Hormones, haemostasis, and the risk of thrombosis
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Citation for published version (APA):
van Zaane, B. (2010). Hormones, haemostasis, and the risk of thrombosis
Chapter 3

Hormones and cardiovascular disease: a shift in paradigm with clinical consequences?


Seminars in Thrombosis and Haemostasis 2009; 35:478-487
**Abstract**

Several endocrine disorders have been associated with an increased risk of cardiovascular disease and mortality. In addition, even subtle hormonal disturbances may modulate the function of cardiovascular organs. In this review, we discuss in detail the contribution of thyroid hormones, cortisol, the somatotropic hormones, and prolactin in the development of cardiovascular disease. We do not only discuss epidemiological evidence on the association between hormones and cardiovascular disease, but also address possible pathophysiological mechanisms underlying this association. In fact, hormones can contribute to the development of cardiovascular disease both indirectly by inducing secondary metabolic changes such as hypertension, insulin resistance or dyslipidaemia; and directly by modulation of cellular pathways that are important in the process of atherosclerotic plaque formation (atherogenesis), plaque instability, and thrombosis. To date, several new therapeutic approaches which focus on the control of hormones at tissue level, independently of their circulating levels, are being developed. This may offer new possibilities for cardiovascular risk reduction.

**Introduction**

Cardiovascular disease (CVD), i.e. myocardial infarction and stroke, is the leading cause of death worldwide. As a consequence of large scale cardiovascular risk factor screening, a great number of patients with an increased CVD risk is identified before the occurrence of ischaemic events. These individuals often participate in primary prevention programmes and are managed with lifestyle measures or pharmacological therapy. Despite these recent and significant improvements with regards to screening and intervention, more than 50% of all cardiovascular events occur in individuals that have escaped primary prevention. Therefore, the search for novel modulators of cardiovascular disease is necessary.

The association between several endocrine disorders and the presence of cardiovascular disease is known already for many decades. Extreme hormone disturbances in case of hormone excess or deficiency may negatively affect the cardiovascular system both indirectly by inducing secondary hypertension, insulin resistance or dyslipidaemia; and directly by interactions with cellular pathways that are important in the process of atherosclerotic plaque formation (atherogenesis), plaque instability and thrombosis. Recent observations indicate that variation in hormone levels occurring within the physiological range is also associated with cardiovascular disease. Within these levels, secondary disease such as insulin resistance or dyslipidaemia does not come into play yet, and this strengthens the concept that several hormones in itself may harm the vasculature. If this is true, controlling hormone levels, either systemically or at tissue level, could become a therapeutic target in the prevention of cardiovascular disease.

Against this background, we here review the available literature on the contribution of a specific subset of hormones in the development of cardiovascular disease, namely a. the thyroid hormones (tri-iodothyronine (T3) and thyroxine (T4), hereafter both referred to as thyroid hormone unless specified otherwise); b. cortisol; c. the somatotropic hormones (growth hormone (GH) and insulin-like growth factor-1 (IGF-1)); and d. prolactin. This review will discuss in detail 1. epidemiological evidence on the association between hormones, both in excess or if deficient as well as at physiological levels, and cardiovascular mortality due to myocardial infarction or stroke; 2. possible pathophysiological mechanisms underlying this association; and 3. perspectives on possible interventions in hormone levels for cardiovascular risk reduction.

**Clinical importance of endocrine disturbances: epidemiological evidence**

Several epidemiological studies suggest an association between thyroid dysfunction, both overt hyperthyroidism and hypothyroidism, and cardiovascular disease. Not surprisingly, given the various designs and settings of the available studies, the reported cardiovascular mortality ratios range from 0.9 to 1.4 and 0.7 to 1.11 for hyper- and hypothyroidism, respectively. The majority of reports, however, indicate an increased mortality in both disease entities, confirming a shaped U-curve for the epidemiological relationship between thyroid disorders and cardiovascular mortality.

