgången av december 2010 hade 149 av dem utvecklat invasiv bröstcancer (d.v.s. cancer med spridningsförmåga), och bland de 471 som dött var bröstcancer dödsorsak i 26 fall.

Till delarbete II användes material från Malmö Kost Cancer Studien (MKCS), som är en stor databas och biobank i Malmö. I början på 1990-talet bjöd man in alla kvinnor i Malmö födda mellan 1923 och 1950 och frågade om de ville delta i ett projekt där man skulle studera sambandet mellan kost och cancer. Totalt deltog 17035 kvinnor. Även här svarade deltagarna på ett frågeformulär och det togs blodprov, som djupfrystes för framtida analyser. Fram till och med december 2006 hade 766 av dessa kvinnor utvecklat bröstcancer. De som fått diagnosen bröstcancer eller uppgett att de blivit behandlade för struma när de gick med i MKCS har uteslutits från det aktuella delarbetet. Efter s.k. matchning av de återstående bröstcancerfallen för jämförelse med de kvinnor i MKCS som 2006 inte hade utvecklat bröstcancer, kom studien att omfatta 676 kvinnor med bröstcancer och 680 friska kontroller.

Sammantaget har kohortstudierna i denna avhandling visat statistiskt signifikanta samband mellan högre blodnivåer av sköldkörtelhormon; totalt T3 och fritt T4, och ökad risk

för kvinnor att utveckla bröstcancer. Vad gäller totalt T3 har det också påvisats statistiskt signifikanta samband mellan högre blodnivåer och en ökad risk att utveckla bröstcancer med prognostiskt ogynnsamma egenskaper och en ökad risk för död i bröstcancer.

Med hänsyn till de erhållna resultaten och idag förekommande sätt att behandla vissa vanliga sköldkörtelsjukdomar är det angeläget att genom ytterligare studier närmare klargöra den biologiska bakgrunden till de påvisade sambanden mellan sköldkörtelhormon och bröstcancer. Ökad kunskap på detta område kan få betydelse både för att förebygga och att behandla bröstcancer, som är den vanligaste cancerformen bland kvinnor.

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Paper I

Prospectively measured triiodothyronine levels are positively associated with breast cancer risk in postmenopausal women

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Abstract

Introduction: The potential association between hypo- and hyperthyroid disorders and breast cancer has been investigated in a large number of studies during the last decades without conclusive results. This prospective cohort study investigated prediagnostic levels of thyrotropin (TSH) and triiodothyronine (T3) in relation to breast cancer incidence in pre- and postmenopausal women.

Methods: In the Malmö Preventive Project, 2,696 women had T3 and/or TSH levels measured at baseline. During a mean follow-up of 19.3 years, 173 incident breast cancer cases were retrieved using record linkage with The Swedish Cancer Registry. Quartile cut-points for T3 and TSH were based on the distribution among all women in the study cohort. A Cox's proportional hazards analysis was used to estimate relative risks (RR), with a confidence interval (CI) of 95%. Trends over quartiles of T3 and TSH were calculated considering a P-value < 0.05 as statistically significant. All analyses were repeated for pre- and peri/postmenopausal women separately.

Results: Overall there was a statistically significant association between T3 and breast cancer risk, the adjusted RR in the fourth quartile, as compared to the first, was 1.87 (1.12 to 3.14). In postmenopausal women the RRs for the second, third and fourth quartiles, as compared to the first, were 3.26 (0.96 to 11.1), 5.53 (1.65 to 18.6) and 6.87 (2.09 to 22.6), (P-trend: < 0.001). There were no such associations in pre-menopausal women, and no statistically significant interaction between T3 and menopausal status. Also, no statistically significant association was seen between serum TSH and breast cancer.

Conclusions: This is the first prospective study on T3 levels in relation to breast cancer risk. T3 levels in postmenopausal women were positively associated with the risk of breast cancer in a dose-response manner.

Introduction

Thyroid disorders and breast cancer both have a post-menopausal peak incidence, and a potential association between hypo- and hyperthyroid disorders and breast cancer has been investigated in a large number of studies during the last decades [1-19]. However, the results have not been conclusive.

Experimental studies have shown that thyroid hormones can have estrogen-like effects in breast cancer, and that thyroid hormone receptors influence both normal breast cell differentiation and breast cancer cell proliferation [2,3]. Several clinical and epidemiological cross-sec-

tional studies have been performed comparing levels of triiodothyronine (T3), thyroxin (T4) and thyrotropin (TSH) in breast cancer patients versus healthy controls. The results have been contradictory, some in favor of higher levels in cases [4-7] other in controls [8] and yet some report no differences [9-15].

It is, however, difficult to conclude from cross-sectional studies whether differences in thyroid hormone levels are associated with different risks of breast cancer, or if breast cancer itself alters thyroid hormone levels. To date, there is only one prospective cohort study on this issue, involving 61 breast cancer cases, where pre-diagnostic levels of TSH and T4 were related to subsequent risk of breast cancer [16]. The present study is a population based, prospective cohort study including 2,696 pre and

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peri/postmenopausal women in whom TSH and T3 levels were measured at baseline. During a follow-up of a total of 51,989 person-years, 173 women were diagnosed with incident breast cancer.

The aim of the present study was to investigate prediagnostic serum levels of TSH and T3 in relation to breast cancer incidence in pre- and peri/postmenopausal women, respectively.

Materials and methods

The Malmö Preventive Project

Originally, 10,902 women participated in the Malmö Preventive Project. The project was established in 1974 when residents in Malmö, a city in southern Sweden, were invited to participate in a health survey. Entire birth cohorts, men and women, were examined until 1992 when the department closed. Approximately 70% of invited subjects participated.

All participants answered a questionnaire concerning socio-demographic information, lifestyle habits, and medical history. Questions on reproductive factors, use of oral contraceptives (OC), and hormonal replacement therapy (HRT), were only included in women screened from April 1983 and onwards (8,051 subjects). There was no information on type of HRT. Body mass index (BMI) (kg/m2) was assessed by a trained nurse on baseline examination. A subject was considered to have a previous history of goiter if the question 'have you been treated for goiter' was answered with 'yes'. There was no available information on type of treatment.

The participants were initially part of a preventive health care project. All former participants were informed about the present study by newspaper as required by the local ethical committee. All participants were offered to be excluded from the present study.

Triiodothyronine (T3) and thyrtropin (TSH) analysis

Blood samples were taken after an overnight fast with the patient in the supine position. The serum samples were analyzed for T3 and TSH in women born in 1928 and 1941 and examined in 1983 and 1984. In women born in 1935 (examined from 1990 to 1992), TSH levels were measured in all, but T3 was only measured in a sub-set of all women. In women born in 1935, T3 was analyzed in those with pathological TSH values, a history of thyroid disease, or those with an enlarged thyroid gland at examination. In addition to this, the attending physician could also decide to analyze T3. The basis for the decision to analyze T3 in an individual subject was not recorded systematically.

T3 was measured by a double anti-body radioimmunoassay (reference interval 0.9 to 3.2 mmol/l) [20]. Only six women had a value above the upper reference limit. TSH was also measured by a double anti-body radioimmuno-

assay [20] in 1983 and 1984 (period I, reference interval <8 mIU/l), whereas a immunoradiometric analysis (IRMA) was used in 1990 to 1992 (period II, reference interval 0.4 to 4.0 mIU/l). Data on coefficients of variation from the laboratory analysis were unfortunately not available.

Study cohort

Among 10,902 women, reproductive data including menopausal status had been assessed in 8,051 subjets. Serum TSH had been measured in 2,944 of these (women born in 1928, 1941 and 1935, respectively). Those with prevalent breast cancer (n = 46), goiter (n = 196) or both of these conditions (n = 6) were identified and excluded from the study.

Finally, 2,696 women constituted our study population. Information on T3 was not available for all women born in 1935, and T3 had been assessed in 2,185 out of all 2,696 women. The present study was approved by the ethical committee at Lund University, Sweden: Dnr 652/2005 and Dnr 501/2006.

Follow-up

Breast cancer cases, invasive and *in situ*, were retrieved up until 31 December 2006 by record linkage with The Swedish Cancer Registry (until 31 December 2005) and, due to a one-year delay in registration, also from its regional branch, The Southern Swedish Regional Cancer Registry (cases diagnosed in 2006). Among 2,696 women, there were 173 incident breast cancer cases. Information on vital status was retrieved from the Swedish Cause-of-Death Registry.

Mean follow-up was 19.3 (5.08) years and total follow-up was 51,989 person-years.

Statistical methods

Quartile cut-points for T3 and TSH were based on the distribution among all women in the study cohort. Due to the change of method of analysis of TSH, separate cut-points were used for samples analyzed before and after this change.

Each woman was followed until the end of follow-up, 31 December 2006, or until she got breast cancer or died. The incidence of breast cancer per 100,000 person-years was calculated in different quartiles of T3 and TSH. A Cox's proportional hazards analysis was used to estimate corresponding relative risks (RR) with a confidence interval (CI) of 95%. Possible confounding factors, that is, established and potential risk factors for breast cancer, were introduced as covariates in a subsequent analysis. Age was entered as a continuous variable and all other factors were entered as categorical variables classified as in Tables 1 and 2 (menopausal status, use of HRT, number of children, use of oral contraception, age at menarche, smoking status, alcohol consumption, BMI, height,

Table 1: Distribution of potential risk factors for breast cancer according to serum T3 level

Factor	T3 quartile				
	1 **N = 494**	**2** **N = 704**	**3** **N = 456**	**4** **N = 531**	**All** **N = 2,185**
	Column percent (T3 levels in italics)				
T3 (mIU/l)	*<1.50*	*1.60 to 1.80*	*1.90 to 2.00*	*2.1 to -4.30*	
Age					
<50	62.1	46.6	26.1	21.5	39.7
>50	37.9	53.4	73.9	78.5	60.3
Premenstrual	60.3	44.2	28.7	23.2	39.5
Peri/postmenopausal	39.7	55.8	71.3	76.8	60.5
HRT in peri/postmenopausal	13.3	14.2	15.4	17.6	15.4
missing	-	-	0.3	-	0.1
Nulliparity	18.2	17.0	13.2	19.6	17.1
missing	-	-	0.4	0.8	0.3
OC-use	4.7	6.0	9.0	11.5	7.6
missing	-	-	0.7	0.9	0.4
Menarche <12 years	15.2	13.4	9.6	11.9	12.6
missing	-	0.4	1.3	1.3	0.7
Smoking					
never	43.5	47.7	47.6	47.1	46.6
current	35.8	33.2	34.0	33.1	34.0
ex	20.6	19.0	18.0	18.8	19.1
missing	-	-	0.4	0.9	0.3
Alcohol consumption					
nothing	14.2	14.1	13.2	19.0	15.1
less than every week	55.3	60.7	62.5	60.1	59.7
every week	30.6	25.1	23.9	19.4	24.7
missing	-	0.1	0.4	1.5	0.5
BMI					
<20	14.8	10.1	7.9	7.5	10.1
≥20 <25	62.3	56.1	53.9	46.5	54.7
≥25 <30	17.0	26.1	27.9	30.5	25.5
≥30	5.9	7.7	10.3	15.4	9.7
Height					

Table 1: Distribution of potential risk factors for breast cancer according to serum T3 level (Continued)

≤ 160	24.1	28.8	28.9	36.5	29.7
>160 ≤ 165	32.6	33.2	30.9	30.7	32.0
>165 ≤ 170	26.7	24.9	25.0	22.0	24.6
>170	16.6	13.1	15.1	10.7	13.7
Education					
<12 years	53.0	53.6	62.5	64.2	57.9
12 years	20.9	27.4	22.1	21.8	23.5
>12 years	21.7	14.9	11.2	10.7	14.5
Missing	4.5	4.1	4.2	3.2	4.0
Married	54.9	54.4	51.3	50.3	52.9
missing	0.8	1.8	7.2	14.5	5.8

Abbreviations: T3, triiodothyronine; HRT, hormone replacementtherapy; OC, oral contraceptives; BMI, body mass index.

educational level and marital status). Trends over quartiles of T3 and TSH were calculated by introducing the quartile number as a continuous variable in the analysis. A *P*-value < 0.05 was considered as statistically significant.

