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**Author:** Debeij, Jan  
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General introduction

Jan Debeij
General introduction

In this thesis the relation between thyroid hormones and the coagulation system will be examined. As an introduction, the hypothalamic-pituitary-thyroid axis, the coagulation system and their interactions will be discussed. A short overview of the literature preceding the research reported in this thesis is about the relation between thyroid hormones, the coagulation system, and risk of bleeding and venous thrombosis.

Thyroid hormones

The hypothalamic-pituitary-thyroid axis is the regulatory system of thyroid hormone action. In the hypothalamus, thyroid releasing hormone (TRH) is secreted and transported to the pituitary gland. Here, TRH stimulates the production of thyroid stimulating hormone (TSH), which in its turn is released in the circulation and affects the functioning of the thyroid gland. The thyroid gland is located in the ventral part of the neck and consists of 2 lobes, connected by the isthmus. The thyroid consists of follicles lined by cuboidal epithelioid cells. The thyroid hormones tri-iodothyronine (T3) and tetra-iodothyronine (T4) are synthesized in the gland under influence of TSH and regulated by negative feedback mechanisms. More T4 than T3 is produced and stored, while T3 is the most active form of the hormone. In several peripheral tissues like the liver, muscles and kidney, T4 is converted into the more active T3 (this is done by 5'-monodeiodination). Thyroid hormones bind to nuclear receptors, the thyroid hormone receptors (THR). When T3 or T4 binds to the THR (T3 has a 10-fold greater affinity for the THR), they either activate or repress gene transcription. Thyroid hormone influences the basal metabolic rate, resulting from influence on the carbohydrate, protein and lipid metabolism, the Na-K pump activity and thermogenesis.

Hyperthyroidism is a condition in which the thyroid gland produces and secretes excessive amounts of the free thyroid hormones T3 or T4. Hyperthyroidism is one cause of thyrotoxicosis—the hypermetabolic clinical syndrome which occurs when there are elevated serum levels of T3 or T4. There are several causes of
hyperthyroidism such as Graves’ disease, toxic adenoma, and toxic multinodular goitre. Thyroiditis (inflammation of the thyroid gland) may also cause hyperthyroidism, but often progresses to gland dysfunction and, thereby to hormone deficiency and hypothyroidism.

Another cause is oral consumption of excess thyroid hormone tablets (surreptitious use of thyroid hormone), as is the rare event of consumption of ground beef contaminated with thyroid tissue, and consequently thyroid hormone (termed "hamburger hyperthyroidism"). Amiodarone, an anti-arrhythmic drug which is structurally similar to thyroxine, may cause either under- or overactivity of the thyroid. Finally, hypersecretion of thyroid stimulating hormone (TSH), which is almost always caused by a pituitary adenoma, is also a (very rare) cause of hyperthyroidism. Major clinical signs of hyperthyroidism include weight loss (often accompanied by an increased appetite), anxiety, intolerance to heat, hair loss, muscle aches, weakness, fatigue, hyperactivity, irritability, polyuria, polydipsia, delirium, tremor, pretibial myxedema and sweating.

Hypothyroidism is a pathologic condition in which the thyroid gland produces inadequate amounts of the thyroid hormones thyroxine (T4) and triiodothyronine (T3). Since iodine is an essential building block to produce T3 and T4, iodine deficiency is the most common cause of hypothyroidism worldwide. In parts of the world where iodine is widely available in food, hypothyroidism is most commonly caused by Hashimoto's thyroiditis; other causes may include an absent thyroid gland or central hypothyroidism due to impaired production of the hypothalamic hormone TRH or the anterior pituitary hormone TSH. Central hypothyroidism may occur following for example injury to these glands from physical trauma, compression by a tumor or vascular insufficiency. Certain medications may cause hypothyroidism, such as lithium-based mood stabilizers, amiodarone and thalidomide. Partial or total removal of the thyroid gland also causes hypothyroidism. Clinical symptoms can be divided in early and late symptoms. Early symptoms are thin, brittle fingernails; dry, itchy skin; weight gain, water retention, myxedema, hair loss, depression, depressed reflexes, hypotonia, muscle cramps, muscle weakness, fatigue, cold intolerance, bradycardia, elevated serum cholesterol. Late symptoms are thinning of the outer
third of the eyebrow, slow speech and hoarse voice, carpal tunnel syndrome and hypotension.

**The coagulation system**

Haemostasis is the process by which blood loss is prevented. It leads to cessation of blood loss to the extravascular space from a damaged vessel. The endpoint of haemostasis is reached when the damage to a blood vessel wall is covered by a platelets and fibrin-containing plug, thereby stopping bleeding. Thereafter repair of the damaged vessel can start.