In patients with Cushing’s syndrome, the mortality rate is four times higher than expected (standardised mortality ratio 3.8; 95% CI 2.5-17.9), with cardiovascular disease being the most
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Possible pathophysiological mechanisms

Hormone disturbances can negatively affect the cardiovascular system both chronically by accelerating the process of atherogenesis and plaque instability; and acutely by precipitating thrombosis. Atherosclerosis is characterised by a runaway pro-inflammatory cascade that starts at the endothelium, in an environment of increased shear stress and augmented oxidative stress. Damaged endothelium has a diminished capacity to generate nitric oxide (NO) that is responsible for cell coping and vasodilatation. As soon as damaged endothelium is established, neo-intima formation and evolution of macrophages towards foam cells could be recognised in early atherosclerotic lesions. Neo-intima formation is characterised by hypertrophic smooth muscle cells in the media layer. Some of these will be freed from their media structure, and differentiate into fibromuscular cells with migration towards the luminal plaque site. These two processes of damaged endothelium and neo-intima formation could cover a period of some decades. In the background of ongoing inflammation and a subsequent increase in calcification of media structures, new vessels (angiogenesis) will grow into the atherosclerotic lesion with luminal extension of the plaque volume. This lessens the integrity of the fibrous cap, calcification of media structures, new vessels (angiogenesis) will grow into the atherosclerotic lesion with luminal extension of the plaque volume. This lessens the integrity of the fibrous cap, and the lesion prone to plaque rupture. Finally, after physical disruption of the fibrous cap, the circulation comes into contact with tissue factor within the lipid core of the lesion. A thrombus is subsequently formed through aggregation of blood platelets and activation of the coagulation cascade causing the most dreaded ischaemic complications. A thrombus is subsequently formed through aggregation of blood platelets and activation of the coagulation cascade causing the most dreaded ischaemic complications. In the following, we will discuss the literature per hormone on each of these individual steps in atherogenesis, plaque instability, and thrombosis, if present. It is well-known that thyroid hormone can indirectly modulate the process of atherogenesis by its effects on lipids, body weight and blood pressure. This is illustrated by the ensuing hypercholesterolaemia and obesity in overt hypothyroidism, weight- and lipid-lowering in overt hyperthyroidism, and hypertension in both. At the hepatic level, thyroid hormone...
stimulates both lipogenesis and lipolysis. In addition, thyroid hormone induces the expression of hepatic LDL receptors and enhances hepatic removal of lipids from the circulation\[^{34}\]. However, there are more interactions of interest. Thyroid hormone can also directly influence atherogenesis through its effects on oxidative stress. Oxidisability of LDL is reported to be increased in hypothyroid patients, normalising after T4 treatment, and thyroid hormone has been shown to decrease the \textit{in vitro} LDL oxidation by endothelial cells and macrophages through altering the intracellular redox systems\[^{35;36}\]. Thyroid hormones also target endothelial cells; T3 that is formed after local conversion of T4 by tissue deiodinases, and possibly T4 itself as well, stimulate endothelium-derived NO synthase (eNOS), causing some of the rapid (nongenomic) vasodilatory effects observed in thyrotoxicosis\[^{31}\]. Based on the above, most effects of thyroid hormone seem to suppress atherogenesis and are thus protective for cardiovascular disease. However, an increasing body of evidence suggests an excess of thyroid hormone to be prothrombotic\[^{37}\]. Thyroid hormone was found to upregulate receptor-mediated transcription of fibrinogen and several other coagulation factors \textit{in vitro}\[^{38}\]. In addition, thyroid hormone-induced upregulation of mRNA expression and protein synthesis of von Willebrand factor (VWF) was observed in human umbilical vein endothelial cells\[^{40}\]. Indeed, high levels of factor VIII (FVIII) and VWF contribute to a hypercoagulable state in hypertrophic patients, whereas lower VWF levels were reported in hypothyroidism\[^{32}\]. Other studies have shown impaired platelet reactivity to ristocetin, adrenalin and collagen in hypothyroidism, the reverse of which might be true in hyperthyroidism, but additional studies are warranted to confirm this\[^{41;42}\]. Figure 1A summarises the pathophysiological mechanisms through which thyroid hormone could modulate atherogenesis and thrombosis.