Breast cancer may be associated with different risk factors in peri/postmenopausal versus premenopausal women. Furthermore, both thyroid conditions and breast cancer have been associated with BMI [21,22]. Following this, all analyses were repeated for pre- and peri/postmenopausal women separately, and stratified for BMI (BMI <25 vs. BMI ≥25). The potential interaction between T3/TSH and menopausal status was tested by adding an interaction term in the Cox analysis. A *P*-value <0.05 was considered indicative of a statistically significant interaction.

It has been reported that the relation between TSH and T3/T4 may be disturbed in breast cancer patients, hence the presence of undiagnosed breast cancer may affect thyroid hormone levels [16,23,24]. Consequently, cases diagnosed within three years after baseline examination and subjects with less than three years follow-up were excluded in an additional analysis. As it is possible that certain combinations of T3/T4 and TSH can be related to an increased risk of breast cancer, different combinations of low (below median) and high (above median) TSH and T3 were examined in relation to risk of breast cancer. Moreover, the risk of breast cancer in relation to T3 quartiles was analyzed in different strata of TSH (below and above the median). Interaction between T3 and TSH was investigated by entering an interaction term in the Cox analysis. T3 was entered as quartiles, and TSH as a dichotomies variable (below/above median).

Since T3 was not measured in the whole group born in 1935, the analysis of T3 was repeated including only women born in 1928 and 1941.

Due to the potential effect of estrogens on thyroxin binding globulin (TBG) concentration, and hence the levels of T3, an additional analysis was executed excluding women treated with HRT or OC.

Results

Advanced age, peri/postmenopausal status, use of OC, HRT and high BMI, were more common in high T3 quartiles (Table 1). With regard to TSH, the highest percentage of peri/postmenopausal women, were in the lowest and highest TSH quartiles, respectively (Table 2).

Overall, the risk of breast cancer was statistically significantly higher in the fourth T3 quartile as compared to the first quartile (Table 3). This association was even stronger for postmenopausal women, also showing a marked dose-response pattern (*P*-value for trend < 0.001). No such associations were seen in premenopausal women. There were no statistically significant interactions between menopausal status and T3 (*P*: 0.48) or TSH (*P*: 0.83).

There was no overall statistically significant association between serum TSH levels and breast cancer (Table 4).

In the analyses stratified for BMI, there was a weak, non-significant association between high T3 levels and breast cancer in the group with BMI over 25, the RR for the fourth vs. the first quartile was 2.41 (0.99 to 5.84), as compared to the corresponding RR in women with BMI below 25, 1.31 (0.72 to 2.41) (other data not shown).

Adjusted RRs were very similar to crude RRs with regard to both T3 and TSH. When women who devel-

Table 2: Distribution of potential risk factors for breast cancer according to serum TSH level

Factor	TSH quartile				
	1 **N = 647**	**2** **N = 528**	**3** **N = 793**	**4** **N = 710**	**All** **N = 2,678**
	Column percent (TSH levels in italics)				
TSH (mIU/l)					
period I	*<1.50*	*1.60 to 2.00*	*2.10 to 3.00*	*≥3.10*	
period II	*<0.90*	*1.00 to 1.30*	*1.40 to 2.00*	*≥2.10*	
Age					
<50	21.5	42.6	40.9	24.9	32.3
>50	78.5	57.4	59.1	75.1	67.7
Premenopausal	24.0	41.9	41.5	27.3	33.6
Peri/postmenopausal	76.0	58.1	58.5	72.7	66.4
HRT in peri/postmenopausal	17.3	20.8	17.2	13.0	16.6
missing	0.2	-	-	-	0.1
Nulliparity	13.9	13.1	18.0	18.2	16.1
missing	1.4	1.9	0.5	0.3	0.9
OC-use	3.7	8.7	8.7	4.4	6.3
missing	1.7	2.1	0.8	0.3	1.1
Menarche <12 years	13.4	12.5	13.0	12.0	12.7
missing	2.3	3.6	1.0	0.6	1.7
Smoking					
never	42.2	41.9	47.9	51.0	46.2
current	36.0	36.9	31.9	29.7	33.3
ex	20.2	19.3	19.7	18.9	19.5
missing	1.5	1.9	0.5	0.4	1.0
Alcohol consumption					
nothing	17.0	12.3	14.2	16.5	15.1
less than every week	59.4	53.2	57.8	60.0	57.8
every week	21.5	30.7	26.5	23.0	25.2
missing	2.2	3.8	1.5	0.6	1.9
BMI					

Table 2: Distribution of potential risk factors for breast cancer according to serum TSH level (Continued)

<20	10.0	11.6	9.1	7.3	9.3
≥20 <25	53.3	50.8	57.9	52.0	53.8
≥25 <30	26.9	27.3	23.8	28.9	26.6
≥30	9.7	10.4	9.2	11.8	10.3
Height					
≤ 160	30.3	26.9	29.5	33.9	30.4
>160 ≤ 165	32.8	29.5	31.8	33.2	32.0
>165 ≤ 170	26.0	27.5	25.3	21.7	24.9
>170	11.0	16.1	13.4	11.1	12.7
Education					
<12 years	58.6	54.4	55.1	61.0	57.4
12 years	23.3	24.6	26.4	22.0	24.1
>12 years	14.2	18.8	14.2	13.2	14.9
missing	3.9	2.3	4.3	3.8	3.7
Married	45.0	49.6	51.2	54.9	50.4
missing	19.6	25.0	7.6	4.2	13.0

Abbreviations: TSH, thyrotropin; HRT, hormone replacementtherapy; OC, oral contraceptives; BMI, body mass index.

oped breast cancer within three years after screening were excluded from the analyses, all results were similar for T3, but for TSH in premenopausal women, the risk in the second quartile was even stronger (Tables 3 and 4).

The analyses of combinations of low and high T3/TSH levels showed a statistically significant association for crude RR in the highT3/highTSH groups, as compared to the low/low category (1.62: 1.01 to 2.60). The association was, however, not statistically significant in the adjusted analysis (1.63: 0.99 to 2.69). The data were also stratified for TSH, below and above median with regard to T3 quartiles. There was a statistically significant risk for breast cancer in the highest T3 quartile (2.69: 1.19 to 6.08) in women with a TSH above the median. The corresponding RR in women with low TSH levels was 1.62 (0.68 to 3.86). There was no statistically significant interaction between T3 and TSH in the whole cohort, in premenopausal or peri/postmenopausal women.

When the analyses were repeated, excluding the women born in 1935, all results were similar, crude RR = 5.56 (1.66 to 18.57) for postmenopausal women in the highest T3 quartile (other data not shown).

Also, the analyses were repeated excluding women treated with HRT and OC, and the results were similar, crude RR = 6.50 (1.99 to 21.2) for postmenopausal women in the highest T3 quartile (other data not shown).

Discussion

This is the first prospective study on T3 levels in relation to breast cancer risk. It shows that T3 levels in postmenopausal women are positively associated with the risk of breast cancer in a dose-response manner.

It may be asked whether breast cancer cases in this cohort are representative of the whole breast cancer population. This cohort mainly comprised middle-aged women and 70% of the women invited to the health examination attended. As we have no information about exposure to the studied risk factors in women outside this cohort, observed incidence rates may not be applicable to all age groups or to the general population. However, as there was a wide distribution of T3 and TSH levels, it was possible to make internal comparisons between subjects with low and high values, respectively. We consider that our estimations of relative risks were not considerably affected by selection bias.

Incomplete follow-up may affect the results. However, the Swedish Cancer Registry and the Swedish Cause-of-Death Registry have been validated and found to have a completeness of about 99% [25].

Subjects with elevated T3 levels and a possible diagnosis of hyperthyroidism may already be within the health care system and due to this the diagnosis of breast cancer might be established earlier than for euthyroid subjects.

Paper I

Table 3: Breast cancer incidence in pre-, peri/postmenopausal and all women in relation to serum T3 levels

Group	T3 (quartile)	Individuals (n)	Breast cancer cases (n)	Person-years	Incidence/100,000	All cases		Cases >3 years following Baseline examination	
						RR (CI: 95%)	RR** (CI: 95%)	RR (CI: 95%)	RR** (CI: 95%)
All	1	494	28	10,418	269	1.00	1.00	1.00	1.00
	2	704	40	14,613	274	1.02 (0.63 to 1.66)	1.12 (0.69 to 1.84)	1.10 (0.66 to 1.83)	0.99 (0.60 to 1.66)
	3	456	31	9,096	341	1.28 (0.77 to 2.13)	1.42 (0.83 to 2.43)	1.37 (0.78 to 2.42)	1.21 (0.70 to 2.10)
	4	531	47	10,091	466	1.75 (1.10 to 2.80)*	1.87(1.12 to 3.14)*	1.98 (1.15 to 3.38)*	1.78 (1.10 to 2.90)*
	P-trend					0.007	0.009	0.007	0.008
Premenopausal	1	298	25	6,376	392	1.00	1.00	1.00	1.00
	2	311	21	6,621	317	0.81 (0.45 to 1.45)	1.00 (0.55 to 1.81)	1.00 (0.53 to 1.87)	0.80 (0.43 to 1.46)
	3	131	7	2,816	249	0.63 (0.27 to 1.47)	0.71 (0.30 to 1.70)	0.79 (0.33 to 1.91)	0.69 (0.33 to 1.61)
	4	123	9	2,610	345	0.88 (0.41 to 1.88)	0.92 (0.40 to 2.15)	1.07 (0.45 to 2.50)	0.96 (0.44 to 2.10)
	P-trend					0.49	0.66	0.94	0.99
Peri/post-menopausal	1	196	3	4,042	74	1.00	1.00	1.00	1.00
	2	393	19	7,992	237	3.24 (0.96 to 10.9)	3.26 (0.96 to 11.1)	2.75 (0.80 to 9.48)	2.90 (0.85 to 9.91)
	3	325	24	6,281	382	5.21 (1.57 to 17.4)*	5.53 (1.65 to 18.6)*	4.63 (1.35 to 15.8)*	4.39 (1.30 to 14.8)*
	4	408	38	7,481	508	6.94 (2.14 to 22.5)*	6.87 (2.09 to 22.6)*	6.14 (1.86 to 20.3)*	6.49 (1.99 to 21.1)*
	P-trend					<0.001	<0.001	<0.001	<0.001

Abbreviations: T3, triiodothyronine; RR, relative risk; RR**, relative risk adjusted for age (continuous), menopausal status (in analysis of all women), use of HRT, nulliparity, use of oral contraception, age at menarche, smoking status, alcohol consumption, BMI, height, educational level and marital status, * $P < 0.05$.