Under physiological conditions, the vessel wall prevents platelet adhesion and clot formation. Haemostasis begins almost instantly after an injury has damaged the endothelial lining of the vessel. This damage exposes subendothelium, which contains collagen and von Willebrand factor, to the blood components. This initiates platelets adhesion and aggregation at the site of injury; primary haemostasis. Almost simultaneously with the initiation of primary haemostasis, secondary haemostasis is activated by tissue factor, also released from the vessel wall. Secondary haemostasis is a complex system of coagulation proteins in the blood plasma. Its goal is to generate large amounts of thrombin when needed, ultimately resulting is the generation of insoluble fibrin from soluble fibrinogen to strengthen the platelet plug and allowing adherence and activation of cells involved in vascular repair.

The process of coagulation was originally regarded as a cascade of one factor activating the next. The coagulation system is classically divided in two pathways, extrinsic and the intrinsic pathway, finally leading to fibrin formation through a common pathway (figure 1). The pathways are a series of reactions, in which a zymogen (inactive enzyme precursor) of a serine protease and its glycoprotein co-factor are activated which subsequently catalyze the activation of a next zymogen. Rather than a cascade, it is a system in which there are multiple positive and negative feedback loops as well as cross-connections between the pathways.

To achieve fast thrombin generation three phases can be identified:
1. Initiation: Following damage to the blood vessel the extrinsic pathway becomes activated. Tissue factor (TF), which is expressed by endothelial cells, subendothelial tissue and monocytes, is exposed to factor VII (FVII) and forms an activated TF-FVIIa complex. TF-FVIIa in its turn activates Factor X. Together with factor V, calcium and phospholipids, factor X forms the prothrombinase complex. This prothrombinase complex activates prothrombin to thrombin.

2. Amplification: Thrombin formation is amplified by the small amount of thrombin generated by the extrinsic pathway in the initiation phase. Thrombin activates the intrinsic pathway by activation of factor XI, factor VIII and factor V. This leads to the production of activated factor IX and activated factor VIII which together with a phospholipid membrane and calcium form a tenase complex, activating factor X to activated factor X, again leading to formation of the prothrombinase complex which produces large amounts of thrombin from prothrombin. Thrombin converts fibrinogen to fibrin, one of the building block of a haemostatic plug. It also activates factor XIII, which crosslinks the fibrin strands to stabilize the clot. The coagulation system remains in a prothrombotic state by the continued activation of FVIII and FIX to form the tenase complex, until it is inhibited by anticoagulant pathways.

3. Inhibition: Following the amplification phase, several inhibiting mechanisms are activated. The activation of factor X (FX) to form FXa by TF-FVIIa is almost immediately inhibited by tissue factor pathway inhibitor (TFPI), stopping the initiation phase. Protein C is activated by thrombin. Together with protein S and phospholipids it degrades activated factor V and activated factor VIII, thereby inhibiting coagulation. Antithrombin is also produced, degrading thrombin, activated factor IX, activated factor X, activated factor XI and activated factor XII, this way also inhibiting the propagation of coagulation. Furthermore, plasmin is generated out of plasminogen to cleave fibrin, inhibiting excessive fibrin formation.

The complete coagulation system maintains a balance allowing rapid formation of a clot upon injury that is limited to the site of injury. Any imbalance can induce a bleeding tendency and a protective effect against venous thrombosis or can lead to a low risk of bleeding with a pro-thrombotic tendency, possibly leading to clot formation.
Venous thrombosis

With an incidence of 1-2 per 1000 person-years, venous thrombosis is the third most common cardiovascular disease in Western society (after acute coronary syndrome and stroke). Clinical manifestations of venous thrombosis are deep venous thrombosis and pulmonary embolism. Deep venous thrombosis is a condition where a thrombus develops in the deep veins of the calf or in more proximal veins, such as the popliteal, femoral or iliac veins. This leads to obstruction of venous drainage of blood. Characteristic symptoms of deep venous thrombosis are a red, swollen, painful leg. Pulmonary emboli develop from deep venous thrombi. A venous thrombus formed anywhere in the body can dislodge from its location and move...
more proximally. The emboli are caught in the first web of smaller vessels they encounter: the lungs. This leads to various symptoms: dyspnea, tachypnea, pleuric pain, cough and wheezing, although the clinical presentation is highly variable. The 30 days case fatality rate for deep venous thrombosis and pulmonary embolism combined is 11% and the 1-year case-fatality rate is 23% [1,2]. In more than 6 percent of all deaths massive pulmonary emboli are found by autopsy. Much research has been done on risk factors for venous thrombosis. These risk factors can be divided in genetic and acquired risk factors. Genetic risk factors include antithrombin deficiency, factor V Leiden mutation, prothrombin 20120A mutation, ABO blood group, protein C deficiency and protein S deficiency. Acquired risk factors include surgery, plaster cast, cancer, long haul travel, pregnancy, and oral contraceptive use [3-6].