Endogenous hypercortisolism or excessive use of exogenous glucocorticoids (time accumulating dose) are atherogenic conditions as they lead to enhanced accumulation of visceral and liver fat with a secondary increase in insulin resistance, hypertension, and dyslipidemia\[^{43}\]. In addition, cortisol can negatively affect endothelial NO availability by glucocorticoid receptor-mediated regulation of gene transcription (genomic actions)\[^{44}\]. Conversely, cortisol can directly decrease oxidative stress through inhibition of nuclear factor-κB (NF-κB) with a diminished release of harmful reactive oxygen species (ROS), and is able to acutely enhance NO production by ways of non-transcriptional activation of endothelial NO synthase (eNOS) (non-genomic actions)\[^{45}\]. Of note, in healthy subjects, short term variation in plasma cortisol concentrations, both at physiological and supraphysiological levels, failed to affect NO-dependent vasomotor function\[^{46}\]. In animal models, application of dexamethasone- or methylprednisolone-coated stents prevented neo-intima formation after local injury, and the latter steroid has also been shown to reduce macrophage density in the locally damaged artery\[^{47;48}\]. Cortisol further suppresses the expression of adhesion molecules on endothelial cells, decreases leucocyte extravasation and migration, and potentiates norepinephrine-induced contractions of vascular smooth muscle cells through glucocorticoid receptors, which limits the extent of inflammation within the vascular wall\[^{49}\]. As such, cortisol exhibits both beneficial and harmful effects on atherogenesis. It is possible that these opposing effects occur at different cortisol levels, with beneficial effects mainly occurring within the physiological range and harm only inflicted by prolonged conditions of cortisol excess. Similar opposing effects may exist in relation to thrombosis. Glucocorticoids are prothrombotic by increasing the hepatic production of coagulation factors\[^{50;51}\]. Also, several \textit{in vitro} and \textit{in vivo} studies have reported increased levels of tissue-type plasminogen activator (t-PA), plasminogen activator inhibitor-1 (PAI-1) and VWF after exposure of glucocorticoids to human endothelial cells or in healthy volunteers, but results have been inconsistent\[^{52;53}\]. Conversely, glucocorticoids have been hypothesised to inhibit platelet activation by downregulating the synthesis of endothelial prostacyclin, although \textit{ex vivo} studies did not confirm this\[^{54;55}\]. Figure 1B summarises the possible targets of cortisol in atherogenesis and thrombosis.