Table 4: Breast cancer incidence in pre-, peri/postmenopausal and all women in relation to serum TSH levels

Group	TSH (quartile)	Individuals (n)	Breast cancer cases (n)	Person-years	Incidence/100,000	All cases		Cases >3 years following Baseline examination	
						RR (CI: 95%)	RR** (CI: 95%)	RR (CI: 95%)	RR** (CI: 95%)
All	1	647	40	11,764	340	1.00	1.00	1.00	1.00
	2	528	42	9,517	441	1.05 (0.70 to 1.58)	1.07 (0.71 to 1.62)	1.28 (0.81 to 2.01)	1.26 (0.80 to 1.97)
	3	793	55	15,829	347	0.93 (0.62 to 1.41)	0.94 (0.62 to 1.42)	1.20 (0.77 to 1.87)	1.19 (0.76 to 1.85)
	4	710	36	14,521	248	0.87 (0.55 to 1.37)	0.80 (0.50 to 1.27)	0.95 (0.57 to 1.57)	1.03 (0.62 to 1.69)
	P-trend					0.46	0.29	0.82	0.94
Premenopausal	1	155	10	3,170	315	1.00	1.00	1.00	1.00
	2	221	18	4,669	386	1.48 (0.76 to 2.90)	1.38 (0.69 to 2.77)	1.91(0.90 to 4.08)	2.02 (0.97 to 4.23)
	3	329	28	6,869	408	0.95 (0.48 to 1.87)	0.94 (0.47 to 1.87)	1.31(0.62 to 2.79)	1.30 (0.62 to 2.72)
	4	194	7	4,213	166	0.81 (0.36 to 1.86)	0.62 (0.26 to 1.46)	0.88 (0.35 to 2.21)	1.11 (0.46 to 2.67)
	P-trend					0.42	0.20	0.64	0.91
Peri/post-menopausal	1	492	30	8,593	349	1.00	1.00	1.00	1.00
	2	307	24	4,848	495	0.86 (0.51 to 1.45)	0.87 (0.51 to 1.47)	0.93 (0.52 to 1.67)	0.94 (0.53 to 1.67)
	3	464	27	8,960	301	0.93 (0.56 to 1.56)	0.94 (0.56 to 1.58)	1.13 (0.65 to 1.98)	1.14 (0.66 to 1.99)
	4	516	29	10,308	281	0.88 (0.51 to 1.53)	0.82 (0.47 to 1.43)	0.89 (0.48 to 1.65)	0.97 (0.53 to 1.78)
	P-trend					0.73	0.57	0.94	0.87

Abbreviations: TSH, thyrotropin; RR, relative risk; RR**, relative risk adjusted for age (continuous), menopausal status (in analysis of all women), use of HRT, nulliparity, use of oral contraception, age at menarche, smoking status, alcohol consumption, BMI, height, educational level and marital status. * $P < 0.05$.

However, general mammography screening was introduced in Sweden already in 1986 and stands for the majority of detection of breast cancer cases [26]. It is unlikely that the thyroid status does affect participation in mammography screening, hence a possible detection bias in hyperthyroid breast cancer patients was probably not important. It should also be noted that most women with T3 levels in the fourth quartile in the present study still had levels within the normal range, and these women did probably not have any symptoms of thyroid disease.

The method of analysis for T3 remained the same throughout the study. Not all women in the study population had information on T3, but our analyses showed no difference in risk of breast cancer in relation to T3 when the group screened in 1990 was excluded. Hence, there was probably no major selection bias due to the inclusion of only some subjects with information on T3 in the later period.

The method of analyses for TSH changed between 1983 and 1990 as did the reference values. This was taken into account in our statistical analysis.

There is a well known circadian and seasonal variation concerning T3 and TSH levels [27]. Tracking of individuals, that is, ranking of individuals over time is, however, quite stable [28]. A true variation over time would most likely have led to a un-differential misclassification of T3 and TSH, and, hence, attenuated observed risks.

The use of total T3 as a marker of T3 status involves an important methodological aspect. Most T3 in the circulation is bound to three transport proteins, thyroxin binding globulin (TBG), transthyretin and albumin. An increased TBG concentration leads to higher levels of total T3, and this is important to consider in studies of breast cancer risk, as exogenous estrogens, that are risk factors for breast cancer, that is, HRT and OC, led to increased TBG binding capacity [29]. In order to exclude the association between total T3 levels and breast cancer risk as only an effect of differences in the use of HRT and OC, we adjusted our analyses for HRT and OC. Moreover, the analyses in postmenopausal women were repeated excluding women that had used HRT or OC. Following that exclusion, the RR for T3, as compared to the first quartile, was in the second quartile 3.11 (0.92 to 10.6), in the third 4.55 (1.35 to 15.3) and in the fourth quartile 6.50 (1.99 to 21.2). Hence, both adjustment for HRT/OC and exclusion of subjects using these medications resulted in similar risk estimates as were seen in the main analysis. This finding, together with the notion that there is no known association between endogenous estrogen levels and TBG [29], makes it unlikely that the results in the present study were caused by differences with regard to TBG levels.

In the questionnaire used to collect data from the participants, there was no information on co-morbidities or medication which may affect the thyroid function, for example, propanolol, salicylates and phenytoin. This is a limitation in the study, but as there is no known association between such factors and breast cancer risk, the effect from uncontrolled confounding was probably small. There was no information on thyroxin use specifically in the questionnaire, which is another limitation. This was handled by excluding women who affirmed any treatment for *goiter*, which probably limited the interference with the results in the present study. However, the current material did not allow any assessment of the risk of breast cancer following thyroxin therapy.

The current study is the largest prospective study to date on T3/TSH levels and breast cancer risk, but the number of cases was limited. Confidence intervals were wide and the statistical power relatively low. This led to an imprecision in the estimates related to T3, and there is a risk that the lack of association between TSH and breast cancer was due to a type II error.

A large number of epidemiological studies have been performed during the last 50 years on benign thyroid conditions in relation to breast cancer [9-14,30-32], and at least seven studies included more than 1,000 breast cancer cases [9-14,30]. However, most previous studies show no association between thyroid condition and breast cancer. A methodological problem is that the great majority of these studies were cross-sectional; hence it may be difficult to establish a potential causal relationship between thyroid disorders and breast cancer. A study by Cristofanilli *et al.*, including 1,136 breast cancer cases, reported that women with previous hypothyroid conditions had an increased risk of breast cancer [30]. Another prospective study including a cohort of 2,775 women found that previous hypothyroidism and the use of thyroid medications was associated with an odds ratio of 3.8 for breast cancer [16]. An indirect evidence of an association between hypothyroidism and breast cancer is offered by studies following women treated for thyroid cancer (that is, by surgery or radio isotopes causing a hypothyroid state). For example, Brown *et al.* used the SEER registry and found an increased risk of breast cancer in women with a previous diagnosis of thyroid cancer [33]. These three studies were prospective, but a methodological problem is that the majority of these patients had been treated with thyroxin. That is, the results may have been confounded by exposure to exogenous thyroid hormones. Moreover, as the above studies used information on thyroid disorders usually obtained by interviews or record-linkage with diagnosis registries thyroid hormone levels were not directly measured. Thus, these studies do not rule out the possibility that sub-clinical hyperthyroid conditions, or even elevated T3 levels within the normal range, may play a role in breast cancer pathogenesis.

Some previous cross-sectional studies have found that high T3 levels are positively associated with breast cancer [1,5,7], among them the largest study to date including 226 cases (mean age 55 years, range 28 to 79) and 166 controls (mean age 46 years, range 17 to 65) [5]. Turken *et al.* examined 150 cases and 100 controls, and the results indicated that high T4 levels were associated with breast cancer [4]. On the other hand, several reports have suggested an association between low T3 [8,24,34] and/or low T4 levels [8] and breast cancer risk. Indeed, the only prospective study found a negative association between T4 levels and breast cancer risk [16]. Finally some studies did not show any associations at all with regard to T3/T4 and breast cancer [17,35,36], the two largest with 356 and 136 cases, respectively [35,7]. High TSH levels have been associated with breast cancer risk in some studies [24,37], but the only prospective analysis to date found a negative association [16]. Yet, most have found no association between TSH levels and breast cancer [3,5,7,8,35,36,38-40]. One study found that TSH is low in cases among postmenopausal women but high in cases among pre-menopausal [1], indicating that menopause status may modify the potential association between thyroid hormones and risk of breast cancer. Autoimmune thyroid diseases (defined as the presence of thyroid peroxidase antibodies; TPOab) have been positively associated with breast cancer risk in several cross-sectional studies [4,17,40]. It is unclear whether the presence of TPOab in serum from patients with breast cancer is related to an increased risk following TPOab related conditions or if it is a general autoimmune response to the malignancy [41]. Indeed, the only prospective study showed that the pre-diagnostic presence of TPOab was not related to the subsequent risk of breast cancer [16]. In our prospective study, breast cancer cases diagnosed within three years after screening were excluded, hence the effect of (sub-clinical) cancer disease on thyroid hormones or TSH, that is, reverse causality, is unlikely.

The presence of thyroid hormone receptors in human breast and breast cancer cell lines has been established [42-45], and the proliferative effect of T3 has been confirmed by various experimental studies on breast cancer. Our findings are in line with these data [1-3,46].

It has been shown that T3 binds and stimulates the estrogen receptor, acting in synergy with estrogen on breast cancer cell lines, potentiating the estrogenic effect and enhancing cell proliferation [47]. The role of estrogen in carcinogenesis of the breast is well known and the possibility that this effect may be even stronger in conjunction with high levels of T3 could in part explain the results in the present study.

Obesity is associated with increased breast cancer risk in postmenopausal women [48]. Estrogen levels are higher in obese compared to normal weight women. The potentiating effect of T3 might further increase the risk

in this subgroup. Although our results did not reach statistical significance, they are in line with this hypothesis.

The significant positive association between T3 and breast cancer is specifically strong in postmenopausal women. Therefore, an imbalance between estrogen and T3 with an increased T3/E2 ratio may be more important than a pure synergistic effect between these two hormones for the risk of developing breast cancer. It has been suggested that this imbalance might enhance breast cancer development [1]. This theory is in accordance with our findings that higher T3 levels in postmenopausal women are positively associated with the risk of breast cancer in a dose-response manner. The present study excluded women with a previous treatment for goiter and there was no information on thyroxin use. An important issue for future studies will be to evaluate the widespread, long-term use of thyroxin treatment in TSH suppressive doses for benign thyroid disease [49,50], and the management of women with subclinical hyperthyroidism [51-53]. Such studies will contribute with important evidence whether or not to treat mild and/or sub-clinical thyroid disorders.

Conclusions

In conclusion, the present prospective cohort study, the first of its kind on prospectively measured T3 levels and breast cancer risk, indicates that high T3 levels in post-menopausal women are positively associated with the risk of breast cancer in a dose-response manner.