The recurrence risk of a venous thrombotic event is much higher than the risk of a primary event. The cumulative incidence of recurrent venous thrombosis has been described to be 4-11% within the first year and 12-30% in the five years after the first event. Incidence rates of recurrence vary between 25 and 46 per 1000 person-years [7-10]. While many risk factors for first venous thrombosis are known, this is not the case for recurrent events. Furthermore, the risk profile for recurrent venous thrombosis is different from that of first venous thrombosis. This is the case for for example thrombophilia, an increased tendency of the blood to clot. Thrombophilia includes deficiencies of the natural anticoagulants antithrombin, protein C, and protein S, as well as carriership of factor V Leiden, prothrombin G20210A, and high levels of factors VIII, IX, or XI, homocysteine, and fibrinogen. While thrombophilia is associated with a 2-10 fold increased risk of a first event, it does not predict risk of recurrence [7,9]. Age, also an important risk factor for a first venous thrombosis, does not influence risk of recurrence [11,12].

**Thyroid hormones and the coagulation system**

The first time a relation between thyroid disease and venous thrombosis was described was in 1913 by Kaliebe [13]. He described a patient with Graves disease and cerebral venous thrombosis and proposed a relation between thyroid hormone
and venous thrombosis. Other case reports followed, again describing subjects with hyperthyroidism and cerebral venous thrombosis, suggesting an effect of thyroid hormone excess on the coagulation system [14-18]. Subsequent studies focused on alterations in levels of coagulation factors in patients with thyroid disease and mostly confirmed that hyperthyroidism was associated with prothrombotic changes.

Squizzato et al. reviewed the available literature [19] until 2007 on thyroid hormones and their effect on the coagulation system. An important conclusion of their study was that there were no high quality papers on this subject. After pooling the available studies of low and medium quality they arrived at the following conclusions: In subjects with elevated levels of thyroxine, high levels of von Willebrand factor and fibrinogen were observed. In subjects with decreased levels of thyroxine, an increased bleeding time was seen together with a prolonged activated partial thromboplastin time and prothrombin time and decreased levels of factor VIII, von Willebrand factor and fibrinogen. Apart from studies into the effect of thyroid hormones on the coagulation system, only one small study had been performed on the relation between thyroxine and the risk of venous thrombosis before the start of this thesis in 2007. This study concerned 50 patients with provoked, 50 patients with unprovoked and 50 controls with no venous thrombosis [20]. No increased prevalence of hyperthyroidism was found in patients with venous thrombosis compared with controls.

The objective of this thesis is to clarify the relation between thyroid hormones, the coagulation factors and their effect on the risk of haemorrhage, first venous thrombosis and recurrent venous thrombosis. The effect of thyroid hormones and thyroid stimulating hormones in the thyroid axis on the coagulation system is not clear in all details. Importantly, it is unknown which coagulation factors are influenced by either TSH of FT4 and which are not. Also, claims have been made on a possible auto-immune effect of anti-thyroid peroxidase antibodies on the coagulation system, and so there could be an effect on the coagulation system of these antibodies. Because large studies concerning the effect of thyroid function on the risk of venous thrombosis and haemorrhage are missing, these risks in patients with aberrant thyroid function remain unknown. Since risk factors for a first venous thrombosis
differ from those of a recurrent venous event, it is also of interest to assess the effect of thyroid function on recurrent venous thrombosis.

Outline of this thesis

In chapter 2, the effect of levels of free thyroxine (FT4) and thyroid stimulating hormone on individual coagulation factors is evaluated in a group of patients treated for thyroid cancer. The effect of levels of free thyroxine on the risk for major bleeding in a population using vitamin K antagonists is described in Chapter 3. In the TROL study, a large Norwegian prospective cohort study, the relation between levels of free thyroxine and risk of venous thrombosis was studied, which is described in Chapter 4. Chapter 5, 6 and 7 describe data from the MEGA study, a population based case-control study in Leiden on the aetiology of venous thrombosis. In Chapter 5, the relation between levels of free thyroxine and risk of thrombosis is studied in more detail and in several subgroups. Furthermore, the relation between free thyroxine and coagulation factors is assessed within controls. Because contradictory findings have been reported on the effect of low levels of free thyroxine on the risk of venous thrombosis, this was assessed in the MEGA study and discussed in chapter 6. In chapter 7 the effect of levels of free thyroxine on the risk of recurrent venous thrombosis is reported, as estimated from the MEGA follow up study. Chapter 8 presents a review of the literature incorporating the findings of this thesis.