A deficiency of GH in adulthood, and as a consequence low circulating levels of IGF-1, is characterised by a disturbed postprandial clearance of triglyceriderich lipoproteins. This is possibly due to the combination of increased VLDL synthesis and less hepatic expression of LDL-receptors, as well as decreased levels of 17-OH-hydroxylase, a major rate limiting enzyme in bile metabolism\[^{56;57}\]. Moreover, an increase of visceral and liver fat with elevated levels of free fatty acids, a marker of insulin resistance, is present among these patients. All these factors contribute to an atherogenic phenotype in adult-onset GH deficiency. Acromegalic patients, however, may present with a more atherogenic profile as well, including higher levels of triglycerides and apoB, and increased insulin resistance\[^{58}\]. The latter may be mediated by suppression of adiponectin by GH\[^{59}\]. Both GH and IGF-1 directly stimulate the production of endothelial NO, and patients with adult onset GH deficiency appear to have reduced NO levels\[^{60}\]. Genes that are linked with cellular processes that control weakening of atherosclerotic plaques, such as apoptosis, are partly related to local expression of IGF-1\[^{61}\]. Indeed, high levels of IGF-1 are associated with a higher plaque stability (that is estimated with plaque echogenicity) in the elderly\[^{62}\]. In imitation of cortisol, GH, and possibly IGF-1 appear to have both beneficial and harmful effects on atherogenesis, and again it is conceivable that these opposing effects occur at different levels. Until now, little is known about the influence of GH and IGF-1 on thrombosis in acromegalic patients. In one study, fibrinogen, antithrombin III (ATIII), t-PA, and PAI-1 were significantly increased in patients with acromegaly compared to controls, whereas protein S activity and TFPI levels were decreased\[^{63}\]. It was therefore suggested that acromegalic patients may be in a potential hypercoagulable and hypofibrinolytic state, which might augment the risk of acute cardiovascular complications, but further research including tests of direct thrombin generation or fibrinolytic capacity is needed to confirm this. A small number of studies have also shown higher levels of fibrinogen, PAI-1 and t-PA in patients with adult-onset GH deficiency patients, with a substantial reduction after 2 years of GH therapy\[^{64;65}\]. Figure 1C shows the possible role of somatotropic hormones in atherogenesis and thrombosis.

Recently, it was suggested that prolactin could contribute to an atherogenic phenotype. Hyperprolactinaemic patients present with a higher insulin resistance index; this may be prolactin-mediated through suppression of adiponectin release by adipose tissue\[^{66;67}\]. No role for prolactin in lipid synthesis has been described\[^{68}\]. However, functional prolactin receptors have been found on cells involved in atherogenesis, such as lymphocytes, monocytes, macrophages and endothelial cells. In fact, prolactin alters the responses of cultured endothelial cells after mechanical injury, by giving rise to abnormally shaped cells at the wound front and a reduced cell adhesion\[^{69;70}\]. Prolactin also stimulates smooth muscle cell proliferation and phagocytosis of apoptotic cells by macrophages\[^{71;72}\]. Recent observations indicate that intact prolactin can promote new vessel formation in metastatic breast cancer.
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In addition, prolactin might also stimulate angiogenesis in the atherosclerotic plaque, thus contributing to plaque instability. Matrix metalloproteinases (MMPs) are involved in remodelling of the atherosclerotic plaque. MMPs can cleave prolactin into its 16 kDa fragments, and these fragments inhibit endothelial cell proliferation and promote apoptosis. In addition, T3 is prothrombotic by enhancing the synthesis of hepatic and endothelium-derived coagulation and fibrinolytic proteins, mainly factor VIII and VWF. These data suggest that both the indirect and direct effects of hormones on the process of atherogenesis, plaque disruption and thrombosis may be numerous, and sometimes opposing. Although preliminary, these results may offer new possibilities in cardiovascular risk reduction.

Figure 1A. Thyroxine (T4) and tri-iodothyronine (T3) are both secreted by the thyroid gland and these hormones are bound to thyroid binding globulin in the circulation. T4 is converted into T3 by local deiodinases expressed in various tissues. The unbound fraction of T3 is the bioactive hormone, and T3 binds to the nuclear thyroid receptor (TR) with high affinity. There are two isoforms of TR, TRα and TRβ. T3 can indirectly attenuate the process of atherogenesis by increased expression of hepatic LDL-receptors, and thereby enhancing hepatic removal of lipids. Thyroid hormone excess also reduces obesity and increases blood pressure and insulin sensitivity. In addition, after T3 enters the endothelial cell, non-genomic actions of T3 enhance the production of nitric oxide (NO) with subsequent vasodilatation. Thyroid hormone decreases LDL oxidation in endothelial cells and macrophages. On the other hand, T3 is prothrombotic by enhancing the synthesis of hepatic and endothelium-derived coagulation and fibrinolytic proteins, mainly factor VIII and VWF. ECM indicates extracellular matrix; NO, nitric oxide; PAI-1, plasminogen activator inhibitor-1; SMC, smooth muscle cell; T3, tri-iodothyronine; T4, thyroxine; t-PA, tissue-type plasminogen activator; TR, thyroid receptor; TRE, thyroid responsive elements; and VWF, von Willebrand factor.