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Paper II

Prospectively measured thyroid hormones and thyroid peroxidase antibodies in relation to breast cancer risk

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Thyroid hormones influence both normal breast cell differentiation and breast cancer cell proliferation and stimulate the angiogenesis of certain cancer forms. Several cross-sectional studies have measured thyroid hormones/autoantibodies in breast cancer ceases *vs.* controls, but it is difficult to determine the cause–effect direction in these studies. Only three prospective studies have reported on the subject so far. The aim of our study was to investigate prediagnostically measured levels of thyroid hormones, thyrotropin (TSH) and thyroid autoantibodies in relation to subsequent risk of breast cancer. The Malmoe Diet and Cancer study examined 17,035 women between 1991 and 1996. Blood samples were collected at baseline and free triiodothyronine (T3), free thyroxin (T4), TSH and thyroid peroxidase autoantibodies (TPO-Ab) levels were measured in 676 cases and 680 controls. Relative risks with 95% confidence intervals were assessed using a logistic regression analysis adjusted for potential confounders. Free T4 levels were positively associated with a high risk of breast cancer, and the OR for women with free T4 levels above *vs.* below the median was 1.40 (1.10–1.77). This association was most pronounced in overweight women (1.51:1.07–2.12). Women with high levels of TPO-Ab had a lower risk of breast cancer, but only the analysis of TPO-Ab as a continuous variable reached statistical significance. Free T4 was in our study positively associated with a high risk of breast cancer. This association was most pronounced in overweight/obese women. Women with a high level of TPO-Ab had a relatively low risk of breast cancer.

Experimental, clinical and epidemiological studies suggest that there is an association between thyroid disorders and breast cancer risk. There have been several record-linkage studies using cohorts of patients with thyroid disorders that have investigated the risk of subsequent breast cancer, but the results are not conclusive.[1–8] Another category of studies

have investigated the association between thyroid hormone levels/autoantibodies and breast cancer risk, the majority of these studies were cross-sectional measuring hormone levels in cases following diagnosis, as compared to controls, and they may suggest a cause–effect relationship, but it is difficult to draw conclusions from cross-sectional studies.[9–23] To date, there are only three prospective studies that have reported on the association between thyroid hormone/thyrotropin (TSH) levels and subsequent risk of breast cancer. The smallest found that low levels of free thyroxin (T4), and high levels of TSH, were associated with an increased risk of breast cancer.[24] Another, investigating only TSH, found no association with breast cancer risk.[25] We have in a previous study found that triiodothyronine (T3) levels were positively associated with breast cancer risk in postmenopausal women.[26] Autoimmune thyroiditis has also been positively associated with breast cancer risk,[10,15,17] though the only prospective study on the presence of thyroid peroxidase autoantibodies (TPO-Ab) found no association with subsequent risk of breast cancer.[24]

Experimental studies have shown that thyroid hormones influence both normal breast cell differentiation[9] and breast cancer cell proliferation.[27–29] Furthermore, thyroid hormones seem to have a stimulating effect on angiogenesis of certain cancer forms.[30,31] Thus, it is possible that relatively high

levels of thyroid hormones are related to the subsequent risk of breast cancer.

The major form of thyroid hormone in the blood is T4, while T3 is the biologically active form[32] in the target organ. The overwhelming part of T3 and T4 is bound to different proteins in the blood and it is the free forms that are biologically active, *i.e.* free T3 (fT3) and free T4 (fT4). The free forms of T4 and T3 inhibit TSH secretion by the pituitary, while TSH in turn increase the levels of fT3 and fT4.

The main hypothesis in our study is that prediagnostic fT3/fT4 levels are positively associated with breast cancer risk and that TSH and TPO-Ab have an inverse association with breast cancer risk. Both age (menopausal status) and obesity are strongly related to both thyroid conditions/hormonal levels and breast cancer risk.[32] Thus it is, possible that these factors may modify the potential association between thyroid hormones and breast cancer risk. These hypotheses were examined among 676 breast cancer cases and 680 controls from the Malmoe Diet and Cancer Study (MDCS).

Material and Methods

The Malmoe Diet and Cancer Study

The MDCS, a population-based prospective cohort study, invited all women in Malmoe born between 1923 and 1950. Recruitment was carried out between 1991 and 1996 and 41% of eligible subjects participated. In all, 17,035 women completed the baseline examination.[32]

The MDCS baseline examination included a dietary assessment, a self-administered questionnaire, anthropometric measuring and the collection of blood samples.[33] A subject was considered to have a previous history of goiter if the question "have you been treated for goiter" was answered with "yes." Potential and established risk factors for breast cancer available from the questionnaire are listed in Table 1. Hormonal replacement therapy (HRT) was assessed as current use according to and open-ended question. Menopausal status was defined using information on previous surgery and menstrual status and the classification of pre-, peri- and postmenopausal women have been described in detail elsewhere.[34]

The Ethical committee in Lund, Sweden, approved the MDCS (LU 51–90), and our study, Dnr 652/2005 and Dnr 23/2007.

Follow-up and identification of breast cancer cases

End of follow-up was on 31 December 2006. Breast cancer cases (cancer *in situ* and invasive) were retrieved by record linkage with The Swedish Cancer Registry up until 31 December 2005 and due to a 1-year-delay in reporting, with The Southern Swedish Regional Tumour Registry for the period 1 January 2006–31 December 2006. Vital status was collected from the Swedish Cause-of-Death Registry. There were 576 prevalent breast cancer cases at baseline, and 766 incident cases were diagnosed during follow-up. Two incident

Table 1. Established and potential risk factors for breast cancer in cases and controls

Factor	Categories	Cases (n = 676)	Controls (n = 680)
		Column percent *Mean (SD) in italics*	
Age at baseline	years	*56.8 (7.2)*	*56.6 (7.1)*
BMI	kg/m²	*25.6 (4.3)*	*25.5 (4.2)*
Educational level	≤9 years	68.0	68.8
	9–12 years	7.0	7.2
	>12 years	25	24
Socioeconomic index (SEI)	Manual	33.3	38.9
	Nonmanual	59.6	52.6
	Self-employed	5.8	8.5
	Missing	1.3	0.1
Alcohol consumption	None	6.7	8.2
	<15 g/day	63.0	62.1
	15–30 g/day	15.5	14.1
	>30 g/day	3.4	2.9
	Missing	11.4	12.6
Smoking status	Never	43.0	42.6
	Ex	27.5	28.1
	Current	29.4	29.3
Marital status	Married	33	31
	Unmarried	67	69
Born in Sweden	Yes	88.3	90.4
	No	11.5	9.6
	Missing	0.1	-
Age at menarche	<12 years	6.8	4.7
	12–15 years	86.2	86.6
	>15 years	5.8	8.1
	Missing	1.2	0.6
Oral contraception	Yes	52.1	50.9
	No	47.9	49.1
Number of children	0	13.2	11.5
	1	19.7	22.4
	2	42.9	40.6
	3	16.9	16.2
	≥4	4.7	7.1
	Missing	2.7	2.4
Menopausal status	Pre	26.3	27.1
	Peri/post	73.7	72.9
HRT-use among peri/post menopausal	Yes	33.9	20.4

cases had not donated blood at baseline; hence, there were 764 eligible incident cases of breast cancer. Mean age at diagnosis among these 764 cases was 64.0 years [standard

deviation (SD): 8.0 years] and mean time from baseline examination to diagnosis was 7.0 years (SD: 3.8 years).

Matching and study population

Matching of cases and controls was originally performed as part of a previous study.[35] Incidence density matching, using age as the underlying time scale, was used to select one control for every case matched on calendar time at inclusion (±15 days), menopausal status (pre- vs. peri-/post-) and age at inclusion (±2 years). In all, 760 case-control pairs were exactly matched according to the above criteria. Age at baseline was relaxed to ±3 years in two pairs and to ±4 years in two pairs.

Controls were originally matched to cases at a 2:1 ratio, but only one control for each case was used in the laboratory analyses. The rationale for matching on two controls was to be able to use another control when there was no serum available for the first. Following sample retrieval, 13 individuals had insufficient amounts of serum. Nine were cases and could not bee replaced, leaving four controls. One prematched control was already part of another case-control pair. In all, three new individuals replaced three original controls. Finally, 1,482 unique individuals were included in the study, corresponding to 1,528 observations (764 case-control pairs). Our study excluded subjects that reported a previous history of thyroid disease and/or use of thyroid medications. Finally, the present analysis included 676 breast cancer cases and 680 controls.

Laboratory analyses

Analyses were performed at the Department of Clinical Chemistry, Malmoe University Hospital according to the instructions of the manufacturers. Following venipuncture in nonfasting subjects, blood was separated into serum and buffy coat and stored frozen at −80°C. Samples were stored between 14 and 19 years and had been through two freeze-thaw cycle before the present analysis of free T3, free T4, TSH and TPO antibodies. Measurement was performed with the Beckman Access® Immunoassay System on a Uni-Cel^{TM}DxI800 from Beckman Coulter, Brea, CA. The normal values for free T3 were 3.5–5.4 pmol/L, free T4 8–14 pmol/L and TSH 0.4–3.5 mIE/L. TPO-Ab status was classified as "positive" or "negative" using a cut-off level of 9 kIU/L.

Statistical methods

The cohort was divided into quartiles and decentiles based on serum levels in controls of fT3, fT4, TSH and TPO-Ab. Quartiles were used to dichotomise T3, T4 and TSH values into groups with high (quartiles 3 and 4) vs. low (quartiles 1 and 2) levels. TPO-Ab was dichotomised as described above. Missing values were coded as separate categories for fT4, fT3, TSH and TPO-Ab, as well as for all other categorical covariates. There were some individuals with missing information on studied factors, and several stratified analyses were performed. Moreover, several cases and controls had to be

excluded as they reported a history of thyroid disease and/or use of thyroid medications. In order to maximise the number of cases and controls that could be included in the statistical analyses, matching was abandoned and adjusted analyses included matching factors was used instead.

Unconditional logistic regression analysis was used to calculate odds ratios with 95% confidence intervals (OR with 95% CI) for breast cancer in different categories of the studied factors. For fT3, fT4 and TSH, the first category was used as reference. However, this was unsuitable for TPO-Ab as most values were very low and the discrimination between the lowest categories was poor. Following this, the decentile analysis was applied for TPO-Ab using decentiles 1–5 as reference. Trend over quartiles and decentiles was calculated by including the quartile/decentile variable as a continuous factor. Finally, all factors were also analysed as continuous variables. First, the distribution was tested using a one-sample Smirnof-Kolmogorov test. All distributions differed significantly ($p < 0.05$) from a normal distribution, and before the logistic regression analysis, the continuous variables were transformed to their natural logarithms. Analyses were first performed adjusted only for matching factors (age at baseline, time of baseline examination and menopausal status) and then also including educational level, socioeconomic index, alcohol consumption, smoking status, marital status, country of birth, age at menarche, use of oral contraception, number of children, HRT use and body mass index (BMI).

To explore potential modifying factors, all analyses were repeated stratified for menopausal status and BMI. Menopausal status was classified as pre vs. peri/post and BMI as <25 vs. =25. Statistical analyses were preformed using SPSS 17.0.

Results

Distribution of established and potential risk factors was similar in cases and controls, except that four or more children was more common in controls than cases, and HRT was more common in cases, Table 1.

Triiodothyronine

There was no association between triiodothyronine (fT3) levels and breast cancer risk in the quartile analysis, nor was there any trend over quartiles, Table 2. This was confirmed in the analyses using decentiles (data not shown). Neither were there any statistically significant associations when fT3 levels were dichotomized in high versus low levels, or in the continuous analysis, Table 4. When stratifying for menopausal status and BMI, there were no statistically significant associations in any strata, Tables 3 and 4. All ORs were similar when adjusting for potential confounders. Overall, there were 12 individuals with serum fT3 levels below the reference values and 21 individuals above.

Thyroxin

In the quartile analysis of fT4 and breast cancer, there was a statistically significant positive trend over quartiles, and the

Table 2. Breast cancer risk in categories of fT3, fT4, TSH and TPO-Ab

Analyte	Categories	Range	Cases (n)	Controls (n)	OR (95% CI)	OR[1] (95% CI)
fT3—quartiles (pmol/L)	1	2.20–4.08	167	155	1.00	1.00
	2	4.10–4.30	164	176	0.87 (0.64–1.17)	0.88 (0.64–1.21)
	3	4.32–4.59	119	147	0.75 (0.54–1.04)	0.78 (0.56–1.09)
	4	4.60–6.40	179	166	1.00 (0.74–1.36)	1.10 (0.80–1.52)
	Missing		47	36	1.21 (0.75–1.97)	1.39 (0.83–2.32)
	p Trend				0.87	0.68
fT4—quartiles (pmol/L)	1	1.0–8.9	96	116	1.00	1.00
	2	9.0–9.9	143	172	1.01 (0.71–1.43)	1.02 (0.71–1.47)
	3	10.0–10.9	177	160	1.34 (0.95–1.89)	1.42 (0.99–2.04)
	4	11.0–18.6	213	196	1.31 (0.94–1.83)	1.41 (0.99–1.99)
	Missing		47	36	1.58 (0.95–2.63)	1.79 (1.04–3.07)
	p Trend				0.03	0.02
TSH—quartiles (mIE/L)	1	0.01–0.85	154	160	1.00	1.00
	2	0.86–1.28	185	164	1.17 (0.86–1.59)	1.08 (0.78–1.48)
	3	1.29–1.91	160	159	1.05 (0.77–1.43)	0.96 (0.70–1.33)
	4	1.92–72.81	129	161	0.83 (0.60–1.15)	0.79 (0.56–1.11)
	Missing		48	36	1.39 (0.85–2.25)	1.41 (0.84–2.35)
	p Trend				0.20	0.14
TPO-Ab—decentiles (kIU/L)	1–5	0.0–0.6	348	328	1.00	1.00
	6	0.7–0.9	69	63	1.07 (0.74–1.54)	1.04 (0.70–1.52)
	7	1.0–1.5	56	60	0.91 (0.62–1.34)	0.90 (0.60–1.36)
	8	1.6–13.0	61	67	0.88 (0.61–1.27)	0.88 (0.59–1.30)
	9	13.7–106.7	53	65	0.78 (0.53–1.14)	0.79 (0.53–1.19)
	10	107.4–1087	45	64	0.68 (0.45–1.01)	0.68 (0.45–1.04)
	Missing		44	33	1.19 (0.86–1.64)	1.29 (0.79–2.12)
	p Trend				0.07	0.15

[1]Adjusted for BMI and age at baseline (continuous), educational level, socioeconomic index, alcohol consumption, smoking status, marital status, country of birth, age at menarche, use of oral contraception, number of children, HRT-use and menopausal status.

third and fourth quartiles had borderline significant high ORs, Table 2. There was also a statistically significant trend over decentiles ($p = 0.046$). In the dichotomised and the continuous analysis of fT4, there were statistically significant positive associations with breast cancer risk, Table 4.

The quartile analysis suggested that the positive association between fT4 levels and breast cancer was most pronounced in postmenopausal women, Table 3, but this association was only borderline significant, and it was not confirmed in the dichotomised or continuous analyses, Table 4. Concerning women with a BMI more than 25, there was a statistically significant association between the third quartile and breast cancer risk, and the fourth quartile showed a non-significant positive association with breast cancer, Table 3. This pattern was confirmed in the dichotomised analysis, but not in the continuous analysis, Table 4. Overall, there were 51 individuals with FT4 levels below the reference values and five individuals above.

Thyrotropin
There was no statistically significant association in the quartile or decentile (trend: $p = 0.11$) analyses of TSH and breast cancer risk, Table 2. This was confirmed in the dichotomised and continuous analyses, Table 4. In women with a BMI more than 25, there was a borderline statistically significant negative association between the fourth quartile and breast cancer risk, Table 3. Overall, there were 43 individuals with TSH levels below the reference values and 52 individuals above.

Thyroid peroxidase autoantibodies
In the decentile analysis, there was a nonstatistically significant negative association between TPO-Ab and breast cancer risk, Table 2. This was confirmed in the dichotomised analysis, and the association reached statistical significance in the continuous analysis, Table 4.

Premenopausal women in the highest decentile had a low risk of breast cancer, but there was no statistically significant

Paper II

Table 3. Breast cancer risk in categories of fT3, fT4, TSH and TPO-Ab stratified for menopausal status and BMI

| | | Menopausal status | | | | BMI | | | |
| | | Pre | | Peri/post | | <25 | | ≥25 | |
Analyte	Quartile	Cases/controls	OR[1] (95% CI)	Cases/controls	OR[1] (95% CI)	Cases/controls	OR[1] (95% CI)	Cases/controls	OR[1] (95% CI)
fT3 (quartiles)	1	52/50	1.00	115/105	1.00	107/84	1.00	60/71	1.00
	2	39/48	0.69 (0.37–1.28)	125/128	0.98 (0.67–1.43)	83/91	0.67 (0.43–1.06)	81/85	1.11 (0.68–1.80)
	3	32/36	0.79 (0.40–1.55)	87/111	0.78 (0.52–1.18)	60/76	0.60 (0.37–0.98)	59/71	0.93 (0.55–1.56)
	4	45/41	1.02 (0.55–1.91)	134/125	1.15 (0.78–1.70)	77/78	0.73 (0.46–1.17)	102/88	1.46 (0.90–2.35)
	Missing	10/9	0.98 (0.33–2.91)	37/27	1.50 (0.87–2.92)	23/19	1.06 (0.50–2.25)	24/17	2.02 (0.94–4.38)
	p Trend		0.93		0.64		0.96		0.26
fT4 (quartiles)	1	26/31	1.00	70/85	1.00	44/50	1.00	52/66	1.00
	2	40/50	0.78 (0.37–1.64)	103/122	1.15 (0.75–1.79)	68/78	1.03 (0.58–1.82)	75/94	1.06 (0.63–1.77)
	3	48/46	1.23 (0.60–2.53)	129/114	1.54 (1.00–2.36)	96/89	1.25 (0.73–2.15)	81/71	1.82 (1.10–3.10)
	4	54/48	1.31 (0.65–2.64)	159/148	1.49 (0.99–2.25)	119/112	1.29 (0.77–2.18)	94/84	1.45 (0.88–2.40)
	Missing	10/9	1.25 (0.39–3.97)	37/27	2.15 (1.14–4.04)	23/19	1.66 (0.74–3.75)	24/17	2.37 (1.10–5.22)
	p Trend		0.19		0.03		0.08		0.13
TSH (quartiles)	1	44/49	1.00	110/111	1.00	83/99	1.00	71/61	1.00
	2	59/53	1.12 (0.61–2.03)	126/111	1.07 (0.72–1.57)	107/87	1.26 (0.81–1.00)	78/77	0.85 (0.52–1.40)
	3	44/44	1.19 (0.63–2.24)	116/115	0.97 (0.65–1.43)	82/86	1.04 (0.65–1.64)	78/73	0.87 (0.53–1.44)
	4	22/29	0.68 (0.32–1.45)	107/132	0.82 (0.56–1.22)	54/56	1.10 (0.64–1.82)	75/105	0.59 (0.36–1.00)
	Missing	9/9	1.03 (0.33–3.22)	39/27	1.65 (0.91–3.00)	24/20	1.48 (0.70–3.11)	24/16	1.57 (0.72–3.39)
	p Trend		0.55		0.28		0.38		0.12
TPO-Ab (decentiles)	1–5	94/92	1.00	254/236	1.00	184/175	1.00	164/153	1.00
	6	19/21	1.04 (0.50–2.16)	50/40	1.09 (0.69–1.74)	37/31	1.23 (0.71–2.12)	32/32	1.01 (0.58–1.78)
	7	17/17	1.03 (0.47–2.23)	39/43	0.81 (0.49–1.33)	27/35	0.72 (0.41–1.28)	29/25	1.22 (0.66–2.25)
	8	16/16	1.03 (0.46–2.30)	45/51	0.75 (0.47–1.18)	33/33	0.95 (0.55–1.65)	28/34	0.74 (0.42–1.31)
	9	18/16	1.19 (0.55–2.57)	35/49	0.71 (0.44–1.16)	30/28	1.04 (0.58–1.85)	23/37	0.65 (0.36–1.18)
	10	5/14	0.31 (0.10–0.92)	40/50	0.78 (0.49–1.24)	17/28	0.63 (0.32–1.23)	28/36	0.72 (0.41–1.26)
	Missing	9/8	1.23 (0.42–3.62)	35/25	1.47 (0.83–2.60)	22/18	1.39 (0.69–2.83)	22/15	1.75 (0.84–3.64)
	p Trend		0.53		0.09		0.40		0.16

[1]Adjusted for BMI and age at baseline (continuous), educational level, socioeconomic index, alcohol consumption, smoking status, marital status, country of birth, age at menarche, use of oral contraception, number of children, HRT-use and menopausal status. BMI and menopausal status were not included in the analysis of respective factor.

trend over decentiles, Table 3. The dichotomous and continuous analyses did not suggest any effect modification by menopausal status on the association between TPO-Ab and breast cancer risk, Table 4. Overall, there were 244 individuals with TPO-Ab levels above the cut off value.

Discussion

fT$_4$ were in our study positively associated with a high risk of breast cancer. This association was most pronounced in overweight/obese women, as compared to normal weight women. Women with a high level of thyroid peroxidase antibodies (TPO-Ab) had a slightly lower risk of breast cancer.

Thyroid hormones and breast cancer

There was a positive association between fT4 and breast cancer in our study. A large number of epidemiological studies have been performed during the last 50 years on benign thyroid conditions in relation to breast cancer,[1–6,8,36–38] and at least seven studies included more than 1,000 breast cancer cases.[1–6,8] The majority of these studies were cross-sectional, many used patients cohorts treated with T4, and the results from different studies were contradictory. It is, hence, difficult using these observations to establish a potential causal relationship between thyroid disorders and breast cancer.