Figure 1B. Cortisol is a steroid hormone that is secreted by the adrenal gland after stimulation of pituitary derived ACTH. In the circulation, cortisol is bound to cortisol binding globulin and its unbound form is the bioactive hormone. Cortisol binds with high affinity to the nuclear glucocorticoid receptor (GR). Indirect effects of increased cortisol levels on atherogenesis are numerous, including enhanced deposition of fat in different tissues with subsequent insulin resistance and secondary dyslipidaemia, and an increase in blood pressure. Conversely, cortisol reduces oxidative stress, suppresses the expression of adhesion molecules, and controls leucocyte extravasation and migration. Interestingly, non-genomic and genomic actions of cortisol on NO synthesis exert an opposite effect. Glucocorticoids may be prothrombotic by increasing the hepatic production of coagulation factors and, possibly, by enhancing the secretion of VWF, PAI-1, and t-PA by endothelial cells. The effect on platelet function is not fully elucidated. ACTH indicates adrenocorticotropic hormone; ECM, extracellular matrix; GR, glucocorticoid receptor; ICAM, intracellular adhesion molecule; NFκB, nuclear factor-kappa beta; NO, nitric oxide; PAI-1, plasminogen activator inhibitor-1; ROS, reactive oxygen species; SMC, smooth muscle cell; t-PA, tissue-type plasminogen activator; VCAM, vascular cell adhesion molecule; and VWF, von Willebrand factor.

disease, probably by endothelial cell proliferation. In addition, prolactin might also stimulate angiogenesis in the atherosclerotic plaque, thus contributing to plaque instability. Matrix metalloproteinases (MMPs) are involved in remodelling of the atherosclerotic plaque. MMPs can cleave prolactin into its 16 kDa fragments, and in vitro these fragments inhibit endothelial cell proliferation and promote apoptosis. This could imply that, within the atherosclerotic plaque, 16 kDa prolactin inhibits local repair mechanisms. Taken together, there is some evidence which indicates that prolactin could be involved in atherogenesis. On the other hand, T3 is prothrombotic by enhancing the synthesis of hepatic and endothelium-derived coagulation and fibrinolytic proteins, mainly factor VIII and VWF. ECM indicates extracellular matrix; NO, nitric oxide; PAI-1, plasminogen activator inhibitor-1; SMC, smooth muscle cell; T3, tri-iodothyronine; T4, thyroxine; t-PA, tissue-type plasminogen activator; TR, thyroid receptor; TRE, thyroid responsive elements; and VWF, von Willebrand factor.

unclear, but enhanced platelet activation in response to prolactin has been described. However, the data on a possible relationship between plasma levels of prolactin and platelet activation are inconclusive.

These data suggest that both the indirect and direct effects of hormones on the process of atherogenesis, plaque disruption and thrombosis may be numerous, and sometimes opposing. Although preliminary, these results may offer new possibilities in cardiovascular risk reduction.
Perspectives on therapeutic approaches for cardiovascular risk reduction