Table 4. Breast cancer risk in continuous and dichotomised fT3, fT4, TSH and TPO-Ab stratified for menopause status and BMI

Analyte	Scale	Range Case/control	Crude OR (95% CI)	All Adjusted OR[1] (95% CI)	Menopausal status (adjusted OR[1]: 95% CI) Pre	Peri-/post	BMI (adjusted OR[1]: 95% CI) <25	≥25
fT3	Dichotomised	2.20–4.30	1.00	1.00	1.00	1.00	1.00	1.00
		4.32–6.40	0.95(0.76–1.19)	1.00 (0.79–1.26)	1.07 (0.67–1.71)	0.91 (0.69–1.19)	0.77 (0.55–1.08)	1.10 (0.79–1.54)
	Continuous (ln)	640/644	0.61(0.20–1.88)	0.68 (0.19–2.40)	0.42 (0.095–1.86)	0.45(0.99–2.02)	0.75 (0.46–1.24)	0.87 (0.15–4.91)
fT4	Dichotomised	1.00–9.90	1.00	1.00	1.00	1.00	1.00	1.00
		10.00–18.60	1.32 (1.06–1.65)	1.40 (1.10–1.77)	1.47 (0.91–2.38)	1.30 (0.99–1.71)	1.24 (0.88–1.75)	1.51 (1.07–2.12)
	Continuous (ln)	629/644	1.88 (0.96–3.69)	2.48 (1.12–5.50)	3.99 (0.71–22.48)	2.17 (0.88–5.32)	2.82 (0.82–9.76)	2.30 (0.80–6.59)
TSH	Dichotomised	0.01–1.28	1.00	1.00	1.00	1.00	1.00	1.00
		1.29–72.81	0.86 (0.69–1.08)	0.85 (0.67–1.07)	0.90 (0.56–1.43)	0.88 (0.67–1.15)	0.95 (0.68–1.32)	0.77 (0.55–1.08)
	Continuous (ln)	630/646	0.90 (0.77–1.06)	0.91 (0.76–1.09)	0.86 (0.61–1.22)	0.95 (0.77–1.17)	0.96 (0.74–1.25)	0.83 (0.64–1.07)
TPOab	Reference limit	Negative: <9.00	1.00	1.00	1.00	1.00	1.00	1.00
		Positive: ≥9.00	0.77 (0.59–1.01)	0.81 (0.61–1.08)	0.77(0.42–1.43)	0.81 (0.58–1.13)	0.88 (0.57–1.37)	0.73(0.49–1.11)
	Continuous (ln)	632/647	0.94 (0.90–0.99)	0.95 (0.90–0.998)	0.96 (0.86–1.08)	0.94 (0.88–0.997)	0.93 (0.85–1.01)	0.95 (0.89–1.03)

[1]Adjusted for BMI and age at baseline (continuous), educational level, socioeconomic index, alcohol consumption, smoking status, marital status, country of birth, age at menarche, use of oral contraception, number of children, HRT-use and menopausal status.

Another category of previous studies have measured thyroid hormones in cases and controls. Turken et al. examined 150 cases and 100 controls and the results indicated that high T4 levels were associated with breast cancer.[10] However, of the three prospective studies to date, one found a negative association between T4 levels and breast cancer risk,[24] another found no association between TSH and breast cancer,[25] and our previous study, the first prospective study on T3 levels in relation to breast cancer risk, found that prediagnostically measured T3 levels were positively associated with breast cancer risk in postmenopausal women.[26] The finding that T3 levels are positively associated with breast cancer risk has also been reported by some cross-sectional studies,[9,11,13] among them the largest study to date including 226 cases and 166 controls.[11] Contrary to this, several reports have suggested an association between low T3[14,18,19] and/or low T4 levels[14] and a high breast cancer risk. Finally, some studies did not show any associations at all with regard to T3/T4 and breast cancer.[15,20,21] A problem in previous cross-sectional studies is that breast cancer per se may change thyroid hormone levels. That is, if breast cancer by some reason leads to lower/higher levels of thyroid hormones, this would lead to a spurious association between low/high hormonal levels and a high risk of breast cancer. Following this, it is difficult to evaluate a potential association between thyroid hormonal levels and breast cancer using results from previous studies, and this may also explain why previous studies have shown contradictory results. Our study indicates that the association between high prediagnostic levels of fT4 and breast cancer may be particularly strong among women with a BMI more than 25. Obesity is associated with increased breast cancer risk in postmenopausal women,[39] and estrogen levels are higher in obese compared to normal weight women.[40] It may be that high fT4 potentiate the association between obesity and breast cancer risk as it has been suggested that estrogens and thyroid hormones may act on the same receptors.[29]

The lack of a positive association between fT3 and breast cancer is somewhat surprising given the results for fT4 and our previous study on total T3.[26] One possible explanation might bee the short half-life of fT3 (~ 30 h) compared to the much longer half-life of fT4 (6–7 days),[32] making the use of fT3 as a marker of thyroid status somewhat unstable as compared to fT4 and total T3.

Thyrotropin and breast cancer

Our study shows no association between TSH and breast cancer risk. Three prospective studies have reported on the potential association between TSH levels and breast cancer risk.[24–26] The smallest found a negative association,[24] while the others did not see any association.[25,26] Concerning cross-sectional studies, at least two have reported on a positive association between high TSH levels and breast cancer risk,[18,22] but most others have found no association.[11,13,14,17,20,21,23,41] The findings on TSH in the present study is in line with the majority of previous studies in that there was no association with breast cancer risk.

Thyroid peroxidise antibodies and breast cancer

There was a negative association between TPO-Ab levels and the risk of breast cancer in our study. Autoimmune thyroid diseases have been positively associated with breast cancer risk in several cross-sectional studies,[10,15,17] but the only previous prospective study, including only 15 cases, showed that the prediagnostic presence of TPO-Ab was not related to the subsequent risk of breast cancer.[24] It is unclear whether the presence of TPO-Ab in serum from patients with breast cancer is related to an increased risk following TPO-Ab related conditions, or if it is a general autoimmune response to the malignancy.[42]

Our study shows a negative association between TPO-Ab and breast cancer risk and is in accordance with our hypothesis. Patients with autoimmune thyroiditis in time, become hypothyroid with pathological levels of TPO-Ab that sustain for years. Hence, elevated levels of TPO-Ab, indicating a hypothyroid state, would be expected to be associated with a protective effect against breast cancer and this may explain the findings in our study.

Methodological issues

It may be asked whether breast cancer cases in this cohort are representative of the whole breast cancer population. This cohort mainly comprised middle-aged women and 41% of the women invited to the health examination attended. As we have no information about exposure to the studied risk factors in women outside this cohort, observed incidence rates may not be applicable to all age groups or to the general population. However, as there was a wide distribution of thyroid hormones, TSH and TPO-Ab levels, it was possible to make internal comparisons between subjects with low and high values, respectively. Hence, we consider that our estimations of relative risks were not considerably affected by selection bias.

Incomplete follow-up may affect the results. However, the Swedish Cancer Registry and the Swedish Cause-of-Death Registry have been validated and found to have a completeness of about 99%.[43]

Subjects with elevated fT4 levels and a possible diagnosis of hyperthyroidism may already be within the health care system and due to this the diagnosis of breast cancer might be established earlier than for euthyroid subjects. However, general mammography screening was introduced in Sweden already in 1986 and stands for the majority of detection of breast cancer cases.[44] It is unlikely that the thyroid status does affect participation in mammography screening; hence, a possible detection bias in hyperthyroid breast cancer patients

was probably a minor problem. It should also be noted that most women with fT4 levels in the fourth quartile in our study still had levels within the normal range, and these women did probably not have any symptoms of thyroid disease.

There is a well-known circadian and seasonal variation concerning T3 and TSH levels.[45] This may have lead to a nondifferential misclassification of fT3, thus leading to an attenuation of observed risks. This may be one possible explanation to the differences between our present study and our previous observations regarding fT3, as T3 in or previous study was measured in blood samples drawn in the morning after an overnight fast. This may be a less important problem for fT4 as is has a considerably less marked circadian rhythm.[45] Short-time variation is low for fT3 and fT4, but may be slightly higher for TSH,[46] which makes it difficult to exclude that such variation attenuated true risks associated with TSH. Concerning long-time variation, tracking of individuals, *i.e.* ranking of individuals over time is quite stable for thyroid hormones and TSH.[47] In any case, a true variation over time would most likely have lead to an undifferential misclassification and, hence, attenuated observed risks.

Our study used prospectively collected blood samples that had been stored between 14 and 19 years. First, it may be considered if frozen samples differ from fresh samples and, second, if prolonged storage affect observed levels. This has been investigated by Männistö *et al.* who found that fT4, TSH and TPO-Ab levels were very similar when analysed in frozen *vs.* fresh serum, but for fT3, these levels were slightly higher in frozen/thawed samples as compared to fresh serum.[48] Concerning storage time, the same author reported that fT3, fT4 and TSH levels were not affected by storage for 14–18 years, but TPO-Ab levels were only stable for up to 14 years and were then followed by a slight increase.[49] That is, freezing/thawing and storage time may have affected the absolute levels of fT3 and TPO-Ab in the our study, but there is nothing to suggest that samples from cases and controls are different, and it is not likely that relative comparisons such as ORs would have been affected.

Conclusions

Free thyroxin levels (fT4) were in our study positively associated with a high risk of breast cancer. This association was most pronounced in overweight/obese women, as compared to normal weight women. Women with a high level of thyroid peroxidase antibodies (TPO-Ab) had a slightly lower risk of breast cancer.

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Paper III

CLINICAL STUDY

Triiodothyronine levels in relation to mortality from breast cancer and all causes: a population-based prospective cohort study

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Abstract

Objective: The potential association between thyroid hormones and breast cancer has been investigated in a large number of studies without conclusive results. This study investigated triiodothyronine (T_3) levels in relation to breast cancer mortality in a population with no breast cancer patients at baseline. An additional aim was to study T_3 levels in relation to mortality from other cancers and all-cause mortality.

Design and methods: This was a population-based prospective cohort study including 2185 women in whom T_3 levels were measured as part of a preventive health project, i.e. before diagnosis in women who later developed breast cancer. Mean follow-up was 24.1 years and record-linkage to The Swedish Cause-of-Death registry identified 471 women who died: 26 out of breast cancer and 182 from other cancers. Mortality was assessed using a Cox's analysis, yielding hazard ratios (HRs), with 95% confidence intervals. Analyses of T_3 as a continuous variable were repeated for pre- and peri/postmenopausal women separately.

Results: T_3 levels were positively associated with the risk of breast cancer-specific death in the age-adjusted analysis: HR for T_3 as a continuous variable was 2.80 (1.26–6.25). However, the crude analysis did not reach statistical significance. Breast cancer mortality was even higher in postmenopausal women: 3.73 (1.69–8.22), but stratified analyses included few events. There were no statistically significant associations between T_3 levels and deaths from other cancers, age-adjusted HR: 1.09 (0.72–1.65) or all-cause mortality (1.25:0.97–1.60).

Conclusions: This study, the first of its kind on prospectively measured T_3 levels, indicates that T_3 levels are positively associated with breast cancer-specific mortality and that this is not related to a general effect on all-cause mortality.

European Journal of Endocrinology **168** 483–490

Introduction

Thyroid disorders are common in women and an association with breast cancer would have a large impact. A large number of cross-sectional studies have investigated the association between breast cancer and thyroid disease, or levels of thyroid hormones, but these studies have been inconclusive (1, 2, 3). Two recent prospective studies have, however, found a positive association between pre-diagnostic thyroid hormone levels and breast cancer risk (4, 5). These studies did not find any corresponding association between TSH and risk (ibid).

In spite of the potentially large clinical relevance, there are only two small studies, including 84 and 47 patients respectively, on thyroid hormones and clinical outcome, i.e. survival following breast cancer, but they found no clear associations (6, 7). A problem in studies

measuring thyroid hormones in breast cancer patients is, however, that the disease *per se*, surgery, or adjuvant treatment may affect hormonal levels.

In order to investigate whether there is a clinically important association between thyroid hormone levels and breast cancer, the most relevant measurement is probably the breast cancer-specific mortality in a population with no breast cancer at baseline; this would reflect the net result of incidence and survival in a population. The only previous study on thyroid conditions and breast cancer mortality to our knowledge is Goldman *et al*. (8), but they found no association. They did, however, not include information on thyroid hormonal levels.

This study is a population-based, prospective cohort study including 2185 women with no breast cancer at baseline and in whom triiodothyronine (T_3) levels were measured as part of a preventive health project,

i.e. before diagnosis in women who developed breast cancer. In a previous study based on this cohort, we have found a positive association between T_3 levels and the risk of breast cancer (4), but it is not known whether this leads to a corresponding increase in breast cancer mortality.

The aim of this study was to investigate serum levels of T_3 in relation to breast cancer mortality in a population with no breast cancer patients at baseline. An additional aim was to study T_3 levels in relation to all-cause mortality.

Materials and methods

The Malmö Preventive Project

Originally, 10 902 women participated in the Malmö Preventive Project. The project was established in 1974 when residents in Malmö, a city in southern Sweden, were invited to participate in a health survey. Entire birth cohorts, men and women, were examined until 1992 when the department closed. Approximately 70% of invited subjects participated (9).

All women answered a questionnaire concerning sociodemographic information, lifestyle habits, and medical history. Questions on reproductive factors, e.g. menopausal status, were only included in women screened from April 1983 and onward. BMI (kg/m^2) was assessed at baseline examination (9). A subject was considered to have a history of goiter if the question 'have you been treated for goiter' was answered with 'yes'. There was no available information on specific type of previous thyroid disorders or type of treatment. The study was approved by the ethics committee at Lund University: Dnr 652/2005 and Dnr 501/2006.

T_3 analysis

Blood samples were taken after an overnight fast with the patient in the supine position. The serum samples were analyzed for T_3 and TSH in women born in 1928 and 1941 and examined in 1983 and 1984. In women born in 1935 (examined from 1990 to 1992), T_3 was only measured in a subset of all women, i.e. those with pathological TSH values, a history of thyroid disease, or those with an enlarged thyroid gland at examination. In addition to this, the attending physician could also decide to analyze T_3 (4). No analysis of thyroxin (T_4) had been performed at baseline in these women. T_3 was measured by a double antibody RIA (reference interval 0.9–3.2 mmol/l) (10). Only six women had a value above the upper reference limit.

Study cohort and follow-up on mortality

Among 10 902 women, reproductive data including menopausal status had been assessed in 8051 subjects.

T_3 had been measured in 2383 women (1161 born in 1928, 907 born in 1941, and 315 born in 1935). Women with prevalent breast cancer ($n=35$), goiter ($n=167$), or both ($n=4$) were identified and excluded from the study. Finally, the study population consisted of 2185 women with information on T_3 and without breast cancer at baseline or a record of goiter. Information on vital status and cause of death was retrieved from the Swedish Cause-of-Death Registry up until 31 December 2010.

Statistical analysis

Quartile cut-points for T_3 were based on the distribution among all women in the study population. T_3 levels were investigated in relation to factors known to be associated with subsequent mortality, i.e. age at baseline, educational levels, alcohol consumption, and BMI. A Kendall's τ-b test was used giving correlation coefficients (τ_b) and corresponding P values. Smoking was compared in relation to T_3 quartiles using a χ^2 test. A Bonferroni's correction was performed for P values in pairwise χ^2 comparisons multiplying the P value with the number of comparisons. A two-sided P value <0.05 was regarded as statistically significant. Missing categories were not included in these tests.

The primary endpoint was breast cancer as underlying cause of death. In addition to this, death from other cancers, death from other causes, and all causes were included in the main analysis. The distribution of the above mortality-related factors was examined in different groups defined by vital status and cause of death. These distributions were tested using the χ^2 test as described earlier for smoking.

Each woman was followed from baseline until the end of follow-up, 31 December 2010, or until she died. Mean follow-up was 24.1 years (S.D. 5.3) and total follow-up included 52 579 person-years. A Cox's proportional hazards analysis was used to estimate hazard ratios (HRs) with a confidence interval (CI) of 95% in relation to T_3 levels. T_3 levels were introduced as a continuous variable in the main analyses. T_3 was not normally distributed ($P<0.001$ using a one sample Kolmogorov–Smirnov test), but to give estimates more readily interpretable, the original T_3 values were used to obtain HRs. However, T_3 values were also transformed using the natural logarithm in order to confirm whether the associations were statistically significant or not. Analyses were adjusted first for age at baseline and in a final model for all potential confounders. The limited number of breast cancer deaths did not allow inclusion of all covariates in the same model, but in relation to this endpoint, age and one additional factor at a time were included in the final model. In this way, a maximum of three variables were included in each model. In tables, only the model with the largest change from the crude HR for T_3 was reported. The primary endpoint, breast cancer as cause of death, was also

analyzed with T_3 as a continuous variable stratified for menopausal status as we, in our previous study on T_3 levels and breast cancer incidence, found that the association was considerably stronger in postmenopausal women compared with premenopausal. The stratified analyses did only allow the inclusion of one additional covariate, and we considered age at baseline to be most important. The stratified analyses were further evaluated by including a term for interaction in the model between T_3 levels and menopausal status. A P value <0.05 was considered as a statistically significant interaction. Finally, breast cancer death was analyzed in relation to different T_3 quartiles where breast cancer-specific mortality was calculated per 10 000 person-years. Corresponding HRs were calculated as earlier.

It is possible that subclinical disease may affect T_3 levels, and in a sensitivity analysis, all analyses were repeated excluding all women who died before 2 years following baseline examination. SPSS version 18.0 (Lund, Sweden) was used for all analyses.

Results

Women in higher T_3 quartiles were relatively old ($\tau_b=0.29$; $P<0.001$), they had a lower educational level ($\tau_b=-0.10$; $P<0.001$), a lower alcohol consumption ($\tau_b=-0.07$; $P<0.001$), and they were more often overweight than women in lower T_3 quartiles ($\tau_b=0.17$; $P<0.001$; Table 1). There was no statistically significant association between T_3 levels and smoking (overall $P=0.82$).

Women who died from cancers other than breast cancer, or from causes other than cancer, were considerably older at baseline compared with women alive at the end of follow-up ($P<0.001$; Table 2). Women who died from causes other than cancer during follow-up had a lower educational level ($P=0.006$), reported a lower alcohol consumption ($P=0.03$), and had a higher BMI ($P<0.001$) than women alive at the end of follow-up. Current smoking was more common among women who died from cancers other than breast cancer, and all causes other than cancer, compared with women alive at the end of follow-up ($P<0.001$).

In this study, we observed a positive association between T_3 levels and the risk of death from breast cancer (Table 3). The crude analysis did not reach statistical significance, but the risk increased following adjustment for age and other potential confounders. This association with mortality from breast cancer was only apparent among postmenopausal women compared with premenopausal. However, the terms for interaction between T_3 levels and menopausal status did not reach statistical significance. All statistically significant results in the continuous analyses were

Table 1 Distribution of potential determinants for mortality according to serum T_3 level. Column percent P values are given in italics.

Factor	T_3 quartile (T_3 range (mIU/l))					Kendall's τ-b coefficient (τ_b; P values)
	1 ($n=494$) (<1.50))	2 ($n=704$) (1.60–1.80))	3 ($n=456$) (1.90–2.00))	4 ($n=531$) (2.10–4.30))	All ($n=2185$) (0.60–4.30))	
Age						
<50 years	62.1	46.6	26.1	21.5	39.7	0.29
>50 years	37.9	53.4	73.9	78.5	60.3	(<0.001)
Education						
Missing	4.5	4.1	4.2	3.2	4.0	–
<12 years	53.0	53.6	62.5	64.2	57.9	-0.10
12 years	20.9	27.4	22.1	21.8	23.5	(<0.001)
>12 years	21.7	14.9	11.2	10.7	14.5	
Smoking						
Missing	–	–	0.4	0.9	0.3	–
Never	43.5	47.7	47.6	47.1	46.6	(0.82)[a]
Current	35.8	33.2	34.0	33.1	34.0	
Ex	20.6	19.0	18.0	18.8	19.1	
P value[b]	Ref.	*1.08*	*1.14*	*1.32*	–	
Alcohol consumption						
Missing	–	0.1	0.4	1.5	0.5	–
Nothing	14.2	14.1	13.2	19.0	15.1	-0.07
Less than every week	55.3	60.7	62.5	60.1	59.7	(<0.001)
Every week	30.6	25.1	23.9	19.4	24.7	
BMI (kg/m²)						
<20	14.8	10.1	7.9	7.5	10.1	0.17
≥20–<25	62.3	56.1	53.9	46.5	54.7	(<0.001)
≥25–<30	17.0	26.1	27.9	30.5	25.5	
≥30	5.9	7.7	10.3	15.4	9.7	

[a]Overall P value from the χ^2 test. Not including missing values.
[b]Pairwise χ^2 tests, with corrected P values (multiplied with 3). Not including missing values.

Table 2 Distribution of potential determinants for mortality according to vital status. Column percent *P* values are given in italics.

Factor	Alive (*n*=1714)	Dead from breast cancer (*n*=26)	Dead from other cancers (*n*=182)	Dead from other causes (*n*=263)	Dead from all causes (*n*=471)	χ^2, *P* value[a]
Age						
<50 years	44.1	65.4	24.2	19.4	23.8	<0.001
>50 years	55.9	34.6	75.8	80.6	76.2	
P value[b]	Ref.	0.09	<0.001	<0.001	–	
Education						
Missing	2.8	0.0	8.2	9.1	8.3	–
<12 years	56.4	69.2	63.2	62.7	63.3	0.003
12 years	24.6	19.2	19.8	19.4	19.5	
>12 years	16.2	11.5	8.8	8.7	8.9	
P value[b]	Ref.	1.53	0.39	0.006	–	
Smoking						
Missing	0.4	0.0	0.5	–	0.2	–
Never	49.5	46.2	35.7	35.4	36.1	<0.001
Current	30.2	23.1	47.8	50.2	47.8	
Ex	20.0	30.8	15.9	14.4	15.9	
P value[b]	Ref.	1.14	<0.001	<0.001	–	
Alcohol consumption						
Missing	0.6	0.0	0.5	–	0.2	–
Nothing	14.2	26.9	12.6	21.3	18.3	0.04
Less than every week	59.9	50.0	63.2	57.0	59.0	
Every week	25.3	23.1	23.6	21.7	22.5	
P value[b]	Ref.	0.57	2.01	0.03	–	
BMI (kg/m^2)						
<20	9.9	7.7	9.3	11.8	10.6	0.004
≥20–<25	56.8	50.0	50.5	44.5	47.1	
≥25–<30	24.6	34.6	29.1	27.8	28.7	
≥30	8.6	7.7	11.0	16.0	13.6	
P value[b]	Ref.	2.13	0.93	<0.001	–	

[a]Overall *P* value from the χ^2 test tested for alive/dead from breast cancer/dead from other cancers/dead from other causes. Not including missing values.
[b]Pairwise χ^2 tests, with corrected *P* values (multiplied with 3). Not including missing values.

confirmed using the logarithmic T_3 value (data not shown). In the quartile analysis, the risk of breast cancer death was higher in the fourth T_3 quartile compared with the first quartile (Table 4). The limited number of events did not allow a quartile analysis stratified for menopausal status.