Several new therapeutic approaches that target endocrine pathways have been developed. Most of these novel compounds do not modulate circulating hormone levels, but instead act locally at the tissue level in order to minimise systemic effects. An example is the development of selective agonists for the nuclear thyroid receptor $\beta_1$-isoform ($TR\beta_1$), which displays preferential lipid-lowering effects without influencing heart rate by $\alpha_1$-isoform stimulation. Accordingly, the $TR\beta_1$-selective agonist KB141 and a similar $TR\beta_1$-selective compound (GC-1) that display selective liver uptake were found to have lipid-lowering, anti-obesity and anti-diabetic effects in several rodent models and monkeys at doses that do not affect heart rate. Recently, the selective thyromimetic compound KB2115 was found to be safe and well tolerated in moderately overweight and hypercholesterolaemic humans and lead to a 40% lowering of LDL cholesterol after only 14 days of treatment. In addition, various tissues express the enzyme 11$\beta$-hydroxysteroid dehydrogenase type 1 (11$\beta$-HSD1), which converts cortisone to cortisol. This enzyme increases the local removal of cortisol and can prevent local hypercortisolism. Consequently, inhibition of 11$\beta$-HSD1 enhances insulin sensitivity in healthy volunteers, reduces blood glucose levels in diabetics and body weight in obese mice.

Tabel 1. Effect of an increase in hormonal levels on distinct aspects of atherothrombosis.

<table>
<thead>
<tr>
<th>Aspects in atherothrombosis</th>
<th>Thyroid hormone</th>
<th>Cortisol</th>
<th>GH/IGF-1</th>
<th>Prolactin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Direct effects</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endothelial dysfunction</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
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<td>+</td>
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<td>+</td>
<td>+</td>
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<tr>
<td>NO production</td>
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<td>0</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Adhesion molecule expression</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>VWF, PAI-1, t-PA</td>
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<td>+</td>
<td>+0</td>
<td>0</td>
</tr>
<tr>
<td>Pro-inflammatory cells</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Smooth muscle cell proliferation</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><strong>Indirect effects</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Obesity</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Insulin resistance</td>
<td>+</td>
<td>+</td>
<td>+0</td>
<td>+</td>
</tr>
<tr>
<td>Blood pressure</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Dyslipidaemia</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
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<tr>
<td>LDL receptor</td>
<td>+</td>
<td>+</td>
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</tr>
</tbody>
</table>

GH indicates growth hormone; IGF, insulin-like growth factor; NO, nitric oxide; VWF, von Willebrand factor; PAI-1, plasminogen activator inhibitor-1; t-PA, tissue-type plasminogen activator; LDL, low density lipoprotein; -, downregulation; +, upregulation; 0, no effect; I, opposing effects; and ?, conflicting effects.

Figure 1C. Growth hormone is secreted by the pituitary and increases hepatic synthesis of IGF-1. Both GH and IGF-1 are bound to binding proteins: GH binding protein and IGF binding proteins, respectively. GH binds with a high affinity to GH receptors and after binding, paracrine and autocrine release of IGF-1 occurs. GH has a lipolytic action on fat deposits in adipose and skeletal tissues with improvement of insulin sensitivity at long term. GH augments the removal of highly atherogenic triglyceride-rich lipoproteins from the circulation, especially in the postprandial phase. IGF-1 induces NO synthesis, promotes smooth muscle cell proliferation, and prevents cell apoptosis. The thrombotic effects of GH and IGF-1 are not clear. Furthermore, IGF-1 augments the removal of highly atherogenic triglyceride-rich lipoproteins from the circulation, especially in the postprandial phase. IGF-1 induces NO synthesis, promotes smooth muscle cell proliferation, and prevents cell apoptosis. The thrombotic effects of GH and IGF-1 are not clear. The thrombotic effects of GH and IGF-1 are not clear.
Conclusions

Several endocrine abnormalities increase the risk of cardiovascular morbidity and mortality. This is not limited to hormone excess or hormone deficiency, but even subtle hormonal disturbances may affect cardiovascular disease outcome. Despite a growing interest, this field is largely unexplored. Compelling evidence exists that hormones in itself, but even not through associated metabolic changes, can modify both the chronic and acute manifestations of cardiovascular disease. Research is currently focused on controlling tissue concentrations of hormones independently of their circulating levels. Although still preliminary, the attempts to target these peripheral tissues may open new windows for the prevention of cardiovascular disease.

References


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