The risk of deaths from cancers other than breast cancer, causes other than breast cancer, and all causes were positively associated with T_3 levels in the unadjusted analyses (Table 3). However, following adjustment for age, and subsequently for all potential confounders, these estimates were close to unity. When

Table 3 Mortality in relation to serum T_3 levels. The model with the largest influence on the HR, compared with the crude model, included alcohol consumption in addition to age at baseline; HR and corresponding *P* values presented in table. All these multivariate models showed statistically significant HRs for T_3.

Cause of death	Subjects in analysis (*n*)	Dead (*n*)	HR (95% CI), crude T_3	*P* value, T_3	HR (95% CI), age-adjusted T_3	*P* value, T_3	HR (95% CI), adjusted[a] T_3	*P*, value T_3
Breast cancer								
All	2185	26	2.22 (0.92–5.37)	0.077	2.80 (1.26–6.25)	0.012	2.82[b] (1.25–6.37)	0.01[b]
Premenopausal[c]	863	14	0.81 (0.17–3.76)	0.784	0.93 (0.20–4.37)	0.930	–	–
Postmenopausal[c]	1322	12	4.30[d] (1.88–9.86)	0.001	3.73[e] (1.69–8.22)	0.001	–	–
Other cancers	2185	182	1.45 (0.99–2.11)	0.055	1.09 (0.72–1.65)	0.685	0.98 (0.64–1.51)	0.937
Other causes	2185	445	1.60 (1.26–2.02)	<0.001	1.17 (0.90–1.52)	0.237	1.07 (0.82–1.41)	0.610
All causes	2185	471	1.63 (1.30–2.05)	<0.001	1.25 (0.97–1.60)	0.087	1.16 (0.89–1.50)	0.280

[a]Adjusted for age at baseline, educational level, smoking status, alcohol consumption, and BMI (continuous).
[b]Adjusted for age at baseline and one factor at a time out of educational level, smoking status, alcohol consumption, and BMI (continuous).
[c]Menopausal status at baseline.
[d]Interaction between T_3 and menopausal status: *P* value=0.07.
[e]Interaction between T_3 and menopausal status: *P* value=0.12.

Table 4 Breast cancer-specific mortality in relation to serum T_3 levels. The model with the largest influence on the HR for the fourth quartile, compared with the crude model, included smoking status in addition to age at baseline; HR and corresponding *P* value presented in table. All these multivariate models showed statistically significant HRs for the fourth T_3 quartile, except for the model including age at baseline and BMI where this association was only borderline significant (*P*=0.05002).

T_3 (quartile)	Subjects (*n*)	Dead from breast cancer (*n*)	Mortality/10 000 person-years	HR (95% CI), crude T_3	HR (95% CI), age-adjusted T_3	HR[a] (95% CI), adjusted T_3
1	494	4	3.2	1.00	1.00	1.00
2	704	10	5.8	1.82 (0.57–5.81)	2.07 (0.65–6.63)	2.07 (0.65–6.61)
3	456	3	2.8	0.91 (0.20–4.05)	1.27 (0.28–5.77)	1.27 (0.28–5.76)
4	531	9	7.4	2.48 (0.76–8.09)	3.61 (1.08–12.1)	3.65 (1.09–12.2)
Total	2185	26	4.9	–	–	–
Overall *P* value	–	–	–	*0.30*	*0.15*	*0.14*

[a]Adjusted for age at baseline and one factor at a time out of educational level, smoking status, alcohol consumption, and BMI (continuous).

women who died within 2 years following baseline were excluded from the analyses, all results were similar (data not shown).

Discussion

This is the first prospective study on T_3 levels in relation to breast cancer mortality. It shows that pre-diagnostic T_3 levels are positively associated with the risk of breast cancer-specific death and that this is not related to a general effect on cancer death or all-cause mortality.

It may be asked whether breast cancer cases in this cohort are representative of the whole breast cancer population. This cohort mainly comprised middle-aged women and 70% of the women invited to the health examination attended (9). As we have no information about exposure to the studied risk factors in women outside this cohort, absolute risks may not be applicable to all age groups or to the general population. However, as there was a wide distribution of T_3 levels, it was possible to make internal comparisons between subjects with relatively low and high values respectively. Hence, we consider that our estimates of relative risks were not considerably affected by selection bias.

Incomplete follow-up may affect the results. However, the Swedish Cause-of-Death Registry has been validated and found to have a completeness of about 97.3% in 2008 (11). Moreover, correctness of cause of death due to malignancies has been shown to be higher than 90% in the Swedish Cause-of-Death Registry (12).

Subjects with elevated T_3 levels may already be within the health care system, possibly because of related symptoms, and due to this, the diagnosis of breast cancer might be established earlier than for euthyroid subjects. If true, this would lead to early diagnosis and an expected decrease in breast cancer mortality in this group. However, this is not likely as the contrary was found in this study. Furthermore, general mammography screening was introduced in Sweden in 1991 and stands for the majority of detection of breast cancer cases (13). It is also unlikely that thyroid status does affect participation in mammography screening. It should

also be noted that most women with T_3 levels in the fourth quartile in this study still had levels within the normal range, and these women probably did not have any symptoms of thyroid disease. It is not likely that the results in this study were due to a detection bias.

The method of analysis for T_3 remained the same throughout the study period and all analyses were performed using a standardized collection of blood samples. T_3 levels were analyzed following blood collection in 1983, 1984 and between 1990 and 1992. There are no recorded values for the coefficient of variance (CV) from the laboratory covering this period. However, reported within-laboratory CV at the time for T_3 were in the order of 9–11% according to a large European analysis including 150 laboratories (14). Following this, we consider that T_3 levels had a good reliability and that misclassification was probably low.

It is also important to consider true variation in T_3 values as there is a well-known circadian and seasonal variation (15). Tracking of individuals, that is ranking of individuals over time, is, however, quite stable (16), and a true variation over time would most likely have led to an undifferential misclassification of T_3 and, hence, attenuated risks.

The current study is the largest prospective study to date on thyroid hormone levels and breast cancer mortality, but the number of deaths due to breast cancer was still very limited. CIs were wide and the statistical power was relatively low. This led to an imprecision in the estimates related to T_3 and breast cancer mortality. This was specifically apparent in the quartile analysis where CI was very wide, and the overall *P* value did not reach statistical significance. Moreover, statistical power was an even more serious problem in analyses stratified for menopausal status, and these results, although statistically significant, should be regarded with caution.

An additional problem related to the limited number of breast cancer deaths was that not all confounders could be included at the same time in the final model for this endpoint. However, the models including age at baseline and one additional factor yielded statistically significant positive associations between T_3 levels and

breast cancer death; this indicates that these factors did not seriously confound the observed association.

Concerning death from cancers other than breast cancer, death from causes other than breast cancer, and all-cause mortality, there were a substantially larger number of events, and we consider that the lack of any strong associations was not merely the result of poor statistical power, i.e. a type II error.

In order to simplify the interpretation of HR obtained in the continuous analysis, we used original values even if they were not normally distributed. However, all statistically significant results in the continuous analyses were confirmed using the logarithmic T_3 value. This is also what would be expected given the high correlation between raw and log-transformed T_3 values.

The potential association between thyroid hormones and outcome following breast cancer has been discussed for more than a century. Several early studies used thyroid hormones/extracts as breast cancer treatment, but the effect was not apparent (2). We have found that no studies were published during the last 50 years on this topic. Goldman *et al.* (8), the only study on thyroid conditions and breast cancer mortality, found no excess risk of breast cancer death in women with thyroid disorders. Smyth *et al.* (17) followed 195 breast cancer patients and they found that positive TPO-Ab status was associated with a favorable survival, i.e. a lower risk of recurrent disease and death. Fiore *et al.* (6) examined 47 patients, among whom 16 died, and also found that positive thyroid antibody status (TPO-Ab and/or Tg-Ab) was associated with a low mortality, i.e. a good survival. These findings on recurrence-free survival and overall survival in relation to TPO-Ab status were not confirmed by Jiskra *et al.* (7), including 84 patients where 11 died. Fiore *et al.* (6) and Jiskra *et al.* (7) also investigated T_4 levels and TSH in relation to overall survival, but they found no clear associations. A problem in the interpretation of the above studies is that thyroid hormones, TSH and TPO-Ab, may indeed be affected by prevalent breast cancer, stress, or treatment. Generally, previous studies have been hampered by a low number of cases and short follow-up, and we have found no study on pre-diagnostic thyroid function and survival following breast cancer. Concerning mortality, no study has investigated thyroid function or T_3 levels in relation to breast cancer-specific mortality in a 'breast cancer healthy' population.

All-cause mortality was associated with relatively high T_3 levels in the crude analysis, but adjusted for age and other potential confounders, all estimates were close to unity. There are several recent meta-analyses and reviews on the potential association between thyroid conditions and all-cause mortality. Overt hyperthyroidism was associated with a slightly increased risk of all-cause mortality (RR = 1.21:1.05–1.38) in a meta-analysis by Brandt *et al.* (18), including more than 30 000 patients. All the included studies were adjusted for age, but none for education, BMI, or alcohol consumption, and only some for smoking. Considering subclinical conditions, subclinical hyperthyroidism was positively associated with all-cause mortality in a meta-analysis of seven cohort studies (HR = 1.41:1.12–1.79) using a general population as reference (19). Interestingly, another meta-analysis including ten prospective studies found no statistically significant association between subclinical hyperthyroidism and all-cause mortality (20). This study excluded subjects with goiter, and even in the highest quartile, most subjects were within the reference limits for T_3. All in all, this makes it difficult to compare our results to studies based on overt thyroid conditions. Some of the previous studies also included patients treated with T_4; that is, these studies may reflect an effect caused by exposure to exogenous hormones.

The biological mechanism explaining the association of T_3 levels and breast cancer-specific mortality is not clear. However, the presence of thyroid hormone receptors in human breast and breast cancer cell lines has been established (21, 22, 23, 24), and the proliferative effect of T_3 has been confirmed by various experimental studies on breast cancer. Our findings are in line with these data (25, 26, 27, 28).

It has been shown that T_3 binds and stimulates the estrogen receptor, acting in synergy with estrogen on breast cancer cell lines, potentiating the estrogenic effect, and enhancing cell proliferation (29). The role of estrogen in carcinogenesis of the breast is well known and the possibility that this effect may be even stronger in conjunction with relatively high levels of T_3 could in part explain the results in this study.

The positive association between T_3 and breast cancer was specifically strong in postmenopausal women. Therefore, an imbalance between estrogen and T_3 with an increased T_3/estradiol ratio may be more important than a pure synergistic effect between these two hormones for the risk of developing breast cancer. It has been suggested that this imbalance might enhance breast cancer development (25). This theory is in accordance with our findings that T_3 levels in post-menopausal women are positively associated with breast cancer mortality. An important issue for future studies will be to evaluate the widespread, long-term use of T_4 treatment in TSH-suppressive doses for benign thyroid disease (30, 31), and the management of women with subclinical hyperthyroidism (32, 33, 34). Such studies will contribute with important evidence whether or not to treat mild and/or subclinical thyroid disorders.

Conclusions

In conclusion, the present prospective cohort study, the first of its kind on prospectively measured T_3 levels, indicates that T_3 levels are positively associated with breast cancer-specific mortality and that this is not related to a general effect on all-cause mortality.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Paper IV